

# Potential of Magnetotactic Bacteria for the Fabrication of Iron Nanoparticles

T. Thuy Minh Nguyen, Manish D. Baviskar and Paul Bernazzani

**Abstract** Magnetotactic bacteria are typically found in soils rich in iron. These prokaryote bacteria have the property of using ingesting atoms of iron and generating magnetosomes of nanoparticles of either diamagnetic or paramagnetic nature within each cell. We report on the use of magnetotactic bacteria for the production of uniform nanoparticles of iron mineral magnetite ( $\text{Fe}_3\text{O}_4$ ). The potential for mass production was investigated along with the molecular, physical, and magnetic properties of the magnetosomes using various growth and microscopy techniques. Results reveal that the magnetic particles are stable and that bacteria growth can be optimized to produce magnetosomes with different magnetic properties, suggesting that the industrial development of these bio manufactures lies in the foreseeable future.

**Keywords** Magnetosomes • Magnetic bacteria • Nanoparticles • Iron

## Introduction

The synthetic production of nanoparticles, the cornerstone of nanotechnology, involves various physical and chemical methods. However, a disadvantage of these methods is the production of toxic byproducts, possibly making them not environmentally safe methods [1]. Nanoparticle synthesis using biological systems would follow green chemistry principles as the reagents are eco-friendly including the reducing agent and the capping agent in the reaction [2, 3].

Biogenic nanoparticles involve natural phenomena that take place in the biological systems. Bacteria are considered as the most potent eco-friendly nanofactories [1]. Magnetotactic bacteria (MTB) are a heterogenous group of aquatic microorganisms that have the ability to orient and migrate along geomagnetic field

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lines, a behavior named magnetotaxis. This property is based on specific intracellular structures, the magnetosomes which, in most MTB are nanometer-sized, membrane bound magnetic particles, composed of the iron mineral magnetite ( $\text{Fe}_3\text{O}_4$ ) or more rarely, greigite ( $\text{Fe}_3\text{S}_4$ ). These magnetosomes are organized in one or more straight chains parallel to the long axis of the cells. Such an arrangement confers a magnetic moment to the cell. In the northern hemisphere these MTB are known to move towards the geographic North Pole, while in the southern hemisphere they are known to migrate towards the geographic south. Magnetotactic bacteria are found in diverse aquatic habitats and include coccoid to ovoid cells, rods, vibrios and spirilla from different water bodies (freshwater, seawater), sediment and soil. Reports of South-seeking magnetotactic bacteria found in Southern hemispheres sediments as well as of samples held in similar magnetic conditions in the Northern Hemisphere which verifies the hypothesis that downward direction is advantageous orientation and upward detrimental for the survival of the magnetotactic bacteria with unidirectional motility [4–6].

Bacterial magnetic nanoparticles have great useful potential in biotechnological and biomedical applications. In this study, a liquid growth medium will be modified for cultivation of a magnetotactic bacterium that has been isolated from sediment sample. These modifications include changes in the type and amounts of vitamin, minerals, carbon sources, etc. Serum bottles and designed air-tight laboratory bottles can be used to create microaerobic conditions in order to develop a method for scale-up experiment.

Magnetotactic bacteria (MTB) synthesize unique nanoparticle structures within magnetosomes. MTB's nanoparticles are coated with a thin organic membrane that results in high and homogeneous dispersions in aqueous solutions compared to artificial magnetic nanoparticles, making them ideal biotechnological materials [7, 8]. It has already been demonstrated, that isolation and axenic cultivation of MTB in pure culture is very difficult. To date only a small number of isolates and an even smaller number of genera and species are available [9, 10]. Recently, pure cultivation of magnetic bacteria in defined medium has provided effective advancement on the application of MTB's nanoparticles. Because of MTB's fastidious culture requirements, growth of most of them on a large scale is extremely difficult. Mass cultivation of MTB for magnetosome production may be one of the most important biotechnological processes in the application of MTB's nanoparticles. We report the investigation of a new approach for large-scale production of magnetotactic bacteria. The main goal of this study was development a method with low temperature, lower energy cost and high yield that may make biogenical magnetic nanoparticles a more economical and energy sustainable process than some of the competing processes.

## **Methodology**

### ***Isolation and Screening of Bacteria***

Samples were collected in sterile flasks and plastic containers for bacteriological analysis. The samples were stored in dark cold room at 40 °C for 28 days. The magnetotactic (MTB) bacteria were isolated using the race track method. Isolated bacteria were tested for magnetic particle synthesis ability.

### ***Optimization and Validation of Growth Condition***

Optimum growth condition and media for each isolates will be optimized by adjusting pH, temperature and Different chemical concentration and oxygen requirement. 250 ml Erlenmeyer flask will be used where 10 ml of Sterile Luria-Bertani (LB) broth liquid media will be used as baseline growth media to identify mixo-tropic growth of magnetic tactic bacteria. Then it will be incubated in Shaker overnight at 30 °C AT 150 RPM. Optical density of culture will be checked after 8 to 10 h at 600 nm using spectrophotometer to determine growth of bacteria.

### ***Optimization of Nano Magnetic Practical Synthesis Process***

Isolated cells were seeded in optimized media incubated at optimum growth condition by period analysis of magnet in different growth condition. Prussian blue assay was used to evaluate the presence of magnetosomes.

### ***Source of Microorganism and Liquid Media Preparation***

Magnetotactic bacteria were isolated from a water/sediment microcosm that was collected from the Marquez Crater. Control cells acquired from ATCC were also used. After magnetic collection and isolation methods, LB media was used for cultivation experiments. A modified liquid medium (MLM) was also used which contained Wolfe's vitamin solution, Wolfe's mineral solution, sodium succinate, yeast extract,  $\text{MgSO}_4 \times 7\text{H}_2\text{O}$ , peptone casein, potassium phosphate buffer (pH 7),  $\text{NH}_4\text{Cl}$ , sodium acetate anhydrous, resazurin, ferric citrate, HCl and the pH was adjusted to 7.0. HCl and vitamin solution were added to the MLM after autoclaving.

## ***Microscopy Studies***

Microscopy, isolated cells of the MTB were diluted by sterilized phosphate buffer solution (pH = 7, 10 mM) and then cells were placed on the surface of glass slides. The morphology and configuration of the MTB and their magnetosomes were investigated with different staining techniques.

## ***Isolation and Purification of Magnetosomes***

The magnetotactic bacteria were harvested by centrifugation (8000 rpm, 15 min, 4 °C) and washed by sterilized phosphate buffer solution (pH = 7.0). Then the precipitated cells were resuspended in 1 N NaOH and boiled for 30 min to lyse the cells. Magnetosomes from the disrupted cells were collected at a graduated cylinder by magnets for 1 h, then the nonmagnetic fluid was removed by aspiration and magnetic nanoparticles washed with buffer. Finally, the magnetosomes attracted to the magnet were carefully suspended in sterilized phosphate buffer solution (pH = 7.0).

Collection of Magnetic bacteria was done based on the cells' swimming response to a magnetic field. The south pole of a permanent magnet was attached outside a jar containing the water and sediment samples, 1 cm above the sediment surface. After 2–4 h 1–2 ml of the water in the bottle near the wall adjoining the magnet was collected with a pipette and transferred to sterile tubes to be used for further studies. "Race track" purification and enrichment of the MTB The modified capillary "race track" (CRT) method was used to purify and enrich the MTB obtained by magnetic collection. A capillary tube (length, 6–9 cm) sealed at one end in a gas flame was filled with medium by means of a long hypodermic syringe and was fitted to the narrow end of a Pasteur pipette. The sample material (magnetically collected cells) was placed on the top of a sterile, wetted cotton plug in the wide mouth end of the pipette that served as a reservoir. The capillary was exposed to a magnetic field produced along it with a permanent magnet for 5 h. The MTB migrated through the cotton plug towards the closed end of the capillary. The tip containing the accumulated MTB was then broken off and with the help of a sterile hypodermic needle the organisms transferred to the sterile enrichment medium taken in test tubes that were incubated at 30–35 °C for about two days. This method was repeated two more times to purify the MTB fully. Isolation of magnetic bacteria. The purified magnetic cells were then isolated by the streak plate method using a magnetic field and preserved (at 40 °C) on the same medium.

## ***Assessment of Culture Magnetism***

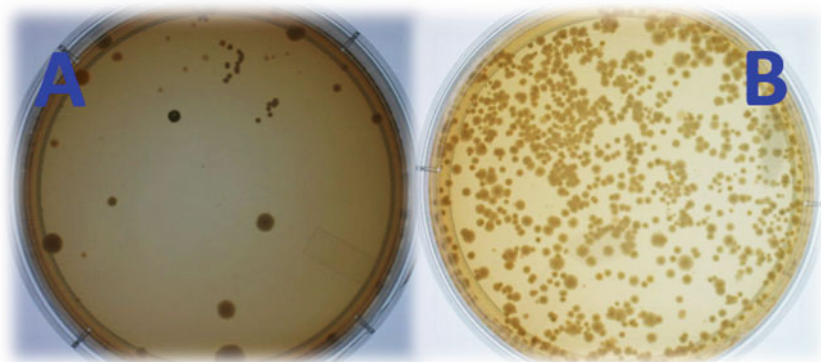
The isolates were tested for their magnetotactic response using the ‘hanging drop technique’ under an optical microscope, with the south pole of a bar magnet being placed some 10 cm distant from the slide. Their magnetic response was also tested in terms of the spreading of their growth on the surface of a semisolid (50:50) LB and MSGM medium [0.8% agar]. The isolates were inoculated in a straight line at the center of the medium in petriplates. The plates were incubated in a magnetic field created by placing the opposite poles of two different bar magnets on either side perpendicular to the line of streaking. The growth pattern after incubation was observed for any spreading towards the magnet poles. The isolated magnetic cultures and a known non-magnetic bacterial culture [*E. coli*] as control were grown in the MSGM medium that contained Ferric Quinate as source of iron. After obtaining sufficient growth (approximately 30–40 mg dry weight), the cell mass was separated by centrifugation at 10,000 g in a research centrifuge. The cell mass thus obtained was dried to constant weight at 105 °C in a hot air oven. Iron content of the cell mass was determined by Infra-red and UV-visible spectroscopy on a Model, using the tri-acid digestion method of iron extraction from the cells.

## **Results and Discussion**

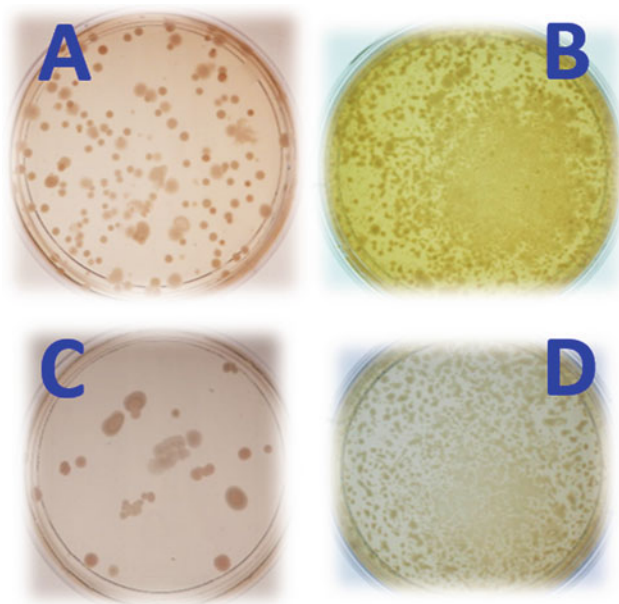
### ***Enrichment and Isolation***

Magnetotactic bacteria (MTB) were successfully enriched from sediment samples obtained from the Marquez crater using the magnetic collection method and purified by the capillary racetrack method. The mixed bacterial culture that was obtained was observed under a microscope and seen to include more than one morphological type. The streak plate method of isolation as well as a total viable count of the original samples were performed to verify the efficacy of the magnetic purification methods. About four different morphological forms of bacteria were obtained as pure cultures and different individual colony characteristic were observed. The bacteria forms included gram positive rod shaped bacteria, gram negative slender rod and gram positive coccus.

The original samples were grown under different media conditions. Figure 1 present the images of Petrie dishes containing the samples as received and following 10 days of incubation in standard media concentration. The growth potential of the samples is observed and optimization can be obtained. Following culture growth, magnetotactic bacteria were isolated using the race track method. Figure 2 shows the microscopy images of bacteria where two distinctive types were identified, one north seeking magnetotactic bacteria and another south seeking. These findings lead to the assessment of the magnetism and of the capacity of the samples to produce magnetosomes.



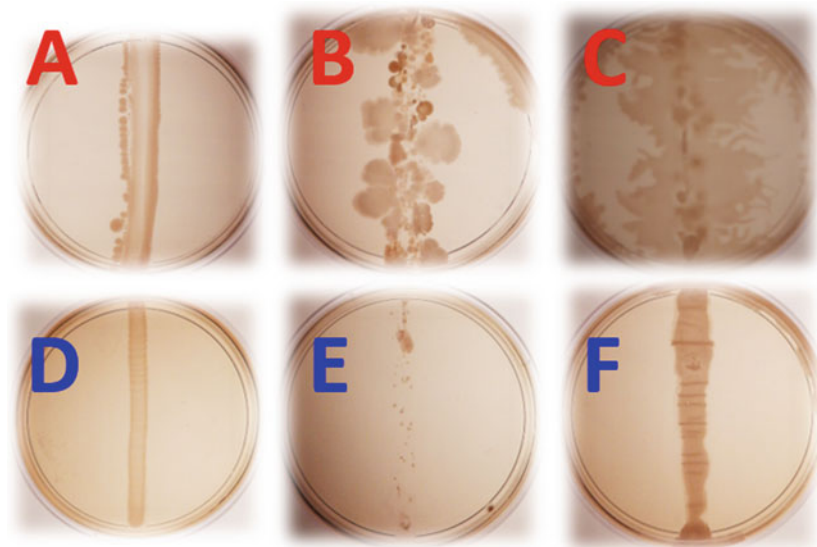
**Fig. 1** Images of sample cultures as obtained in different conditions. Original sediment (panel A), following 10 days of incubation (panel B)



**Fig. 2** Images of north (panels A and B) and south (panels C and D) seeking bacteria following 2 days (panels A and C) and 10 days (panels B and D) incubation times

### ***Assessment of Culture Magnetism and Iron Analysis***

The capability of the magnetotactic bacteria to orient themselves while under the influence of a magnetic field was evaluated by exposing the sediment containing the bacteria to both north and south poles of a magnet. Figure 3 compares the incubation

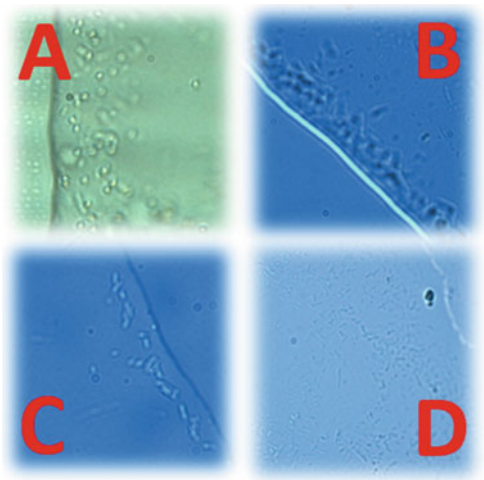


**Fig. 3** Images of bacterial growth when exposed to (panels **A**, **B** and **C**) and in the absence of an external magnetic field (panels **D**, **E**, and **F**). Panels **A** and **D** represent the control *E. coli* samples while panels **B** and **E** represent the cultures after 2 days of incubation and panels **C** and **F** represent the cultures after 10 days of incubation

of the bacteria when exposed to a magnetic field and under normal conditions. Bacteria were plated on a line going up and down on the figure, while the magnetic field is in a left to right orientation. Nonmagnetic cells, *E. coli*, were used as a control. The figure reveals that the sample containing both south and north seeking magnetotactic bacteria results in cell growth that expand toward left and right directions compared to control samples and those without exposure to a magnetic field. This result supports the fact that the prominent growth of magnetosome containing cells was achieved.

To further evaluate the magnetic properties of the cultures, the mixture before isolation as well as the individual isolated cultures were tested by the hanging drop technique and the semisolid medium method. Figure 4 presents the images of the hanging drop technique. Both, the mixed culture as well as the isolated organisms showed a response as expected. In the hanging drop observation. The cultures aligned parallel to each other along the magnetic field lines and showed a migration towards the South Pole and north pole of the magnet wherever it was placed. On the semisolid medium too, the MTB when placed in a magnetic field showed pronounced migration towards the South Pole referred to as ‘south seeking’, while in the absence of magnetic field they did not show any migration. The non-magnetic, motile control bacterial culture [*E. coli*] meanwhile did not show any migration towards any pole and grew as a straight line as inoculated but magnetic motile control *Magnetospirillum magnetotacticum* (ATCC-3163) shows orientation.

**Fig. 4** Images of the magnetic migration by the hanging drop method. The different images represent north (panels **A** and **B**) and south (panels **C** and **D**) seeking bacteria following 2 days (panels **A** and **C**) and 10 days (panels **B** and **D**) incubation times



**Table 1** Optimization parameters for growth of magnetosome containing bacteria

Isolates	Temperature optimization		Growth on liquid media (600 nm) after 3 h incubation (a.u.)	
	Range temperature (°C)	Optimal T (°C)	LB broth	Magnetic spirillum growth medium
A1-N	4–45	30	0.78	0.52
B1-N	4–45	30	0.83	0.39
A2-N	21–45	37	0.88	0.59
B2-N	21–45	37	0.83	0.32
A1-S	4–45	30	0.98	0.41
B1-S	4–45	30	0.76	0.29
A2-S	21–45	37	0.90	0.38
B2-S	21–45	37	1.27	0.24

Along with the temperature and media optimization data, these results and the morphological characters of the isolates are presented in Table 1. The last column represents the iron content of each isolated bacterial strain. The unambiguous correlation between the magnetic response and the iron content of the cells is clearly evident. It can also be seen that the magnetic bacteria accumulated up to more iron within their cells as compared to the non-magnetic cell. These results suggest that the growth of magnetosome containing bacteria can be optimized to yield promising amounts of cells. The next step would be the scale up of the optimized growth to yield manageable amount of nanoparticles.



## Conclusion

Magnetosome containing magnetotactic bacteria have been isolated from sediment samples. Partial growth optimization revealed that the temperature range, incubation temperature and type of media were critical for the efficient growth, but that these could be tailored. The presence of visible magnetic clusters that were influenced by the presence or absence of a magnetic field confirmed the nature of the nanoparticles that the magnetosome contain.

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