

Video Article

Growing Magnetotactic Bacteria of the Genus *Magnetospirillum*: Strains MSR-1, AMB-1 and MS-1

Lucas Le Nagard¹, Viviana Morillo-López², Cecile Fradin¹, Dennis A. Bazylinski²

¹Department of Physics & Astronomy, McMaster University

²School of Life Sciences, University of Nevada Las Vegas

Correspondence to: Cecile Fradin at fradin@physics.mcmaster.ca

URL: <https://www.jove.com/video/58536>

DOI: [doi:10.3791/58536](https://doi.org/10.3791/58536)

Keywords: Biology, Issue 140, Magnetotactic bacteria, magnetotaxis, *Magnetospirillum*, AMB-1, MS-1, MSR-1, Magnetosomes, Oxic-anoxic interface.

Date Published: 10/17/2018

Citation: Le Nagard, L., Morillo-López, V., Fradin, C., Bazylinski, D.A. Growing Magnetotactic Bacteria of the Genus *Magnetospirillum*: Strains MSR-1, AMB-1 and MS-1. *J. Vis. Exp.* (140), e58536, doi:10.3791/58536 (2018).

Abstract

Magnetotactic bacteria are Gram-negative, motile, mainly aquatic prokaryotes ubiquitous in freshwater and marine habitats. They are characterized by their ability to biomineralize magnetosomes, which are magnetic nanometer-sized crystals of magnetite (Fe_3O_4) or greigite (Fe_3S_4) surrounded by a lipid bilayer membrane, within their cytoplasm. For most known magnetotactic bacteria, magnetosomes are assembled in chains inside the cytoplasm, thereby conferring a permanent magnetic dipole moment to the cells and causing them to align passively with external magnetic fields. Because of these specific features, magnetotactic bacteria have a great potential for commercial and medical applications. However, most species are microaerophilic and have specific O_2 concentration requirements, making them more difficult to grow routinely than many other bacteria such as *Escherichia coli*. Here we present detailed protocols for growing three of the most widely studied strains of magnetotactic bacteria, all belonging to the genus *Magnetospirillum*. These methods allow for precise control of the O_2 concentration made available to the bacteria, in order to ensure that they grow normally and synthesize magnetosomes. Growing magnetotactic bacteria for further studies using these procedures does not require the experimentalist to be an expert in microbiology. The general methods presented in this article may also be used to isolate and culture other magnetotactic bacteria, although it is likely that growth media chemical composition will need to be modified.

Video Link

The video component of this article can be found at <https://www.jove.com/video/58536/>

Introduction

Magnetotactic bacteria (MTB) represent a wide range of Gram-negative prokaryotes ubiquitous in freshwater and marine aquatic habitats¹. These bacteria share the ability to produce magnetic crystals made of either magnetite (Fe_3O_4) or greigite (Fe_3S_4), which are in most cases assembled into chains inside the cells. This particular structural motif is due to the presence of several specific proteins acting both in the cytoplasm of the bacteria and on the lipid membrane that surrounds each crystal². Each individual crystal and its surrounding membrane vesicle is called a magnetosome and is ranging in size from about 30 to 50 nm in *Magnetospirillum* species³. Because of the chain arrangement of magnetosomes, these bacteria possess a permanent magnetic dipole moment that makes them align passively with externally applied magnetic fields. Therefore, these bacteria actively swim along magnetic field lines, acting as self-propelled micro-compasses presumably to more effectively locate the most favorable conditions (e.g., O_2 concentration) for growth.

An interesting property of MTB is their ability to regulate both the chemistry and the crystallography of their magnetosome crystals. Most strains produce relatively high purity crystals of either magnetite or greigite, although some biomineralize both minerals⁴. In all cases, the bacteria are able to precisely control the size and the shape of their single magnetic domain crystals. This explains why a great amount of research is undertaken to develop a better understanding of how MTB perform this biomineralization process. Understanding this process might allow the researchers to tailor-make magnetic nanocrystals for many commercial and medical applications.

A substantial obstacle to extensive research on MTB has been the difficulty of growing them in the laboratory. Most species, including the strains used in this work, are obligately microaerophilic when grown with O_2 as a terminal electron acceptor. This explains why these bacteria are most often found at the transition zone between oxic and anoxic conditions (the oxic-anoxic interface, OAI). This clearly shows that MTB have precise O_2 concentration requirements which obviously needs to be taken into account when devising growth media for these organisms. Moreover, the great existing diversity of MTB implies that different strains will need different types of chemical gradients and nutrients to achieve optimal growth.

In this work, we describe the methods for growing three of the most widely studied MTB: *Magnetospirillum magneticum* (strain AMB-1), *M. magnetotacticum* (MS-1) and *M. gryphiswaldense* (MSR-1). These species phylogenetically belong to the *Alphaproteobacteria* class in the *Proteobacteria* phylum, are helical in morphology and possess a polar flagellum at each end of the cell. We provide the protocols for growing

strain MSR-1 in both liquid and O₂ concentration gradient semi-solid media, based on previously published medium recipes^{5,6}. We also present a detailed protocol for growing strains AMB-1 and MS-1 in modified Magnetic Spirillum Growth Medium (MGS^M)⁷.

Protocol

1. Installation of the N₂ Station

NOTE: Choose the inner diameter of the tubing so that it can be connected to the gas tank with minimum leakage and so that the cylinder of a 1 mL plastic syringe tightly fits in this tubing. An illustration of the complete N₂ gassing station is provided in **Figure 1**.

1. Safely install a N₂ gas tank close to a bench on which there is enough space to set up the N₂ station (a length of approximately 50 cm).
2. Connect to the tank a piece of tubing long enough to reach the area where the station will be built. If necessary, apply Teflon tape at the output of the tank to avoid any leakage.
3. To build a station capable of bubbling five bottles of medium at the same time, cut four pieces of tubing of approximately 5 cm in length.
4. Assemble the pieces of tubing in a line with three three-way T-shaped plastic fittings. Connect one end of this line to the piece of tubing at the output of the N₂ tank through an extra T-shaped fitting. Add a 90° elbow fitting at the other end.
5. Use tape to attach the structure to a horizontal metal rod placed approximately 30 cm above the bench.
6. Connect five pieces of tubing (approximately 20 cm in length) to the free outputs of the fittings installed in Step 1.4.
7. Remove the pistons from five 1.0 mL plastic syringes and cut the larger end of these syringes (*i.e.*, the opposite side to the needle), keeping only the graduated part. Fill these syringes with cotton, not too tightly.
8. Insert the syringes in the 20 cm long vertical pieces of tubing and use soapy water to ensure that there is no leakage when N₂ is flowing.
9. Remove the caps of five 25 G needles (0.5 mm x 25 mm) and insert these needles into the 10 cm pieces of thin tubing. Ensure that the needles tightly fit in the tubing.
CAUTION: There is a risk of stabbing during this step. Do it slowly and carefully.
10. Attach the needles prepared in Step 1.9 to the syringes of the N₂ station. Ensure that N₂ is flowing through all five lines and keep the station on stand-by.

2. Growth Medium Preparation

NOTE: It is possible to adjust the amount of medium prepared, as explained in Steps 2.1.2, 2.2.2 and 2.3.8. The amount of water and chemicals used just needs to be proportionally adjusted. The role of all medium components is described in **Supplementary Table 1**.

1. Preparation of liquid growth medium for MSR-1
 1. Prepare a 10 mM ferric citrate solution by adding 0.245 g of ferric citrate to 100 mL of distilled deionized water. Heat and stir to dissolve, until a yellow to orange clear solution is obtained. Autoclave the solution using a standard cycle (at least 15 min exposure at 121 °C) and then store this stock solution at room temperature in the dark.
NOTE: Discard the ferric citrate solution when a precipitate becomes obvious.
 2. In a beaker containing 1 L of distilled deionized water, add the following in order while stirring: 1.0 mL of the trace mineral supplement solution, 0.1 g of KH₂PO₄, 0.15 g of MgSO₄·7 H₂O, 2.38 g of HEPES, 0.34 g of NaNO₃, 0.1 g of yeast extract, 3.0 g of soy bean peptone, 4.35 mL of potassium lactate (60% w/w solution) and 5 mL of the 10 mM Fe(III) citrate stock solution.
NOTE: Adjust the quantities proportionally if a smaller amount of growth medium is needed. For a N₂ station capable of bubbling five bottles at the same time, such as the one built in Step 1 of this protocol, prepare 300 mL of medium.
CAUTION: Trace mineral and Fe(III) citrate stock solutions need to be kept sterile. To avoid contamination, use standard sterile technique when using them (flame open tops of bottles using a Bunsen burner) and use sterile pipette tips for dispensing. Store the mineral solution in a refrigerator at 4 °C.
 3. After the addition of all chemicals, adjust the pH to 7.0 with 1 M NaOH solution. Dispense the freshly prepared medium into 125 mL serum bottles. Pour 60 mL of medium in each bottle.
 4. Bubble N₂ into the medium for 30 min to remove the dissolved O₂, using the small tubing connected to the N₂ station described in Step 1. Place a butyl-rubber stopper on top of each bottle, leaving a small opening to allow the excess gas to exit the bottle.
CAUTION: Foam might form while bubbling with N₂. Adjust the gas flow accordingly to avoid foam production.
 5. Crimp seal each bottle with the prepared stopper and an aluminum seal. The aluminum seal ensures that the bottle remains sealed during the rest of the protocol.
 6. Disconnect the needles and the thin tubing from the N₂ station and replace them with clean needles (1 inch, ≤ 23G). Adjust the valves of the N₂ tank so that a gentle continuous flow of gas exits the tank (about 50 mL/min).
Note: Needles larger than 23G may leave permanent unsealable holes in the stopper.
 7. Insert one of the needles connected to the N₂ tank into a bottle of medium, through the rubber stopper. Immediately insert another clean needle into the same bottle. Repeat this step for the other bottles and let N₂ flow for about 30 min to replace the air in the bottles by N₂.
 8. Disconnect one bottle from the N₂ station by removing the corresponding needle. Wait for a few seconds until the pressure in the bottle of medium decreases to atmospheric pressure and remove the second needle. Repeat this step for all remaining bottles.
CAUTION: To prevent O₂ from re-entering the bottles of growth medium after Step 2.1.4, perform Steps 2.1.4 - 2.1.8 in quick succession. If all bottles cannot be connected to the N₂ station at the same time, proceed with Steps 2.1.4-2.1.8 for the first set of bottles and then repeat these steps for the remaining bottles.
 9. Autoclave the bottles. Let them cool down to room temperature overnight and store them at room temperature afterwards.
2. Preparation of liquid growth medium for AMB-1 and MS-1

1. Prepare a 10 mM ferric quinate solution. First dissolve 0.19 g of quinic acid in 100 mL of distilled deionized water, then add 0.27 g of $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$. Stir to dissolve, until a dark red, clear solution is obtained. Autoclave the solution using a standard cycle (at least 15 min exposure at 121 °C).
NOTE: Store the ferric quinate solution at room temperature in the dark as a sterile stock solution. Discard the solution when a precipitate becomes obvious.
 2. In a beaker containing 1 L of distilled deionized water, add the following in order while stirring: 10.0 mL of the vitamin supplement solution, 5.0 mL of the trace mineral supplement solution, 0.68 g of KH_2PO_4 , 0.848 g of sodium succinate dibasic hexahydrate, 0.575 g of di-Sodium tartrate dihydrate, 0.083g of Sodium acetate trihydrate, 0.45mL of 0.1% aqueous Resazurin, 0.17 g of NaNO_3 , 0.04 g of ascorbic acid and 3.0 mL of the 10 mM Fe(III) quinate stock solution.
NOTE: Adjust the quantities proportionally if a smaller amount of growth medium is needed. For a N_2 station capable of bubbling five bottles at the same time, such as the one built in Step 1 of this protocol, prepare 300 mL of medium.
CAUTION: Vitamin, trace mineral and Fe(III) quinate stock solutions need to be kept sterile. To avoid contamination, use standard sterile technique and sterile pipette tips when dispensing. Store the mineral and vitamin solutions in a refrigerator at 4 °C.
 3. After the addition of all chemicals, adjust the pH to 6.75 using 1 M NaOH solution.
 4. Refer to Steps 2.1.3-2.1.9 for the rest of the protocol.
3. Preparation of semi-solid growth medium for MSR-1
1. Prepare a 0.5 M phosphate buffer solution pH 7.0 by dissolving 3.362 g of K_2HPO_4 and 4.178 g of KH_2PO_4 in 100 mL of distilled deionized water. Check if the pH is 7.0 and adjust the pH slightly with KH_2PO_4 or NaOH if needed. Store the solution in a sealed glass bottle (preferable to plastic in order to avoid oxygen exchange).
 2. Prepare 100 mL of a 0.02 M hydrochloric solution. Add 0.2 g of $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ to this solution and stir to dissolve in order to obtain a 10 mM iron chloride solution. Store the solution in the dark in a sealed glass bottle.
 3. Prepare a 0.8 M sodium bicarbonate solution by dissolving 6.72 g of NaHCO_3 in 100 mL of distilled deionized water. Store the solution in a sealed glass bottle.
 4. Autoclave the solutions prepared in Steps 2.2.1 - 2.2.3 using a standard cycle (at least 15 min exposure at 121 °C). Store them as sterile stock solutions in the dark.
 5. In a beaker containing 1 L of distilled deionized water, add the following in order while stirring: 5 mL of the trace mineral supplement solution, 0.2 mL of 1% aqueous resazurin solution, 0.4 g of NaCl, 0.3 g of NH_4Cl , 0.1 g of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.05 g of $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 1 g of sodium succinate, 0.5 g of sodium acetate, 0.2 g of yeast extract and 1.6 g of agar.
 6. Cover the beaker with aluminum foil and autoclave the solution prepared in Step 2.3.5.
 7. Just before the end of the autoclave cycle, prepare a fresh 4% L-cysteine·HCl·H₂O solution by dissolving 0.8 g of L-cysteine·HCl·H₂O in 20 mL of distilled deionized water. Neutralize the solution to pH 7.0 with 5 M NaOH solution.
CAUTION: it is important that the cysteine solution is prepared fresh to avoid oxidation of the cysteine. Store the mineral solution in a refrigerator at 4 °C.
 8. After autoclaving, let the medium cool down to 50–60 °C and bring the beaker under the flame of a Bunsen burner. Remove the aluminum foil and quickly add the following in order while gently stirring: 0.5 mL of the vitamin solution, 2.8 mL of the sterile phosphate buffer stock solution, 3 mL of the sterile iron chloride stock solution, 1.8 mL of the sterile sodium bicarbonate stock solution and 10 mL of filter-sterilized cysteine solution.
NOTE: Adjust the quantities proportionally if a smaller amount of growth medium is needed. It is usually convenient to prepare a batch of 120 mL of medium.
CAUTION: All solutions must remain sterile for future use. Perform Step 2.3.8 using standard sterile technique and sterile pipette tips when dispensing.
 9. After the addition of all chemicals, transfer the warm medium into 16 mL sterile screw-cap Hungate tubes. Transfer 12 mL of the medium into each tube and seal the tubes.
CAUTION: To avoid contamination, perform Step 2.3.9 under the flame of a Bunsen burner. Perform it before the agar solidifies, while the medium is at 40 °C or above.
 10. Leave the tubes undisturbed for several hours until the agar solidifies and the OAI becomes apparent, materializing as a pink to colorless interface, approximately 1 to 3 cm below the surface of the medium.
NOTE: The medium should slowly turn colorless in the tube. The amount of time needed for this transition is variable and depends on the amount of O_2 initially dissolved in the medium.

3. Inoculation of MTB

NOTE: The cultures of strains AMB-1, MS-1 and MSR-1 can be obtained commercially (**Table of Materials**).

CAUTION: Perform all the following steps in sterile conditions, under the flame of a Bunsen burner.

1. Inoculation of strains MSR-1, AMB-1 and MS-1 in liquid medium
 1. Seal an empty 125 mL serum bottle with a butyl-rubber stopper and an aluminum crimp seal. Insert two needles in the bottle through the stopper, and connect a syringe prepared as in Step 1.7 to one of them. Connect the syringe to a cylinder of O_2 through the same type of tubing as the one used for the N_2 station.
 2. Let O_2 flow through the bottle for about 30 min, to ensure that all air in the bottle is replaced by O_2 . Remove both needles, allowing for a slight overpressure in the bottle and then autoclave. Allow the bottle to cool down to room temperature before use.
NOTE: To save time, perform Steps 3.1.1 and 3.1.2 during the medium preparation and autoclave the bottle along with the medium or the stock solutions.
 3. Sterilize the tops of the stoppers of both the fresh medium bottle and the O_2 bottle by applying a few droplets of 70% ethanol solution on top of them and passing them through the flame of a Bunsen burner.
 4. Using a sterile syringe and a needle, extract 1 mL of O_2 from the O_2 bottle and transfer it into the fresh medium bottle. Make sure that the needle tightly fits on the syringe during this step to avoid any air in the syringe.

5. If using the inoculum from another culture grown in a glass bottle, sterilize the stoppers of both the fresh medium bottle and the older culture bottle by applying a few droplets of 70% ethanol solution on top of them and passing them through the flame of a Bunsen burner. If using the inoculum from a tube of frozen culture, just let it warm up to room temperature with the tube sealed under the flame of a Bunsen burner.
 6. If using the inoculum from another culture grown in a glass bottle, inoculate 1 mL of the older culture into the fresh medium. If using the inoculum from a frozen stock, inoculate only 0.1 mL to dilute the glycerol or dimethyl sulfoxide (DMSO) used in the freezing process. In both cases, use a sterile needle and a sterile syringe.
 7. Incubate the culture at 32 °C and inoculate it into fresh medium after 4 to 7 days.
2. Inoculation of MSR-1 in O₂ gradient semi-solid medium
 1. Verify that the tube of fresh medium displays a well-defined OAI, materialized by a pink to colorless interface.
 2. If the inoculum is coming from another O₂ concentration gradient semi-solid culture, harvest the bacteria by pipetting 50 µL of the culture with a sterile pipette tip placed on the band formed by the bacteria. Slowly inoculate these bacteria at the OAI in the fresh medium (pink/colorless interface), avoiding disturbing the interface. If the inoculum is coming from a frozen culture, proceed in the same way with 100 µL of inoculum instead.
 3. Seal the tube and let the bacteria grow between 25 °C and 30 °C. Transfer into fresh medium using the same procedure before the band of bacteria reaches the surface of the medium.

4. Observation of the Bacteria

1. Sterilize the stopper of the culture bottle by applying 70% ethanol solution on top of it and passing it through the flame of a Bunsen burner. For semi-solid medium, open the tube under the flame of a Bunsen burner. Use a sterile needle and a sterile syringe to extract the bacteria.
2. Use the hanging drop method⁸ to ensure that the bacteria are both magnetic and motile.
NOTE: Phase contrast microscopy gives great results but is not mandatory. Magnifications ranging from 10X to 60X are suitable.
3. Use transmission electron microscopy (TEM) to observe the cell structure and the magnetosomes in detail⁸.

Representative Results

Successful preparation of the growth media can be assessed as follows. At the end of the process, clear solutions (*i.e.*, free of any precipitate) should be obtained (this is true for both the liquid media and the O₂ gradient semi-solid medium). A picture displaying the expected aspect of MSR-1 liquid medium before inoculation can be seen in **Figure 2a**. A successful O₂ concentration gradient semi-solid medium is signaled by the formation of an OAI after a few hours, indicated by the presence of 1–3 cm of pink medium at the top of the tube (**Figure 3**, tube A). The rest of the medium should be colorless. An all-pink medium before inoculation is a sign of complete oxidation of the resazurin and therefore of an unsuccessful preparation of the O₂ concentration gradient.

Successful growth in liquid medium can be checked about 48 h after inoculation (or after a few days if the inoculum is coming from a frozen culture) simply by examining the cultures for turbidity using a light source or a spectrophotometer. Successful growth is confirmed by turbidity proving that the bacteria actually grew (**Figure 2b**). If the medium remains clear after 48 h, the bacteria have not grown. For a liquid medium culture growing normally, the presence of bacteria synthesizing magnetosomes can be checked after 48 h by placing the bottle of medium on a magnetic stir plate and by illuminating it with a flashlight. At a low stirring speed, the medium should "flash", looking alternately darker and brighter depending on the orientation of the stirring magnet with respect to the position of the experimentalist. For a non-magnetic culture, the intensity of light scattered by cells towards the experimentalist will remain constant.

Successful growth in semi-solid medium is indicated by the formation of a microaerophilic band of bacteria at the pink/colorless interface. Due to the consumption of O₂ by the bacteria and the changes in the O₂ gradient, the band will slowly migrate to follow the OAI (**Figure 3**, tube B).

The hanging drop method enables the observer to check that the bacteria are both magnetic and motile. After a few minutes, MTB should concentrate at the edge of the hanging drop, proving that they actively swim along the magnetic field lines (**Figure 4a**). To ensure that the cells are magnetic, flip the orientation of the magnet sitting next to the drop. The bacteria should start swimming towards the opposite edge (**Figure 4b**). Finally, the shape of the magnetosomes can be checked with TEM. Cuboctahedral crystals of about 30–45 nm in diameter should be observed⁹. These crystals are normally arranged in one long chain or multiple shorter chains in the species studied here (**Figure 5**).

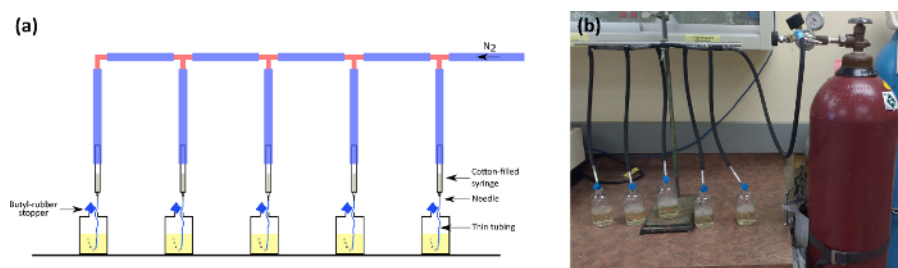


Figure 1: Illustration of the N₂ gassing station. (a) Schematic representation. (b) Picture of the setup. A successful station will have the appropriate length of large and thin tubing so that the end of the thin tubing described in Step 1.9 of the protocol remains immersed in the medium during the gas bubbling step. The flow of gas should also be adjustable at all times, to ensure that all O₂ is removed and that the medium does not spill. [Please click here to view a larger version of this figure.](#)

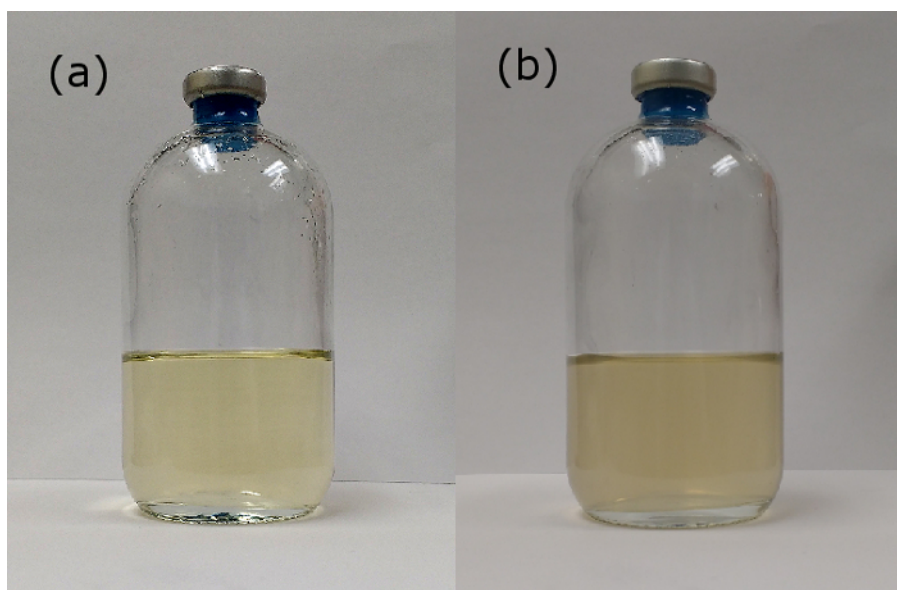


Figure 2: Bottles of liquid growth medium before and after inoculation. (a) Clear MSR-1 medium before inoculation. (b) Turbid MSR-1 medium after 3 days of successful growth. [Please click here to view a larger version of this figure.](#)

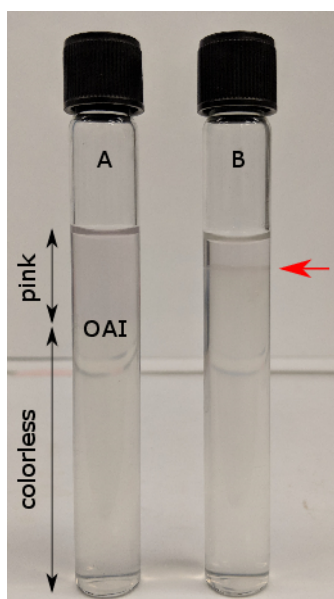


Figure 3: Tubes of O_2 gradient semi-solid medium before (tube A) and 10 days after (tube B) inoculation. The band of bacteria in tube B is indicated by a red arrow.

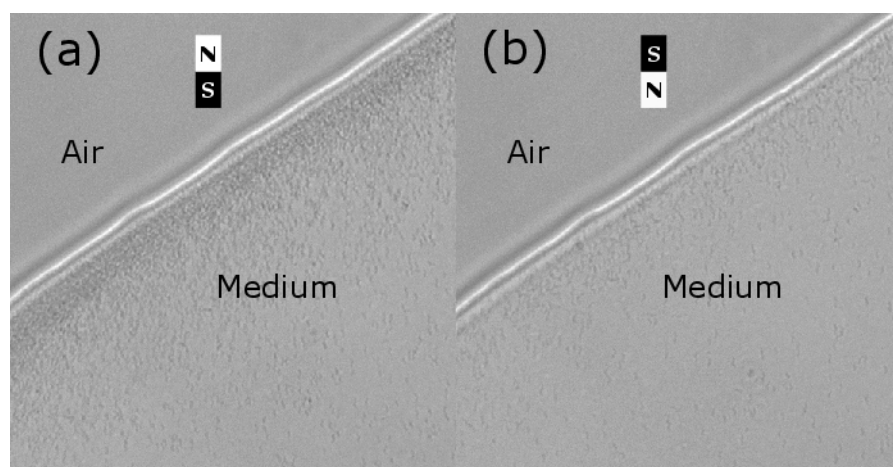


Figure 4: Typical results of a hanging drop experiment at 20X magnification using phase contrast microscopy. (a) MTB swim towards the south pole of a magnet and accumulate at the liquid/air interface. **(b)** 1 min after flipping the magnet, most cells have left the field of view.

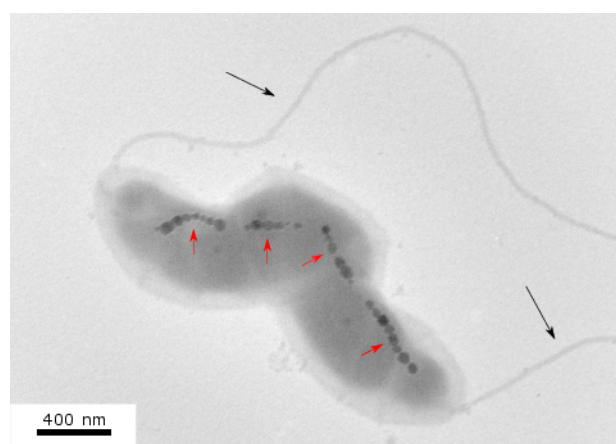


Figure 5: TEM observation of an AMB-1 cell. Magnetosome chains are indicated by red arrows. The two flagella are indicated by black arrows.

Supplementary Table 1. [Please click here to download this table.](#)

Discussion

The specific O_2 concentration requirements of MTB make them non-trivial to grow in the laboratory. A key step of the protocol for liquid medium is the initial removal of all O_2 from the medium in order to control the final concentration by adding a definite volume of O_2 , just before inoculation. It has been shown that MSR-1 grows under almost fully aerobic conditions, however, the magnetism of the cells is drastically reduced. The results from the same study showed that strains AMB-1 and MS-1 do not grow under fully aerobic conditions⁶. For the semi-solid culture of MSR-1, all O_2 is first reduced by the cysteine solution and then the O_2 concentration gradient appears, leading to the formation of the OAI. If a culture does not grow well or is not magnetic, the O_2 concentration should be the first thing to check.

Semi-solid growth medium is an interesting choice for isolating unknown MTB or growing the species that are highly sensitive to both O_2 and redox gradients, as it ensures the existence of stable gradients and allows the bacteria to position themselves at the location offering the best growth conditions. Liquid medium is more convenient to work with when higher volumes are needed (e.g., for DNA extraction), or when further analysis needs to be conducted on the cells (e.g., magnetosome studies) as it allows for an easier separation of the cells from the growth medium. The agar in semi-solid medium might indeed interfere with the cell harvesting technique.

In some cultures, the cells sometimes become non-motile, likely due to spontaneous mutations. Non-motile cells survive and grow in the growth medium since they do not need to swim to find nutrients necessary for their survival. The standard race-track method¹⁰ can then be used to recover a motile culture since only motile cells can swim down the race-track. The loss of the magnetic phenotype can also happen in the culture even if the O_2 concentration is optimal, again due to spontaneous mutations¹¹. The best solution in that case is to start a new culture by inoculating frozen stocks of the wild-type bacteria into fresh medium. Alternatively, the race-track technique can also allow the recovery of a magnetic culture.

It is essential to avoid contamination of the culture by other organisms and therefore most of the protocol must be performed using sterile techniques. Manipulating under the flame of a Bunsen burner usually gives satisfactory results in keeping aseptic conditions. However, if the culture is grown in a contamination-prone environment, additional precautions should be taken, such as working in a laminar flow hood when

preparing the medium and inoculating the bacteria. It should be noted that the risk of contamination is higher in semi-solid medium as the tubes need to be opened every time the cells are removed.

These protocols enable the growth of enough bacteria to perform many different types of experiments, such as physical studies based on optical microscopy^{12,13}, electron microscopy imaging^{14,15}, X-ray spectromicroscopy analyses¹⁶, genomic and protein studies^{17,18}. If necessary, higher concentrations of bacteria in suspension can be achieved by centrifugation. In addition, magnetosomes can be extracted and purified for further applications from cell pellets or dense cell suspensions obtained by centrifugation¹⁹.

Growth media formulations are usually based on the same governing principles (see **Supplementary Table 1** for a summary of the list of the role of each component in the growth media described here). A carbon source must be available to the bacteria, via organic acids (e.g., succinate, acetate, tartrate, lactate) or inorganic compounds such as bicarbonate. The choice of a suitable carbon source depends on the metabolism of the bacteria. Nitrogen present in DNA and proteins, and phosphorus (DNA, membranes) are also required and provided by NaNO₃, NH₄Cl and KH₂PO₄ in the recipes described here. A buffer (HEPES, phosphate buffer) is necessary to resist pH changes. The trace mineral supplement solution contains cofactors for enzymes, and the vitamins can be used by the bacteria as coenzymes or as functional groups for certain enzymes. Yeast extract and soy bean peptone provide a source of amino acids and salts used for protein production. Since MTB are redox sensitive, one or several reducing agents must often be added to the medium (e.g., cysteine, ascorbic acid, lactate). A major source of iron (e.g., ferric citrate, ferric quinate, iron chloride) is needed in the medium for biomineralization. Finally, a small amount of O₂ is required for microaerophilic bacteria as terminal electron acceptor. Obligate anaerobic MTB use other compounds such as nitrate or nitrous oxide instead.

The principal limitation of these protocols is that there is no guarantee that they will work for other species of MTB. Strains AMB-1, MS-1 and MSR-1 are all freshwater MTB and therefore the recipes described in this article cannot be used to grow marine magnetospirilla such as *Magnetospira thiophila* strain MMS-1, *Magnetospira* strain QH-2, or other marine MTB, which require higher salt concentrations. However, to grow other strains of MTB, and for the isolation of new strains, the general methods presented in this article for both liquid and semi-solid media can be used to prepare media with different recipes.

Disclosures

The authors have nothing to disclose.

Acknowledgements

We thank Richard B. Frankel for his help with MTB cultures, Adam P. Hitchcock and Xiaohui Zhu for their support while setting up the MTB cultures at McMaster University, and Marcia Reid for training and access to the electron microscopy facility (McMaster University, Faculty of Health Sciences). This work was supported by the Natural Sciences and Engineering Research Council of Canada (NSERC) and the US National Science Foundation.

References

1. Blakemore, R. P. Magnetotactic bacteria. *Annual Reviews in Microbiology*. **36** (1), 217-238 (1982).
2. Uebe, R., Schüller, D. Magnetosome biogenesis in magnetotactic bacteria. *Nature Reviews Microbiology*. **14** (10), 621 (2016).
3. Faivre, D., Schuler, D. Magnetotactic bacteria and magnetosomes. *Chemical Reviews*. **108** (11), 4875-4898 (2008).
4. Bazylinski, D. A. *et al.* Controlled biomineralization of magnetite (Fe₃O₄) and greigite (Fe₃S₄) in a magnetotactic bacterium. *Applied and Environmental Microbiology*. **61** (9), 3232-3239 (1995).
5. Lefèvre, C. T. *et al.* Diversity of magneto-aerotactic behaviors and oxygen sensing mechanisms in cultured magnetotactic bacteria. *Biophysical Journal*. **107** (2), 527-538 (2014).
6. Heyen, U., Schüller, D. Growth and magnetosome formation by microaerophilic Magnetospirillum strains in an oxygen-controlled fermentor. *Applied Microbiology and Biotechnology*. **61** (5-6), 536-544 (2003).
7. Blakemore, R. P., Maratea, D., Wolfe, R. S. Isolation and pure culture of a freshwater magnetic spirillum in chemically defined medium. *Journal of Bacteriology*. **140** (2), 720-729 (1979).
8. Oestreicher, Z., Lower, S. K., Lin, W., Lower, B. H. Collection, isolation and enrichment of naturally occurring magnetotactic bacteria from the environment. *Journal of Visualized Experiments*. (69) (2012).
9. Pósfai, M., Lefèvre, C., Trubitsyn, D., Bazylinski, D. A., Frankel, R. Phylogenetic significance of composition and crystal morphology of magnetosome minerals. *Frontiers in Microbiology*. **4**, 344 (2013).
10. Wolfe, R. S., Thauer, R. K., Pfennig, N. A 'capillary racetrack' method for isolation of magnetotactic bacteria. *FEMS Microbiology Ecology*. **3** (1), 31-35 (1987).
11. Schübbe, S. *et al.* Characterization of a spontaneous nonmagnetic mutant of Magnetospirillum gryphiswaldense reveals a large deletion comprising a putative magnetosome island. *Journal of Bacteriology*. **185** (19), 5779-5790 (2003).
12. Nadkarni, R., Barkley, S., Fradin, C. A comparison of methods to measure the magnetic moment of magnetotactic bacteria through analysis of their trajectories in external magnetic fields. *PloS One*. **8** (12), e82064 (2013).
13. Waisbord, N., Lefèvre, C. T., Bocquet, L., Ybert, C., Cottin-Bizonne, C. Destabilization of a flow focused suspension of magnetotactic bacteria. *Physical Review Fluids*. **1** (5), 053203 (2016).
14. Komeili, A., Vali, H., Beveridge, T. J., Newman, D. K. Magnetosome vesicles are present before magnetite formation, and MamA is required for their activation. *Proceedings of the National Academy of Sciences of the United States of America*. **101** (11), 3839-3844 (2004).
15. Scheffel, A. *et al.* An acidic protein aligns magnetosomes along a filamentous structure in magnetotactic bacteria. *Nature*. **440** (7080), 110 (2006).
16. Zhu, X. *et al.* Measuring spectroscopy and magnetism of extracted and intracellular magnetosomes using soft X-ray ptychography. *Proceedings of the National Academy of Sciences of the United States of America*. **113** (51), E8219-E8227 (2016).

17. Schüler, D. Molecular analysis of a subcellular compartment: the magnetosome membrane in *Magnetospirillum gryphiswaldense*. *Archives of Microbiology*. **181** (1), 1-7 (2004).
18. Kolinko, I. *et al.* Biosynthesis of magnetic nanostructures in a foreign organism by transfer of bacterial magnetosome gene clusters. *Nature Nanotechnology*. **9** (3), 193 (2014).
19. Hergt, R. *et al.* Magnetic properties of bacterial magnetosomes as potential diagnostic and therapeutic tools. *Journal of Magnetism and Magnetic Materials*. **293** (1), 80-86 (2005).
20. Lefevre, C. T. *et al.* Novel magnetite-producing magnetotactic bacteria belonging to the Gammaproteobacteria. *The ISME Journal*. **6** (2), 440 (2012).
21. Williams, T. J., Lefèvre, C. T., Zhao, W., Beveridge, T. J., Bazylinski, D. A. *Magnetospira thiophila* gen. nov., sp. nov., a marine magnetotactic bacterium that represents a novel lineage within the Rhodospirillaceae (Alphaproteobacteria). *International Journal of Systematic and Evolutionary Microbiology*. **62** (10), 2443-2450 (2012).
22. Zhu, K. *et al.* Isolation and characterization of a marine magnetotactic spirillum axenic culture QH-2 from an intertidal zone of the China Sea. *Research in Microbiology*. **161** (4), 276-283 (2010).