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Isolation Of Magnetotactic Bacteria Used In Wastewater Treatment From Lakes Aquatic Environment

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Abstract
This research presents detection and isolation of magnetotactic bacteria (MTB) from two lakes habitats, utilizing the established methodologies to improve isolation rates, with on sampling, enrichment and purification methods. Environmental samples from two lakes in Algeria were collected and cultured. Microscopy observation identified movement of MTB and growth occurred consistently in purifying medium at the oxic-anoxic transition zone which indicated MTB activity. Obtained results of transmission electron microscopy (TEM) confirm the presence of magnetotactic bacteria in the two lakes, which are being identified for biotechnological applications.
Keywords: *Magnetotactic bacteria, Magnetosomes, MTB, Transmission Electron Microscopy (TEM), Wastewater treatment.*

INTRODUCTION

Magnetotactic bacteria (MTB) are a prokaryote bacteria widely distribute in aquatics environements [1]. They possess magnetosomes which are an intracellular organelles composed of membrane-bound iron crystals, enabling them to align and move along magnetic field lines [2]. They play crucial roles in biogeochemical cycling and have promising applications in medical diagnostics such as targeted drug delivery and magnetic hyperthermia, environmental bioremediation of heavy metals and pollutants and nanotechnology [3, 4-8].
MTB are a group of Gram-negative prokaryotic microorganisms living in the sediments of aquatic environments that have the unique ability to orient themselves and migrate along the lines of the Earth's geomagnetic field [9]. This unique ability is based on the presence of intracellular nanocrystals composed of iron oxide or sulfide [10]. MTB are either microaerophilic or anaerobic that can produce iron oxide nanoparticles, composed of magnetite (Fe_3O_4) or greigite (Fe_3S_4) surrounded by a lipid membrane, in varying amounts [11].Magnetotactic bacteria were first described by medical doctor Salvatore BELLINI in Pavia, Italy in the late 1950s after he noticed bacteria in bog sediments consistently orienting themselves toward the magnetic north, which he went on to name "magnetosensitive bacteria" [12]. In the 1970s, Richard BLAKEMORE independently rediscovered MTB in Massachusetts and was the first to recognize that these bacteria had magnetosome structures within their cells and could orient themselves and sense magnetic fields [13].MTB come from an aquatic environment, typically found in lakes, water column, and sediments, where redox gradients exist, making lakes an ideal platform for detection and purification. Magnetotactic bacteria have important biotechnological applications, in aquatic environments, they become part of iron and sulfurbiogeochemical cycling by making magnetosomes, intracellular magnetic crystals that are involved in sedimentation of metals, capable of filtering and trapping some pollutants, making them good candidates for bioremedation of contaminated environments[14-16]. In this context, the objective of this research is to detect, for the first time, the presence of these bacteria in two different lakes, as well as to isolate and identify them microscopically.

MATERIALS AND METHODS

· In order to separate, investigate and identifying the diversity of magnetotactic bacteria, samples from two different environmental source were taken (Djebel El Ouahch lake, El Meridj lake) of Constantine City, Algeria. The study is focused on bacteria from sediments, due to the fact that the sediment samples could still be observed and magnetically sorted up to five years after their sampling and storage in the laboratory [17].
· One litre bottles are used, filled to around 20-30% of their volume with sediment of the waters sources Djebel El Ouahchlake and El Meridj lake, then stopped up with water. Air bubbles are excluded from the sample bottles.

The maximum number of MTB is obtained when the top five centimetres (5 cm) of sediment are collected [18].

Detecting MTB in water and aquatic sediment samples is due to their magnetotactic behaviour, which in turn is due to their permanent magnetic dipole moment. A drop of water or sediment is placed on a cover slip, then inverted and placed on a small rubber ring, on a microscope slide [13,19].

- Optical microscopic preparation is performed using the hanging drop detection technique [20, 21], to observe MTB collected. The identification is done via the optical microscope (magnification ×100 with oil immersion).
- After initial microscopic screening for possible MTB, samples from two freshwater lakes were cultured in two media for purification and selective growth of magnetotactic bacteria (MTB) : Standard Liquid Medium and semi-solid Magnetospirillum Growth Medium (MSGM) as both can support the aerobic and chemolithoautotrophic nature of MTB [22, 23]. The purification of magnetotactic bacteria (MTB) from environmental sources, is important for enrichment and to eliminate the influence of non-magnetic bacteria.
- As a next step in characterizing the bacteria enriched from environmental samples, GRAM staining was performed on some selected cultures with visible growth, particularly from MSGM tubes or liquid media with increased evidence of bacterial growth.
- Finally, MTB are identified morphologically by transmission electron microscopy (TEM) [24].

RESULTS AND DISUSSION

Sample collection

The efficiency of magnetotactic bacteria (MTB) collection depends largely on the choice of a site with various physicochemical and ecological criteria for their growth and development. For this reason, these microorganisms primarily inhabit aquatic environments where sharp redox gradients are established, particularly at the interface between oxic anoxic transition zones (OATZ) of aquatic sediments, particularly freshwater environments, estuaries, and shallow marine environments, the components that comprise these specific locations allow for low oxygen levels and support the cycling of iron and sulfur important for magnetosome formation [20]. Ideal conditions required for MTB will occur in shallow, quiet waters that have stable sediment layers, a steady supply of iron, and moderate organic matter. Furthermore, magnetotactic bacteria are typically found in temperate conditions, with optimal growth at 20to 30 °C [25].

Detection of MTB

The detection of magnetotactic bacteria (MTB) in environmental water and sediment samples relies primarily on their magnetotaxis behavior, induced by their permanent magnetic dipole moment [12,19].Bacterial growth is monitored by optical microscopy and by detection of the magnetic response using a magnetic bar [26] (Figure 1).

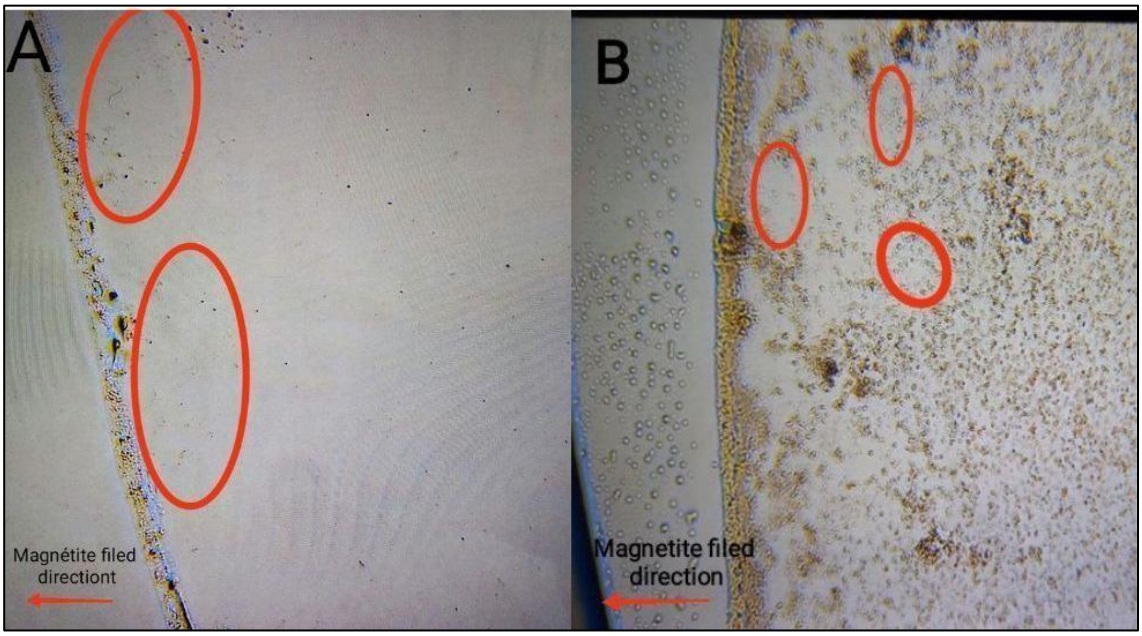

Figure 1: Optical microscopy observation of samples from Djebel El Ouahchlake (A) and El Meridj Lake (B),(magnification X 40) in the presence of a magnetic field.

These initial results coincide with what Faivre and Schüler (2008) [15] also observed in similar experiments leading to a rapid and linear placement of the MTB within the field lines. Lefèvre and Bazylinski (2013)[20] specify a method involving the use of simple devices, such as optical microscopes associated with a controlled magnetic field, allowing both the detection of MTBs and the observation of their motility dynamics. To complete, a set of collective self-organization phenomena observed in confined systems, such as drops or microfluidic channels, is consistent with the work of Waisbord et al. (2016) [27] who highlighted hydrodynamic interactions between magnetotactic cells. These organize themselves collectively according to the magnetic field and the physical properties of the medium. Comperatively with previous studies that show similar magnetotactic reactions in a variety of aquatic systems. For example, (Lefèvre et al., 2011) [23] performed experiments using freshwater pond sediments and reported directional movement of MTB when placed in magnetic fields and showed similar results with Brazilian freshwater lakes. The known motility towards magnetic sources is a unique characteristic that is used when both isolating MTB and with early identification of MTB in mixed microbial communities. The active movement of the bacteria from both lakes indicates that the oxic-anoxic transition zone, which is typically found in sediments or low-oxygen water layers, was well addressed in the samples that were collected, thus creating the ideal conditions for MTB abundance (Lefèvre and Bazylinski, 2013[20].Additionally, the occurrence of magnetotactic bacteria in both lakes supports previous studies which found general occurrences of magnetotactic bacteria in a wide variety of aquatic environments, and that these bacteria can be enriched and observed using a simple magnetic enrichment process (Spring et al., 1993) [28].

Purified cultures and GRAM staining microscopic observations

The enriched cultures were observed using an optical microscope at 40× magnification, with and without the application of an external magnetic field. In the preparations from the semi-solid MSGM medium, the observed cells present a elongate and cocci morphology, motile and oriented in the presence of a magnet, confirming their magnetotactic behavior. This behavior was clearly observed in the samples from both sites, and the directional movement was more pronounced (Figure 2). In the absence of a magnetic field, the observed movements were random. This motility confirmed that the purification and enrichment of magnetotactic bacteria were successful.

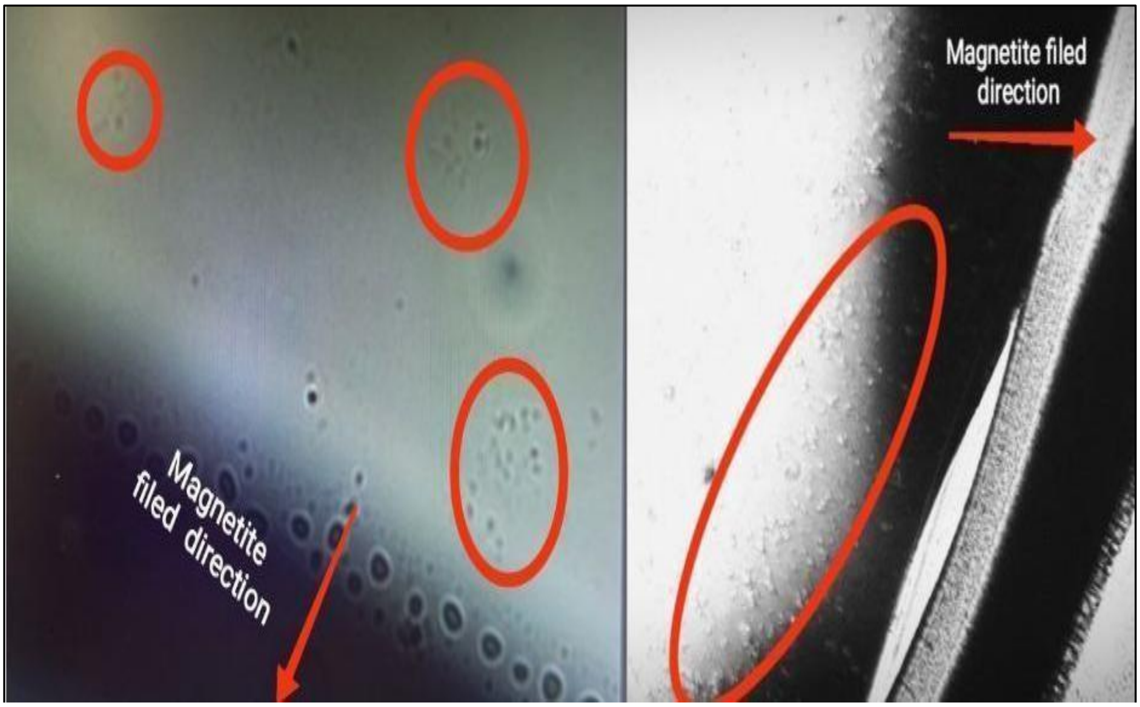

Figure 2: Optical microscopy observation of enriched cultures from the semi-solid MSGM medium in the presence of a magnetic field.

These results are consistent with the work of Blakemore (1975)[13], who was the first to demonstrate the oriented motility behavior of MTB in the presence of a simulated Earth's magnetic field. Furthermore, more recent work by Lefèvre et al. (2011)[23] also confirmed that bacteria with coccoid morphology, such as Magnetococcusmarinus MC-1, exhibit very rapid motility and marked magnetotactic behavior when cultured under appropriate conditions, particularly on semi-solid media that promote redox gradients. Furthermore, observations made on cultures in standard liquid media revealed lower cell density and less obvious magnetotactic behavior. This corroborates the studies of Pósfai et al. (2013)[29], who showed that the efficient cultivation of MTB requires very specific conditions, particularly a stable oxygen gradient, which is difficult to maintain in homogeneous liquid media. It also supports research by Lefèvre and Bazylinski (2013)[20], who stated that magneto-aerotactic behavior the alignment of a bacterium and magnetotactic mobility in the presence of the preferred oxygen concentration while under magnetic influence is the main characteristic of MTB. It is also evidence that semi-solid MSGM medium is effective in creating the oxic–anoxic transition zone (OATZ) that MTB favor, owing to their microaerophilic lifestyle [30].Thus, these results highlight the effectiveness of semi-solid MSGM medium not only to enrich MTB, but also to facilitate their detection and behavioral study by optical microscopy, making it a fundamental tool in the isolation and characterization of these specialized microorganisms.

Transmission electron microscopy (TEM) observations

Microscopic observation of purified bacteria by electronic transmission (TEM) showed the presence of a morphological variety of bacteria possessing magnetosomes and phosphate reserves (Figure3). This confirms the presence of magnetotactic bacteria in this water lakes source, which are currently being identified (Figure 3).

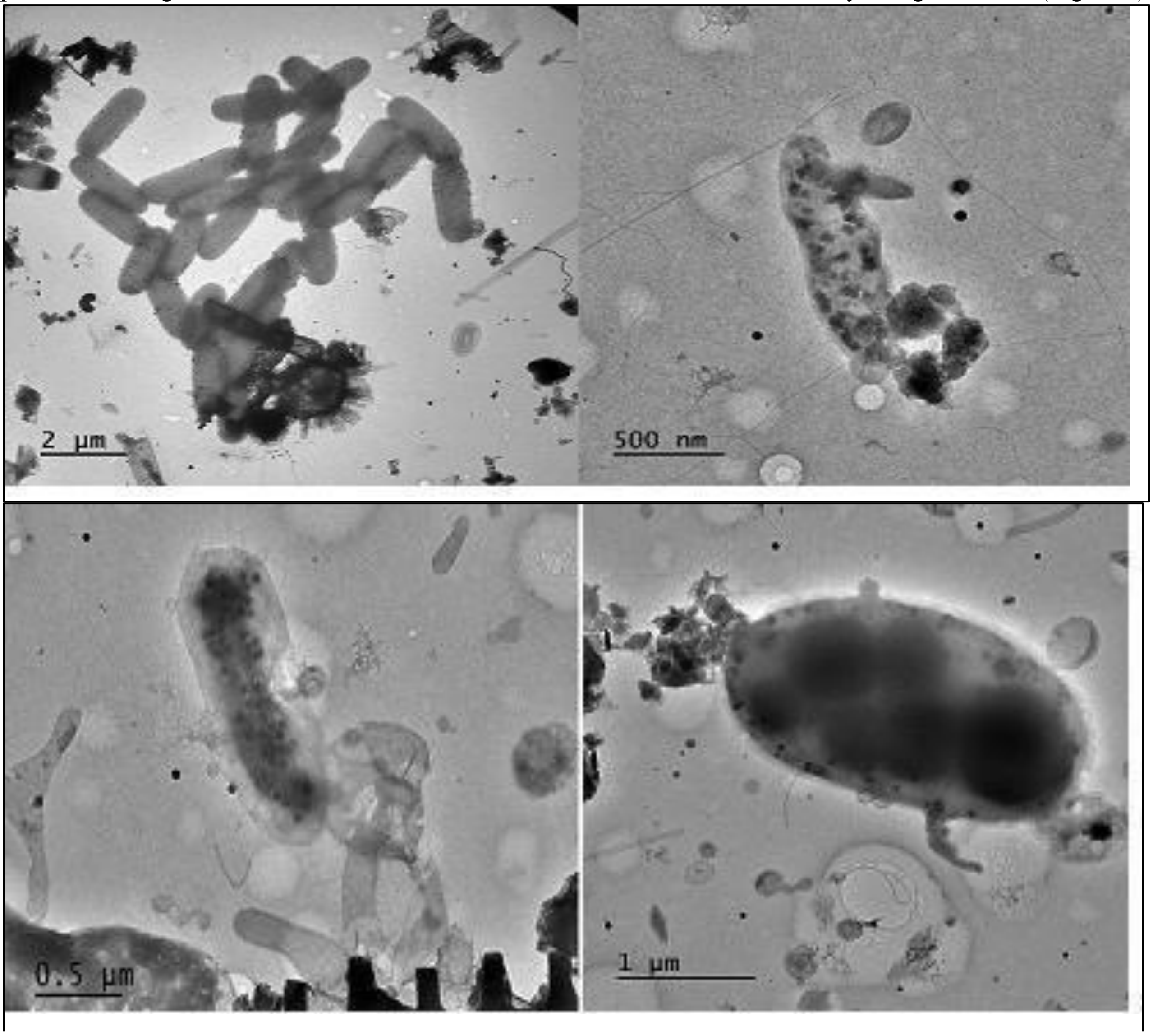

Figure 3: Transmission Electron Microscopy (TEM) observations of Magnetotactic bacteria isolated.

CONCLUSION

This study successfully demonstrate the presence of magnetotactic bacteria (MTB) in water of two Algerian lake ecosystems, which are a model with many benefits for researching biomineralization, bioremediation and different biotechnological applications.

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