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With deep gratitude and love, I dedicate this humble work to all those who supported me throughout my long academic journey.

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أنوار

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It was neither a short journey nor an easy path, but I made it. All praise is due to Allah who made the beginnings smooth and brought us to the end with His grace and generosity. I dedicate this success, first, to my ambitious self. It began with a dream and ended with an achievement. Then, to everyone who walked with me through my academic journey may you always remain my everlasting support.

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(My beloved father)

To the joy of my life and the love of my soul the greatest blessing from Allah to me — the one who carried my name in her prayers day and night, who lit up my path with love, kindness, and warmth; who was a cloud pouring love, giving, and sacrifice. She was, after Allah, the main reason I am who I am today **LaaibSaliha**.

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Abstract :

Magnetotactic bacteria (MTB) are widespread in aquatic environments. They possess magnetosomes, which are intracellular organelles composed of iron crystals, allowing them to align with and move along magnetic field lines. MTBs have several iron and sulfur biogeochemical applications, particularly in medical diagnostics, such as targeted drug delivery and magnetic hyperthermia. In the environment, they play a crucial role in the biogeochemical cycle and in the bioremediation of heavy metals and organic pollutants, as well as in nanotechnology. This study presents a theoretical review and an experimental study of MTB in aquatic environments, based on environmental samples from two lakes in the city of Constantine. Microscopic observation and purification using standard and semi-solid MSGM liquid media confirmed the presence of MTBs in the samples taken from both lakes, though molecular identification is still required.

Keywords: Magnetotactic bacteria, MTB, magnetosomes, magnetotaxis, lake.

Résumé

Les bactéries magnétotactiques (BMT) sont des bactéries largement répandues dans les milieux aquatiques. Elles possèdent des magnétosomes qui sont des organites intracellulaires composés de cristaux de fer, leur permettant de s'aligner et de se déplacer le long des lignes des champs magnétiques. Les BMT ont plusieurs applications biotechnologiques, notamment en diagnostic médical, telles que l'administration ciblée de médicaments et l'hyperthermie magnétique. A l'environnement, elles jouent un rôle curial au cycle biogéochimique et à la bioremédiation des métaux lourds et des polluants organiques, ainsi qu'en nanotechnologies. Ce travail de mémoire présente une revue théorique et une étude expérimentale des BMT en milieux aquatiques, à partir des échantillons environnementaux de deux lacs de la ville de Constantine. L'observation microscopique et la purification sur milieu liquide standard et semi solide MSGM ont permis de confirmer la présence des BMT dans les échantillons des deux lacs prélevés, dont une identification moléculaire est nécessaire.

Mots-clés : Bactéries magnétotactiques, BMT, magnétosomes, magnétotaxie, lac.

الملخص

البكتيريا المغناطيسية (MTB)هي بكتيريا منتشرة على نطاق واسع في البيئات المائية . تمتلك عضيات داخل الخلايا مكونة من بلورات الحديد تسمى المغنيطوزومات, مما يسمح لها بالاصطفاف و التحرك على طول خطوط المجال المغناطيسي.

للبكتيريا المغناطيسية العديد من التطبيقات في المجال التكنولوجيا الحيوية , خاصة في التشخيص الطبي مثل توصيل الأدوية المستهدفة و العلاج بالتحمية المغناطيسية .أما في البيئة ,فإنها تلعب دورا حاسما في الدورة البيوجيوكيميائية وفي المعالجة الحيوية للمعادن الثقيلة و الملوثات العضوية و كذلك استخدمت في تقنيات النانو.

يقدم هذا العمل الدراسي مراجعة نظرية و تجريبية للبكتيريا المغناطيسية في البيئات المائية ,انطلاقا من عينات بيئية مأخوذة من بحيرتين في مدينة قسنطينة . بحيث أكدت الملاحظة المجهرية و التنقية باستخدام الوسط السائل القياسي و الوسط شبه صلب (MSGM) وجود هذه البكتيريا في العينات المأخوذة من كلا البحيرتين مع ضرورة إجراء تحديد جزيئي لها.

الكلمات المفتاحية :الكتيريا المغناطسية,المغنيطوزومات, الانجذاب المغناطيسي , البحيرة.

List of abbreviations

ccw	counterclockwise
cw	clockwise
DNA	deoxyribonucleic acid
FISH	fluorescence in situ hybridization
GC	guanine-cytosine
M-A	magnetic aerotaxis
MRI	magnetic resonance imaging
MPI	magnetic particle imaging
MTB	magnetotacticbacteria
MAI	Magnetosome Island
MSGM	*Magnetospirillum*Growth Medium
NS	north seeking
Nm	nanometer
OAI	oxic-anoxic interface
OATZ	oxic-anoxic transition zones
RNA	Ribonucleic acid
SS	south seeking
TEM	transmission electron microscopy

Table des matières

List of figures

List of tables

Introduction

Magnetotactic bacteria (MTB) are aquatic prokaryotes that can align themselves with the magnetic field of the earth due to intracellular magnetosomes which is a membrane bound magnetic nanocrystals. MTB 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" (Bellini, 2008). In the 1970s, Richard BLAKEMORE independently rediscovered MTB in Massachusetts and was the first to recognize that MTB had magnetosome structures within their cells and could orient themselves and sense magnetic fields (Blakemore, 1975).

They 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. While isolating and purifying MTB is difficult because of low abundance and their fastidious growth requirements. Magnetotactic bacteria (MTB) have important biotechnological applications. In aquatic environments, they become part of iron and sulfur biogeochemical 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.In the biomedical field, they have been used in MRI, as therapeutic drug vectors, and in magnetic hyperthermia a novel technique aimed at killing tumor cells through heat (Faivre and Schüler, 2008; Yan et *al.*, 2012; Alphandéry et *al.*, 2011).

The research presented the detection and purification of MTB from lake habitats, utilizing the already established methodologies to improve isolation rates. The work follows three chapters: the first chapter with a brief overview and introductory description of MTB biology and ecology; the second chapter is about materials and methods with on the sampling and enrichment and purification methods used, finally, the third chapter describes the results of the study along with conclusions and future vie.

Chapter I
Literature review

1. Definition

Magnetotactic bacteria 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 (Montei et*al*., 2015). This unique ability is based on the presence of intracellular nanocrystals composed of iron oxide or sulfide (Belaud,S . 2024).

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 (Hossain et *al*., 2024).

2. Types of MTB

Magnetotactic bacteria (MTB) exhibit two main types of magnetotactic organization depending on the orientation of their magnetosome chains (magnetic nanocrystals) and their response to the Earth's magnetic field: polar and axial (Bazylinski et *al*., 2014).

The polarity of magnetotactic bacteria (MTB) refers to their preferred swimming direction under oxic conditions. In the Northern Hemisphere, most MTB exhibit north-seeking (NS) behavior, swimming northward along the inclination of Earth's geomagnetic field in the oxic-anoxic interface (OAI). In contrast, in the Southern Hemisphere, most MTB display south-seeking (SS) polarity, moving southward and downward under similar oxic conditions(Frankel et *al*., 1997). However, according to Leão et *al* (2016) there is caswher a minority of MTB in each hemisphere exhibit the opposite polarity. In one notable case, the majority of a specific MTB population in the Northern Hemisphere is south-seeking (SS), swimming southward (upward) in response to high oxygen concentrations.

Unlike polar MTBs, axial bacteria reverse their movement (forward/backward) while remaining magnetically aligned (Bazylinskiet*al*., 2014).

3. Ecology of MTB

3.1. Natural habitat

These bacteria are primarily found in stratified aquatic environments, such as marine sediments, lakes, and intertidale zones, where they occupy specific ecological niches, frequently at the interface between oxic and anoxic zones (Bazylinski et *al*., 2004).

MTB has varied habitat preferences are a result of its physiological and metabolic adaptations. In freshwater settings, for example,*Magnetospirillum* species, such *Magnetospirillummagnetotacticum,* are frequently found and flourish in microaerobic conditions close to the OAI (Lefèvre et *al*., 2013). Conversely, marine MTB, like those of the genus *Magnetococcus*, are frequently found in saline or brackish habitats, such as estuaries and coastal sediments, where they must traverse sharp redox gradients (Lefèvre et *al*., 2012). Recent studies have further expanded our understanding of MTB ecology, highlighting their adaptability to extreme environments and their potential role in global iron cycling (Hossain et *al*., 2024). Some MTB, such as *Desulfovibriomagneticus*, are sulfate-reducing bacteria that inhabit anoxic zones of aquatic sediments, using sulfur compounds as electron acceptors (Sakaguchi, Burgess and Matsunaga 1993). Greigite-producing MTB, such as those in the *Desulfamplus* genus, are typically found in sulfidic environments, where they contribute to sulfur cycling. (Lefèvre et *al*., 2012)

Gaining knowledge of MTB's ecological dynamics helps one better understand microbial adaption tactics and how they affect environmental processes more broadly (Faivre and Schüler, 2008).

3.2.Interactions of MTB

Magnetotactic bacteria (MTB) possess magnetosomes, intracellular organelles containing magnetic minerals such as magnetite (Fe_3O_4) or greigite (Fe_3S_4), which allow them to align along geomagnetic fields and interact with their environment (Bazylinskiand Frankel, 2004). Found mostly at the oxic-anoxic interface of aquatic environments, MTB are responsible for the biogeochemical iron and sulfur cycles (Lefèvre and Bazylinski, 2013). Through magnetotaxis, they orient along chemical gradients to obtain maximum access to energy and nutrients (Lin et *al*., 2017). They also coexist with other microbes such as *Magnetospirillum*and*Magnetococcus* that are involved in the breakdown of organic matter as well as the recycling of nutrients in aquatic sediments (Jogler and Schüler, 2009). Through the establishment of synergistic partnerships with methanogenic archaea and sulfate-reducing bacteria, they become involved in ecological processes as well as structuring microbial communities (Frankel and Bazylinski, 2009).

Methanogenic archaea associate symbiotically with sulfur-reducing and iron-oxidizing bacteria under anoxic conditions, promoting mutual metabolic gains. For example, *Desulfovibriomagneticus* lives in association with sulfur-oxidizing bacteria to balance redox in sediments (Sakaguchi et *al*., 2002). These organisms influence sediment geochemistry and vertical metal distribution through the precipitation of magnetic minerals (Faivre and Schüler, 2008). MTBs are also a food source for microbial predators such as nematodes and protists, thus transferring their iron-rich biomass to aquatic food webs (Lefèvre et *al*., 2012). Understanding these interactions emphasizes their inherent ecological relevance and future applications in the fields of environmental and biomedical engineering.

3.3.Influence of MTB on Biogeochemical Cycles: Iron and Sulfur

Magnetotactic bacteria (MTB) play a significant role in biogeochemical cycles owing to their involvement in iron (Fe) and sulfur (S) transformations. These microbes that produce magnetic iron crystals, such as magnetite (Fe_3O_4) and greigite (Fe_3S_4), predominantly inside intracellular magnetosomes, to enhance swimming along geomagnetic fields (Hossain et *al*., 2024; Bazylinski and Frankel, 2004). The biomineralization process thus directly influences S and Fe cycling in aquatic systems by regulating redox processes and promoting mineral precipitation (Lefèvre and Bazylinski, 2013). These metabolic processes, including chemolithotrophy and sulfur reduction, alter sulfur speciation and nutrient fluxes (Faivre and Schüler, 2008) (Hossain et *al*., 2024), so MTB thrive in stratified habitats such as sediments and oxic-anoxic interfaces.

Recent developments underscore the ecological and evolutionary importance of MTB. For instance, fossilized magnetosomes (known as magnetofossils) discovered in ancient sediment layers, such as Late Cretaceous deposits (approximately 85 million years ago), illustrate their enduring role in iron cycling (Chang et *al*., 2023). Genomic investigations indicate that conserved magnetosome genes (for instance, mam, mms) are present across various MTB lineages (such as *Proteobacteria* and *Nitrospirae*), pointing to an ancestral origin associated with redox gradients (Hossain et *al*., 2024; Uzun et *al*., 2022). Furthermore, MTB play a role in environmental cleanup by capturing heavy metals (for example, Cr and As) through the process of mineral incorporation, highlighting their practical applications (Hossain et *al*., 2024) (Amor et *al*., 2020).

3.4.Adaptation of magnetotactic bacteria to extreme environments

Magnetotactic bacteria (MTB) possess remarkable adaptations to inhabit extreme environments like oceanic hydrothermal vents, anoxic sediments or hypersaline lakes. Their ability to produce magnetosomes, magnetic nanoparticles of magnetite (Fe_3O_4) or greigite(Fe_3S_4), provides an evolutionary advantage; these structures - arranged in chains - give a sense of alignment to Earth's magnetic fields at micro and macro scales, allowing them to navigate away from the ambient conditions to seek optimal oxygen or nutrient zones separated by narrow chemical gradients (Lefèvre and Bazylinski, 2013). MTB also develop resistance to oxidative and pH stress in extreme environments through specialized enzymatic systems or cell membranes facilitated by polyunsaturated lipids, which confer additional levels of protection during both anaerobic and aerobic metabolism (Jogler et *al*., 2011). The MTB genome is also enriched with DNA repair and metabolic regulation associated genes, said to confer an adaptive plastic response to high temperatures or hydrostatic pressures. (Lin et *al*., 2014). In addition, certain strains, such as *Magnetococcusmarinus*, exploit low iron environments by inducing specific transporters and recycling metal ions (Li et *al*., 2017). These multi-faceted adaptations all underline ecological roles in biogeochemical cycles and the biotechnological potential of MTB in bioremediation and synthesizing nanomaterials.

4. Diversity of MTB

4.1.Morphology diversity

There are over 50 species of MTB that have been discovered in various natural environmental conditions and according to Lefèvre and Bazylinski (2013), these bacteria can take on a variety of shapes, such as spirille (*Magnetospirillummagneticum*), vibrion (*Desulfovibriomagneticus*), coque (*Magnetococcusmarinus*), or multicellular formations referred to as the MMP (many-celled magnetotactic prokaryote) like *CandidatusMagnetoglobusmulticellularis*(Figure 1) .

Among this diverse shapes of MTB observed, magnetotactic cocci were the dominant morphotype and could be categorized into two types a typical cocci that appeared to have peritrichous flagella, and those characterized by having a drop-shaped form and one bundle of flagella located at the thin end of the cell. Transmission electron microscopy (TEM) analys revealed that the magnetosomes formed by those magnetotactic cocci are magnetite (Fe_3O_4) with octahedral crystal habit.

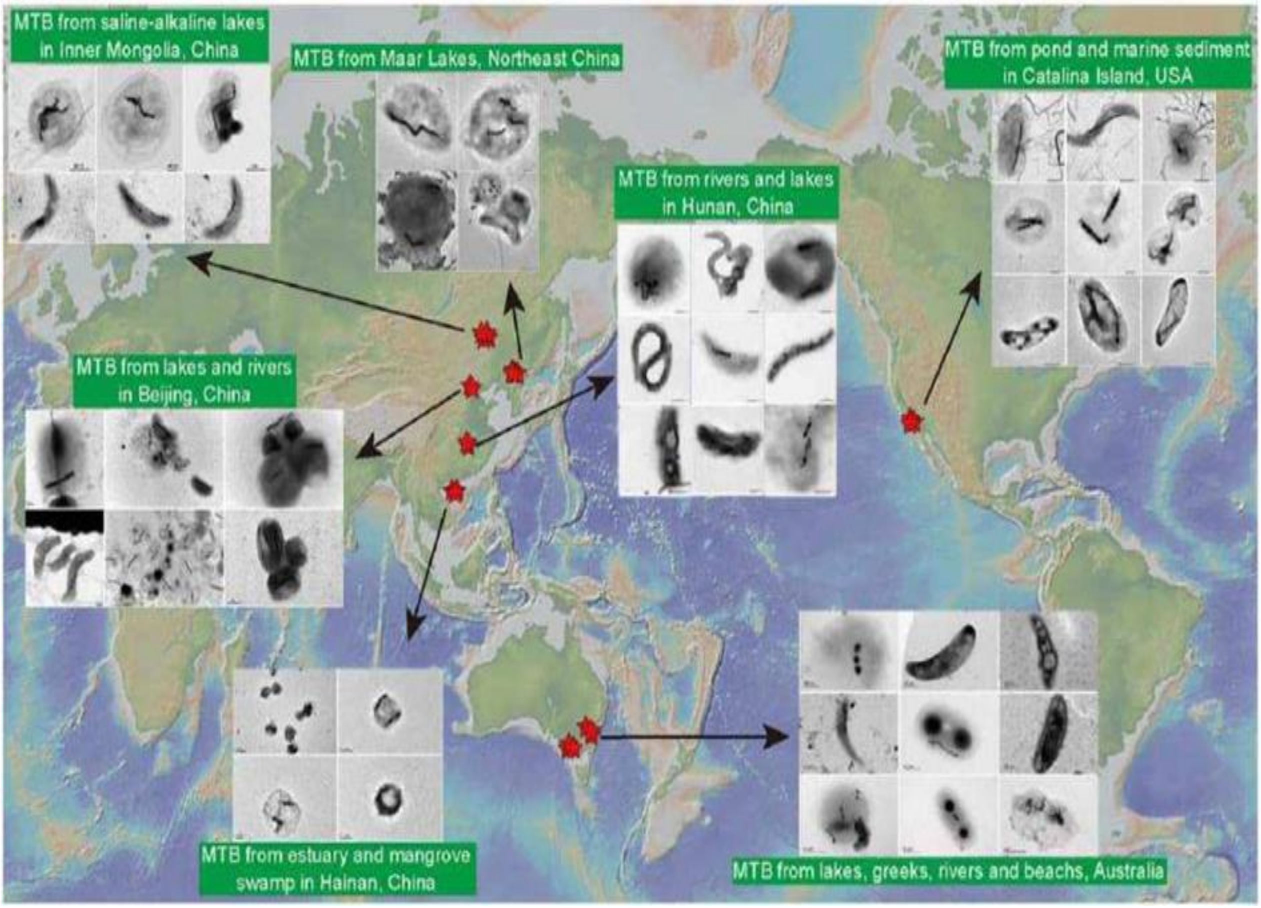

Figure 1: Various morphotypes of MTB found in different locations (Lin et *al*., 2017).

4.2.Physiology diversity

Magnetotactic bacteria (MTB) exhibit remarkable metabolic diversity, shaped by their adaptation to extreme environments and varying oxygen requirements. As outlined by Lefèvre and Bazylinski (2013), MTB can be classified into two major metabolic groups: chemoorganoheterotrophs, which utilize organic compounds for carbon and energy, and chemolithoautotrophs, which oxidize inorganic compounds like sulfur or hydrogen. Complementing this categorization, Faivre and Schüler (2008) emphasize that MTB's metabolic strategies are further influenced by their oxygen tolerance, with species ranging from microaerophiles (e.g., *Magnetospirillum*) to obligate anaerobes or facultative anaerobes. This dual flexibility metabolic and oxygen-related enables MTB to thrive in diverse ecological niches, particularly aquatic sediments characterized by steep oxygen and nutrient gradients. Physiologically, their microaerophilic or anaerobic tendencies align with their iron metabolism, as MTB internalize iron (Fe^{2+} or Fe^{3+}) to biomineralize magnetite (Fe_3O_4) or greigite (Fe_3S_4) nanocrystals within magnetosomes. Though energetically costly, this process is critical for magnetotaxis, allowing MTB to navigate Earth's magnetic fields and locate optimal conditions. Together, their metabolic versatility, oxygen adaptability, and iron biomineralization underscore MTB's ecological success and their pivotal role in biogeochemical cycles, particularly iron cycling.

4.3.Phylogenetic diversity

The active magnetotactic behavior of MTB makes it very easy to magnetically remove and at least partially purify them from water or sediment samples. The main methods for identifying novel MTB populations have been cultivation-based methods and 16S rRNA gene-targeting investigations, including as denaturing gradient gel electrophoresis, fluorescence in situ hybridization (FISH), restriction fragment length polymorphism, and clone library sequencing (table1).

Table 1: Year-Wise Identification of MTB from Various Parts ofthe World (Yadav et *al*., 2025).

Year	Name of the MTB	References
1975	*Magnetospirillummagnetotacticum*	Frankel .2009
1978	*M. marinus*	Kirschvink JL.1980
1981	*M. magnetotacticum MS-1*	Yan L et *al*.,2012
1988	*Magnetovibrio MV-2*	Lefèvre Christopher T et *al*.,2013
1991	*Magnetospirillummagneticum* *M. magneticum AMB-1* *Magnetovibrio MV-1*	Chen H et *al*., 2018
1993	*Magnetovibrio MV-4*	Lefèvre Christopher T et *al*., 2013
1994	*Magnetotacticbacteriumstrain WD-1*	Goswami P et *al*., 2022
1996	*M. bacteriumstrain HM-1*	Goswami P et *al*., 2022
1999	*Magnetospirillumsp. MSM-4*	Le Nagard L et *al*., 2018
2002	*D. magnetous RS-1*	Byrne ME et *al*., 2010

2003	*Magnetotacticbacteriumstrain YN-1*	Uzun M et *al*., 2020
2007	*M. gryphiswaldense*	Amann R et *al*., 2006
2010	*M. biokemorii*	Xie J et *al*., 2009
2016	*Magnetogaba australis IT-1*	MorilloVet*al*., 2014
2016	*Magnetospirillum QH-2*	Lin W et *al*., 2018
2016	*γ-proteobacterium BW-2* *γ-proteobacterium SS-5* *Magnetospirathiophila MMS-1*	Araujo A et *al*., 2015
2016	*CandidatusDesulfamplusmagnetomortis BW-1* *δ-proteobacterium ML-1* *δ-proteobacterium ZZ-1* *δ-proteobacterium AV-1*	Araujo A et *al*., 2015
2016	*Magnetovoidstrain MO-1*	Yang C et *al*., 2012
2016	*Magnetotacticbacteriumstrain TH-1*	Lefèvre Christopher T et *al*., 2013
2016	*Herbaspirillumsp. TK-2*	arquesACQet*al*., 2015
2016	*Magnetotacticbacteriumstrain MG-1*	Gareev KG et *al*., 2021
2016	*M. bacteriumstrain MG-2*	Lefèvre CT et *al*., 2013
2016	*M. bacteriumstrain YSC-1*	Jun G et *al*., 2006

Notes:"Al." likely abbreviates Alphaproteobacterium (common in MTB taxonomy).References are preserved as in the original tables; entries without references are marked with "-".Consolidation merges duplicate entries (e.g., 2016 entries from both tables) without altering names or references.

Using these methodologies, MTB discovered to date are phylogenetically connected to the Alpha, Delta, Gamma and Eta classes of *Proteobacteria*phylum, as well as the *Nitrospirae* phylum (Lin et *al*., 2014). But there have been new findings that show, previously unknown and uncultured magnetotactic bacteria (MTB) also from the *candidate* phylum *Omnitrophica*(formerly candidate division OP3), the candidate phylum *Latescibacteria*(formerly candidate division WS3), and the phylum *Planctomycetes*(Figure 2and 3). (Lin et *al*., 2018)

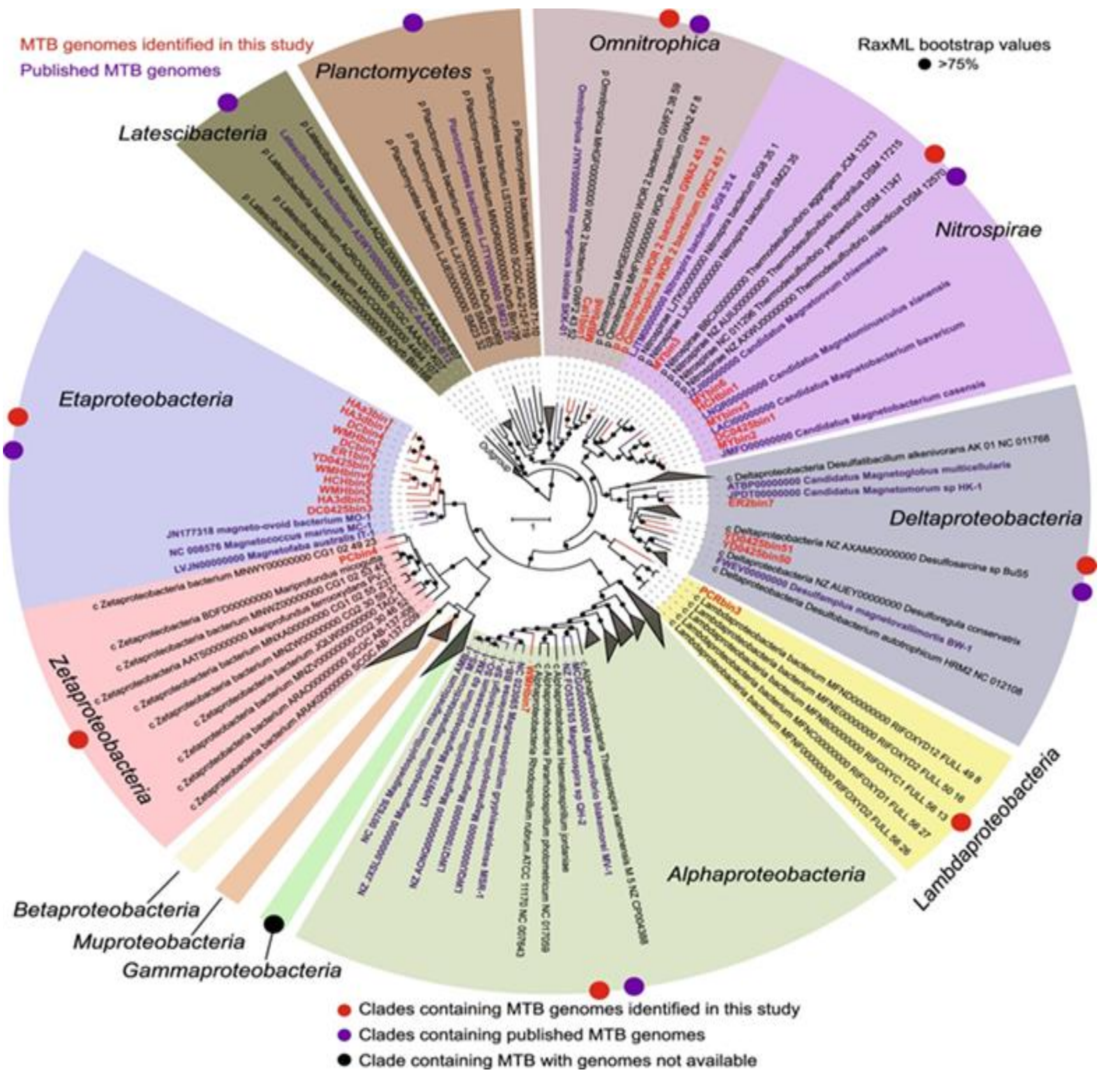

Figure 2: Maximum likelihood phylogeny of MTB genomes. Phylogenomic tree based on concatenated alignment (3973 amino-acid positions) of up to 400 ubiquitous conserved proteins identified with PhyloPhlAn. Archaeal genomes (accession numbers CP001719 and CP000678) were used as the out-group. Red and purple denote MTB genomes from this study and from published MTB genome sequences, respectively. Bootstrap values are indicated with black circles (>75% support from 100 resamples) (Lin et *al.*, 2018).

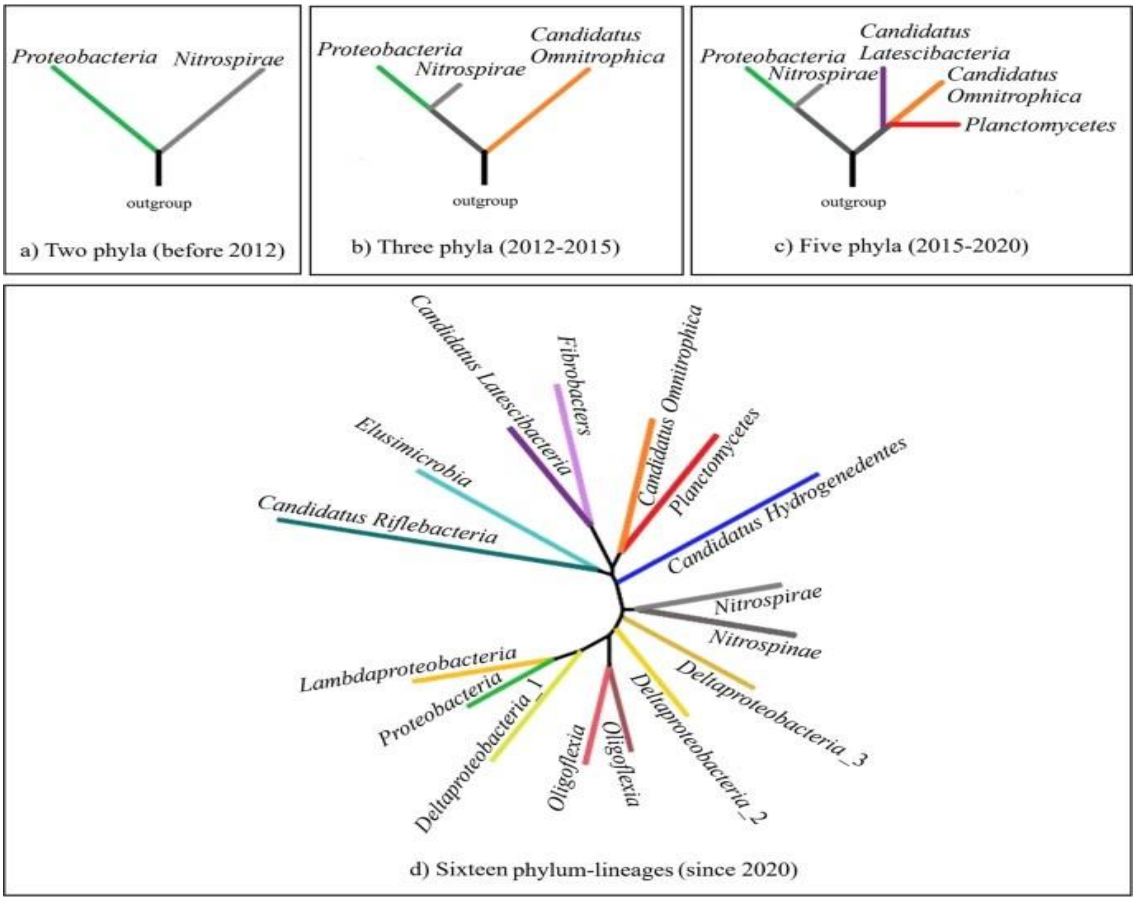

Figure 3: a) Before 2012, only two phyla (*Proteobacteria* and *Nitrospirae*) were identified to contain MTB. b) Between 2012 and 2015, an additional branch for the *CandidatusOmnitrophica* phylum was added. c) Subsequently, two bacterial phyla (*CandidatusLatescibacteria* and *Planctomycetes*) were added to the MTB tree of life based on the presence of magnetosome gene clusters in their genomes. d) The latest addition to the MTB tree has expanded its branches to a total of sixteen bacterial phylum-level lineages. All taxonomic groupings are based on the NCBI taxonomy (Pranami et *al*., 2022).

The best known MTB species belong to the classes *Alphaproteobacteria*, *Deltaproteobacteria*, and *Gammaproteobacteria*, with notable genera such as *Magnetospirillum*,*Magnetococcus*, and*Desulfovibrio*. For example, *Magnetospirillummagicum* and *Magnetospirillumgryphiswaldense* are widely studied species that thrive in freshwater environments, while *Magnetococcusmarinus* is a marine species. In addition, some MTB, such as those of the genus *Desulfovibrio*, occur in sulfate-rich environments, such as salt marshes and estuaries (Figure 4) (Lefèvre and Bazylinski, 2013).

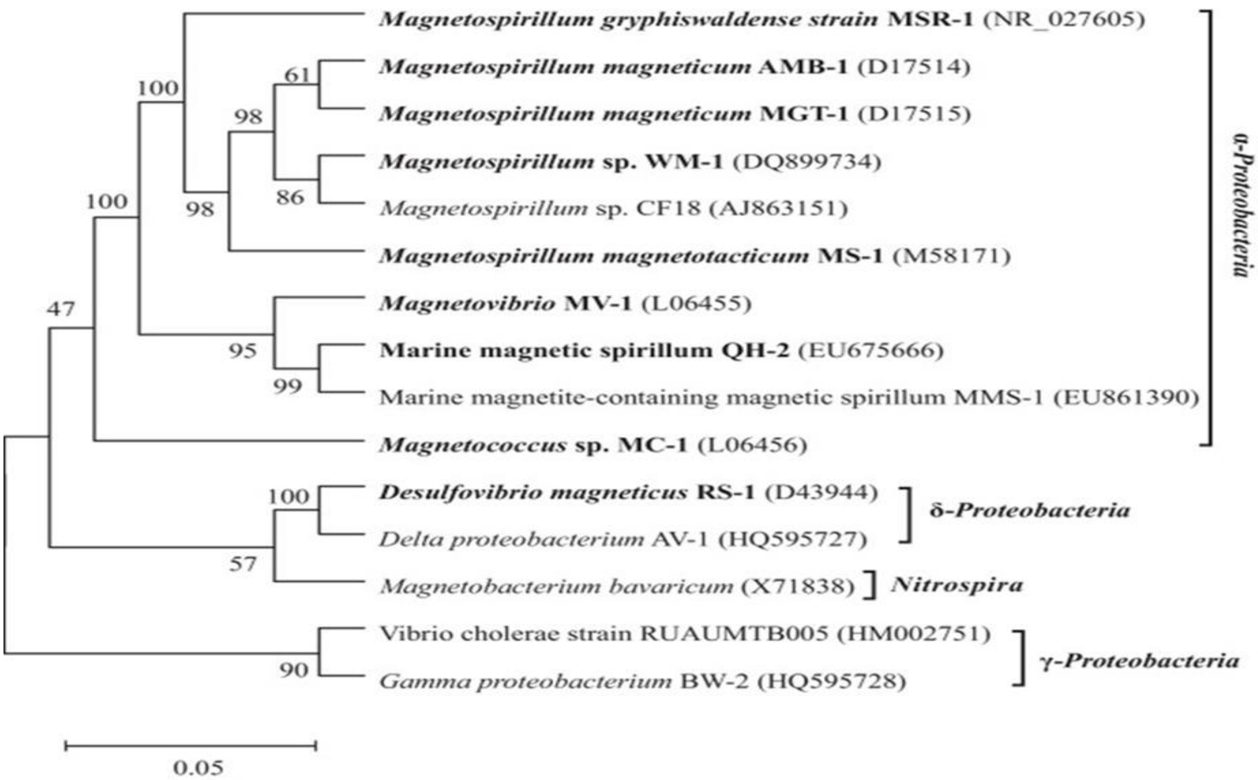

Figure 4: Phylogenetic relationships of MTB. Pure cultures MTB are indicated in bold characters (Yan et *al*., 2012).

4.4.Genotypic diversity

The genotypic diversity of Magnétotactic Bacteria (MTB) reflects their complex evolution and adaption to a variety of environments. Comparative genomic studies, such as those conducted by Jogler et *al*. (2009), have shown that the genes responsible for magnétosome development are frequently grouped together in a geographical area known as the "Ilotmagnétosome." Despite being conserved across many MTB species, this gene lot exhibits significant variation in its structure and content, reflecting unique adaptations to different ecological niches.

For example, some species have different genes that encode the membrane proteins of magnetosomes, while other species have additional genes that regulate the size and form of magnetite cristaux. The horizontal gene transfer events that permitted the genes of the "lot magnetosome" to proliferate among other bacterial strains also had an impact on this genetic diversity. The broad biological range of MTB and their capacity to colonize a range of habitats, including soft water and marine sediments, are explained by these horizontal transfers. In conclusion, MTB's genetic diversity serves as a centralized mechanism for the biomineralization of magnesium and illustrates their evolutionary flexibility and adaptation to shifting environmental situations (Jogler and Schüler 2009).

Magnetotactic bacteria move through their environment by rotating their flagella, helical structures powered by a rotary motor anchored in their cell membrane. Their movement is guided by the Earth's magnetic field, which aligns their internal chain of magnetosomes, making them act like self-propelled compass needles.

5. Motility of magnetotactic bacteria

5.1.Navigation Device

Magnétotactic bacteria have a sophisticated flagellary motility system that enables them to move about their environment efficiently. A rotating motor powered by proton-motrice force activates the flagelles, which are made of the protein flagelline (Frankel et *al*., 2009). According to Faivre and Schüler (2008), these structures enable BMT to move in a helical motion at speeds of 40 to 100 µm/s. The alignment of magnétosomes coordinates the rotation of flagelles, which alternate between CW and CCW sensing, forming an internal chain that functions as a biological boussole (Faivreet*al*.,2008). This combination of flagellary motility and magnetotaxie enables BMT to precisely place itself at the oxique-anoxique interface (OAI).

The motor apparatus of these bacteria exhibits remarkable structural diversity some species, such as *Magnetospirillum*, possess a single flagellum or bundle of flagella at one cell pole, while others, such as *Magnetobacteriumbavaricum*, are equipped with two opposing flagella at each pole according to the work of (Lefèvre et *al*., 2014)(Figure 5).

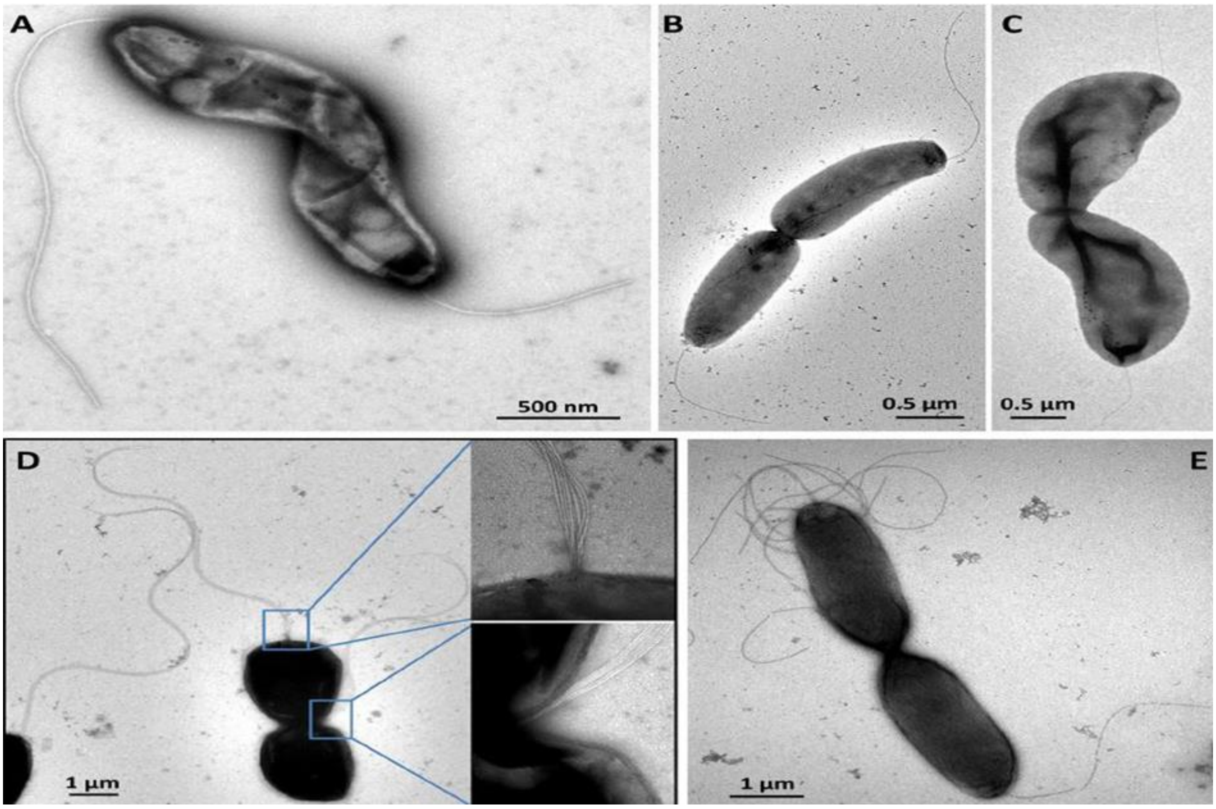

Figure 5: Structural inheritance of asymmetric magnetotactic bacteria and related organisms during cell division. Transmission electron micrographs of dividing cells of *Magnetovibrioblakemorei* strains MV-1 (A) and LM-1 (B) of the *Alphaproteobacteria* class, strain ZZ-1 of the *deltaproteobacteria*class (C), and strain BW-2 (D) and *Pseudomonas brassicacearum* (E) of the *Gammaproteobacteria*class. All these bacteria but P. *brassicacearum* are magnetotactic. Cells are stained with 1% uranyl acetate to show the positions of their flagella (Lefèvre et *al*., 2015).

5.2.Magnetotaxi

One of the most remarkable examples of microbial navigation in the natural world is magnetotaxis, a phenomenon in which magnetotactic bacteria (MTB) align and travel along geomagnetic field lines through a sophisticated biological compass system. To generate a magnetic dipole moment, these microorganisms produce magnetosomes internal, membrane-bound nanocrystals composed of either magnetite (Fe_3O_4) or greigite (Fe_3S_4), which are meticulously arranged in linear chains (Bazylinski et *al*.,2004);(Lefèvre et *al*., 2013). In chemically stratified aquatic environments, particularly at the oxic-anoxic transition zones, this biological magnetic sensor enables MTB to accurately detect and maintain their position at optimal oxygen levels (Hossain et *al*., 2024).

The mechanism of magnetotaxis involves the alignment of magnetosome chains with the Earth's magnetic field (around 50 μT) and the propulsion provided by flagella, resulting in a directed movement known as magneto-aerotaxis (Jogler et *al*., 2009). Two main behavioral patterns have been recognized: polar magnetotaxis, where bacteria swim consistently toward the north or south based on their hemisphere, and axial magnetotaxis, which permits movement in both directions along magnetic field lines (Amor et *al*., 2020). This navigation strategy confers significant evolutionary advantages, enabling bacteria to quickly locate optimal growth conditions while conserving energy that would otherwise be expended on random searching (Chang et *al*., 2023).

The environmental impact of magnetotaxis is significant, as magnetotactic bacteria (MTB) are essential for the biogeochemical cycling of iron and sulfur due to their mineral-forming activities (Hossain et *al*., 2024) ;(Uzun et *al*., 2022). Their magnetosome chains frequently survive as magnetofossils within sediment layers, which are critical indicators of ancient habitats and potential biosignatures in the pursuit of extraterrestrial life (Lefèvre and Bazylinski, 2013). The ongoing influence of this ancient microbial trait in driving scientific and technological progress is demonstrated by the current applications of magnetotaxis, which include targeted drug delivery, environmental remediation, and advancements in nanotechnology (Bazylinski and Frankel, 2004); (Faivre and Schüler, 2008). Magnetotaxis research is a dynamic interdisciplinary field that integrates microbiology, biophysics, and materials science, while also offering critical insights into the development of biological sensing systems.

5.3.Interaction with other navigation systems

5.3.1. Magneto-aerotaxis

Magnetotaxisis the specific term used to describe the directional motility of BMT in the Earth’s magnetic field. Aerotaxis is crucial for the survival of BMT, as it allows them to identify and migrate along oxygen gradients over long distances. Most BMT are microaerophiles or strict anaerobes that require precise levels of (O_2) for their energy metabolism, usually leading to the reduction of sulfur or iron (Frankel et *al*. 2007). The chemotactic sensors of these bacteria are specialized MCPs, notably Methyl-Accepting Chemotaxis Proteins, whose local oxygen variations are detected and act in the regulation of the direction of movement by modulating flagellar rotation (Winklhofer et *al*., 2020). Compared to other aerotactic bacteria. BMTs use magnetotaxis as a complementary strategy for alignment within seconds with the geomagnetic field and for efficient vertical navigation to low-O2 habitats without prolonged random exploration (Smith et *al*., 2006). Experiments in microcosms, i.e., smaller-scale enclosed environments, have shown that some BMTs, even in the absence of magnetosomes, the non-magnetic mutants, maintain a residual ability to migrate toward microaerobic niches (Rahn-Lee et *al*., 2015). This, although perhaps imperfect, separation of aerotaxis via magnetotaxis underlines the relative independence of the two mechanisms,this fine detector of gas gradients reflects an evolutionary adaptation to linear environments, where competition for resources is based on precise position (Lefèvre et *al*., 2011). The study of aerotaxis in these bacteria also offers perspectives for bioengineering, particularly in the design of bio-inspired environmental detection systems (Schüler, 2008).

Behavioral analysis of individual cells within microaerobic bands has allowed us to distinguish two distinct mechanisms of magnetic aerotaxis (M-A): the polar and axial models.

a. Polar magneto-aerotaxis

The Polar (M-A) model illustrates a bimodal system in which the direction of flagellar rotation (either clockwise or counterclockwise) determines the manner in which bacteria migrate in response to gradients in their environment. When the concentration of oxygen (O_2) rises above some optimal value, ccw (counterclockwise) spin of the flagella causes those cells to move along magnetic field lines to areas of low O_2. When cells enter suboptimal conditions, which can be as simple as too little O_2, the bacteria enter State 2, where the flagella spin cw (clockwise), resulting in backward movement back to areas with higher O_2 concentrations. This regulatory mechanism may not function by sensing specifically molecular O_2 concentration, but instead via redox potential, or energy state with the cell, to adapt successfully to changing chemical environments (Figure 6) (Frankel et *al*., 2007).

b. Axial Magneto-Aerotaxis

The magnetic axis model (M-A) shifts to a temperature sensing device or the bacteria that replaces the long ligatures to continue emphasizing an assessment of oxygen (O_2) concentration within environmental conditions. With this transition while also complementing a number of non-magnetic chemical bacteria, it makes it possible to obtain a near instantaneous comparison with the actual concentration of O_2 and a better cell. This condition of the variation of the temperatures of these concentrations more readily can cause inversion of the senses of rotation of the flagella (cw or ccw,) adjusting to the migration direction the long magnetic field (Frankel et *al*., 2007). When cells are aligned to the optimal O_2 zone (versus trop whether it is high or low concentrations), it increases the potential for inversion that ultimately would favor a return to a better optimal range the concentration band for O_2. This reaction dynamic mechanism allows for a spatially efficient environment for efficient redox strategies microenvironments, thus optimizing for survival by gut-generated metabolites (Figure 6) (Schüler, 2008) (Spormannet*al*., 1984).

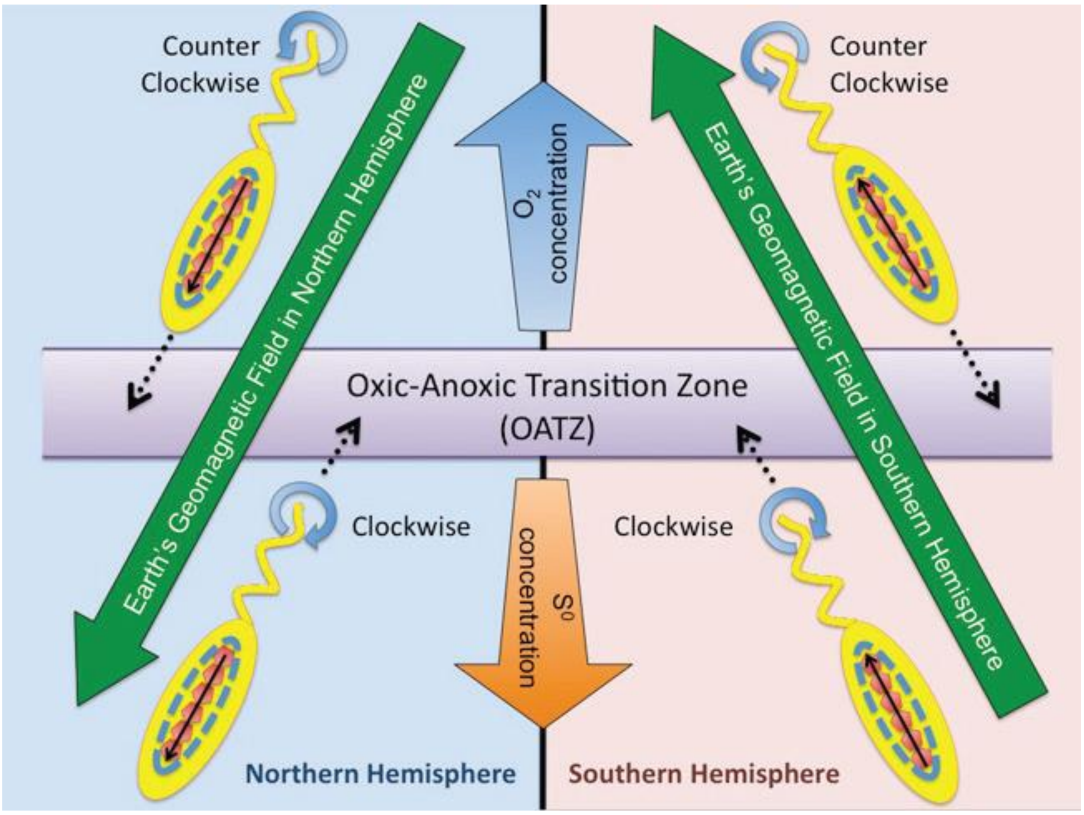

Figure 6: Principle of operation of the swimming of magnetotactic bacteria in their natural environment, following the direction of the magnetic field lines in the two hemispheres (chen et *al*., 2010).

5.3.2. Magneto-chemotaxis

Bacteria that have the ability of Magnetotaxis, also known as magnetotactic bacteria (MTB), not only use magnetotaxis to determine their spatial orientation, but also use chemotaxis to detect and respond to chemical gradients in a complementary way that enhances their navigation through their environment. The bacteria perceive chemical signals such as oxygen or sulfur compounds using chemoreception proteins, which sense those molecules and regulate flagellar rotation to direct movement toward metabolically favorable environments (Frankel et *al*., 2007). Chemo-tactic behavior in MTB is not isolated from magnetotaxis, as the Earth's magnetic field orients their movement along vertical axes, but instead, it is functionally integrated with it because chemo-tactic signaling modulates the position of MTB along chemical gradients, especially in terms of the horizontal dimension of positioning (Smith et *al*., 2006), in a way that magnetotaxis could not account for. In conclusion, the hybrid behavior of MTB involves using the directionality of magnetotaxis and the specificity of chemotaxis to efficiently locateoxic-anoxic interfaces, or the boundary between oxic and anoxic areas, which are important ecological niches in stratified, oxygen-poor sediments (Bazylinski et *al*., 2013). For instance, *Magnetospirillummagneticum* AMB-1 uses magnetotaxis to descend into sediment layers and chemotaxis to determine where the microaerobic zone it requires in order to carry out its physiological processes is located (Lefèvre et *al*., 2014).According to genomic studies, *Magnetospirillumgryphiswaldense* exhibits co-regulation of genes associated to chemotaxis (e.g., cheA, cheY) and magnetosome biogenesis (e.g., mam, mms), indicating the evolutionary relationship between the two systems (Rahn-Lee et *al*., 2015).It is interesting to note that the chemotactic behavior is retained even in mutants deficient in magnetosome synthesis, suggesting that chemotaxis can work under certain environmental conditions separately and can be vital for the existence (Monteil et *al*., 2019). This two-tier navigation system demonstrates an adaptive reaction to complex redox environments where the efficient localization of the optimal metabolic zones provides a competitive advantage (Lefèvre et *al*.,2011). In addition, the sensitivity of MTB to both magnetic and chemical signals promises interesting applications in biosensor and bioremediation technology (Schüler, 2020).

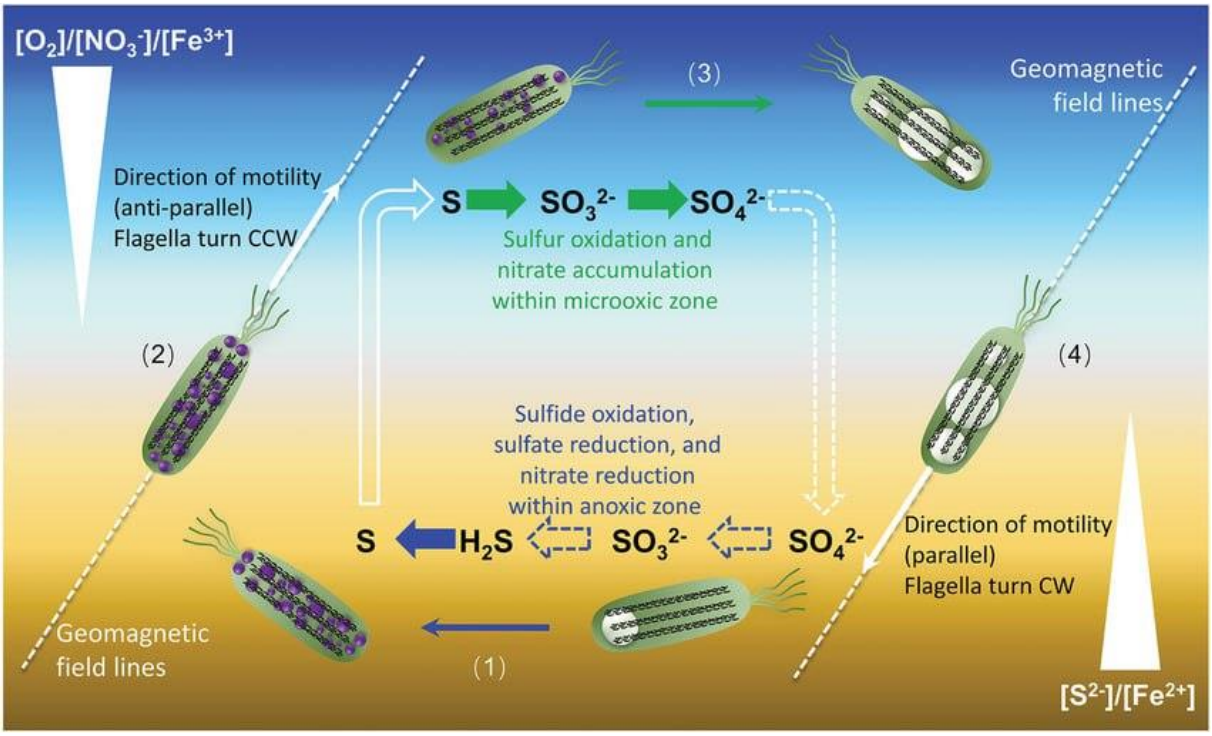

Figure 7: Hypothetical model for magnetotaxis in the sulfur cycle of MTB (northern hemisphere) (Jinhua et *al*.,2020).

5.3.3. Photo-taxis

The directed movement of organisms in response to light stimuli, is a behavioral mechanism that is increasingly gaining popularity in certain magnetotactic bacteria (MTB) as an additional complement to their systems of magnetotaxis and chemotaxis (Bidaud.2023). Although magnetotaxis enables these bacteria to swim along geomagnetic field lines and chemotaxis draws them to attractive flowers, phototaxis offers an additional layer of environmental sensitivity, especially in stratified aquatic environments with sharp light gradients (Bidaud. 2023). It has been suggested in a number of studies that some MTB may be able to sense light and, perhaps, use the light cues as a simple strategy to get to places where the physical factors being light, oxygen and redox states are able to control the distribution of those species (Mathon.2022). The molecular basis of phototaxis in MTB is not well described; nonetheless, it is proposed that light-sensing photoreceptors, for example flavoproteins and rhodopsins, may be alike in function to other phototactic bacteria (De Lanauze, 2013). Pairing phototaxis with magnetotaxis enables phototactic bacteria to have better control of the vertical direction, regulating their redox and chemical gradients, in situ, while at the same time changing their position with relation to the intensity of light, which in turn will allow them to modulate metabolic states (Cité des énergies, 2024). The above-described multi-modal navigation system demonstrates the extraordinary flexibility of MTB that allows them to constantly and quickly adapt their movement to fluctuating environmental conditions and, thus, to become ecologically successful in diverse microbial communities.

5.3.4. Redox-taxis

Redox-taxis are the most well developed navigation system in the case of Magnetotactic Bacteria (MTB), which cocultivate with other bacteria in very sophisticated and efficient ways, thus leading to the survival of the whole consortium in the ecology of stratified water bodies. The modus operandi underlying redox-taxis allows these protozoa not only to track but also to respond to electrochemical potential (Eh) gradients produced by redox interfaces, and therefore, they are able to distinguish the ecotones located either oxic or anoxic condition (OATZ) most effectively. The redox-taxis, in contrast to chemotaxis and aerotaxis, reflect a more global view of the redox level of the environment and can be measured by internal sensors such as cytochromes or iron-sulfur proteins (Taylor et *al*., 1999).

This type of navigation is of utmost importance, especially when it comes to in vivo localizations where magnetotaxis could passively align cells along the geomagnetic field lines. Besides ther1/oxygen-heliotaxis is used at the same time, the cells, e.g., *Magnetospirillumgryphiswaldense*, can move directionally towards microaerophilic microenvironments where low concentrations of oxygen and reduced fermentation substrates co-exist (Frankel et *al*., 2007; Lefèvre and Bazylinski, 2013).

This is one of the ways in which organisms, such as redox, etc., can adapt to different environments and evolutionary aspects of the migration process in colonizing the in the environment, which is characterized by change and competition (Schüler, 2020).

6. Magnetosome biogenesis

6.1.Magnetosomes

The movement of magnetotactic bacteria (MTB) is determined by specialized prokaryotic organelles called magnetosomes, which are intracellular, membrane-bound compartments containing nanometer-scale crystals of magnetic iron minerals typically magnetite (Fe_3O_4) or, in some species, greigite (Fe_3S_4) (table 2) (Bazylinski and Frankel, 2004; Liu et *al*., 2023)(Figure 8). The formation of these magnetosomes occurs at sites in the cytoplasmic membrane, and they are arranged in rows within the cell (with a long axis)(Jogler and Schüler, 2009). It has been realized that bacterial magnetosome is true prokaryotic organelle, with a comparable degree of complexity as its eukaryotic counterpart (Schüler 2008).Magnetosomes enable magnetic orientation similar to a “biological compass” and guide bacteria to areas of low oxygen concentration (microaerophilic or anaerobic) by a mechanism called magnetotaxis (Bazylinski and Frankel, 2004). Its formation requires sophisticated cellular compartmentalization work in which precursor vesicles of size, morphology and crystal alignment are finely controlled to avoid their toxicity (Komeili, 2007). Ecologically, they participate in its biogeochemistry of iron in aquatic sediments; and, technologically, its magnetic properties are exploited in innovative ways, particularly in nanomedicine (targeting of drugs) or in heavy metal decontamination (Frankel et *al*., 2003). These applications are based on their intrinsic stability and biocompatibility, superior to those of synthetic magnetic nanoparticles.

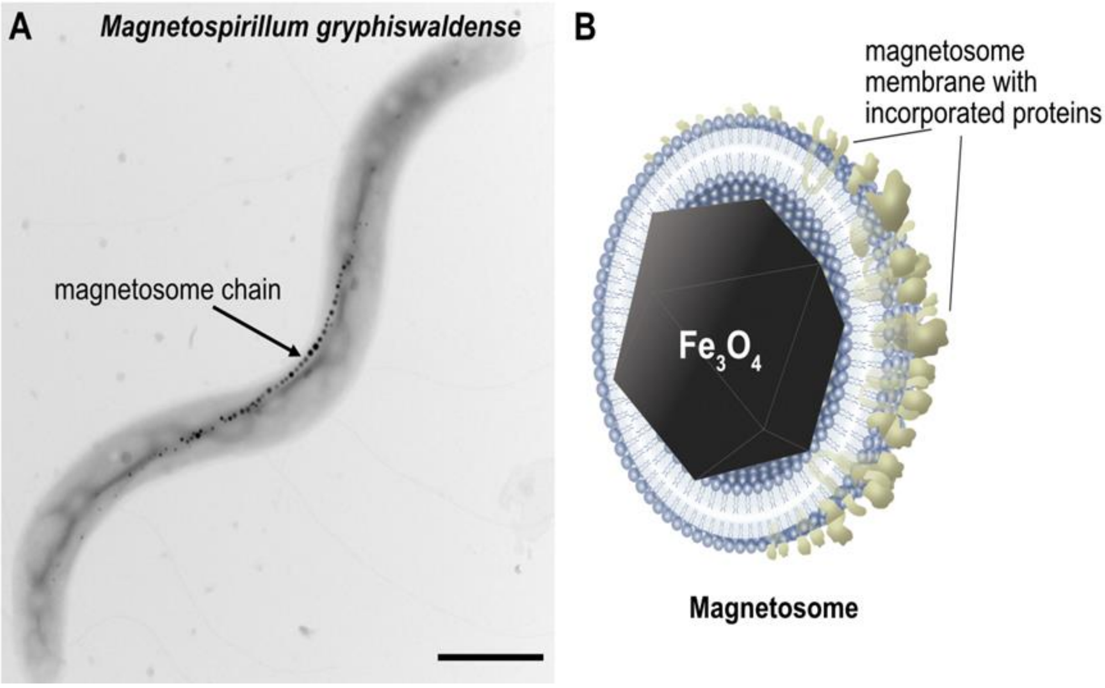

Figure 8: Magnetosomes of magnetotactic bacteria: sophisticated magnetic organelles and promising nanomaterial. (A) Model organism *Magnetospirillumgryphiswaldense*. Scale bar: 1 µm. (B) Schematic of a magnetosome (Marina Dziuba., 2023).

Table 2: Summary of the main MTB strains and their magnetite crystal structure (Hossain et *al*.2024).

Strain	Magnetosome mineral	Phylogenetic affiliation	Habit	Magnetosome elongation axis	Avg. length (nm)	Width/ length	References
Magnetospirillu mmagnetotactic umstrain MS-1	Magnetite	*Alpha-proteobacteria*	Cuboctahedral	**	43	0.9	Devouard et *al*., 1998;Buseck et *al*., 2001; Kobayashi et *al*., 2006
Magnetospirillu mmagneticumstr ain AMB-1	Magnetite	*Alpha-proteobacteria*	Cuboctahedral	**	45	0.85	Li et *al*., 2009
Magnetospirillu mgryphiswaldens estrain MSR-1	Magnetite	*Alpha-proteobacteria*	Cuboctahedral	**	33	0.91	Scheffel et *al*., 2006; Faivre et *al*., 2008
Magnetospirathi ophilastrain MMS-1 (MV-4)	Magnetite	*Alpha-proteobacteria*	Elongated, Octahedral	[111]	22–85	0.85	Meldrum et *al*., 1993a;Devou ard et *al*., 1998
blakemoreistrain MV-1	Magnetite	*Alpha-proteobacteria*	Elongated, Octahedral	[111]	60	0.65	Meldrum et *al*., 1993a
Uncultured MMP	Greigite	*Delta-proteobacteria*	Equidimensional ,	** and [100]	60–90	0.86	Pósfai et *al*., 1998a,b

			Irregular;Elongated, Irregular				
Ca. Desulfamplusmagnetomortisstrain BW-1	Greigite	*Delta-proteobacteria*	Equidimensional, irregular	ND	33	0.96	Lefèvreet*al*.,2011c
Unculturedrods	Greigite	ND	Equidimensional, irregular	** and [100]	60	0.9	Kasama et*al*.,2006
Ca. Magnetomorumlitorale	Greigite	*Delta-proteobacteria*	Elongated, bullet	ND	91	0.44	Wenteretal.,2009

6.2.Magnetosome formation

Magnetotactic bacteria (MTB) intracellularly compose structures called magnetosomes, which are magnetic iron minerals: magnetite (Fe_3O_4) or greigite (Fe_3S_4) surrounded by a phospholipid bilayer membrane. These organelles allow MTB to move in an Earth's magnetic field by a mechanism called magnetotaxis. Under microaerophilic conditions, MTB cultures produce nanoscale magnetite or greigite crystals. Typically, these structures range in size from 25 to 100 nm and exhibit different morphologies, the most frequently observed of which are octahedral cubic, elongated rhombic, bullet-shaped, and other types of crystallization. These intracellular structures within the cell are usually organized into single or multiple linear chains dissociated by the magnetosome membrane. This is both the organ that ensures biomineralization and functional specificity through its proteins which create a unique biochemical microenvironment and confer properties to bacteria, indicated in (Table 3).

Table 3: A list of proteins from MTB related to magnetosome formation (Ren et *al*., 2023).

Function	**Protein**	**Molecularweight (kDa)**
Membrane invagination	MamB	31.9
	MamM	34.5
	MamL	8.6
	MamI	7.2
	MamQ	30
	MamY	40.9
Proteinrecruitment	MamA	46.8

Iron transport	MamB	31.9
	MamM	34.5
	MamH	45.7
	MamZ	70.5
	MamT	18.9
Iron oxidation and reduction	MamZ	70.5
	MamP	29
	MamT	18.9
	MamX	28.2
	MamE	73.5
Crystal nucleation	MamE	73.5
	MamM	34.5
	MamO	66.3
Crystal growth	Mms5	5.79
	Mms6	12.7
	MamR	9.3
	MamD	30
Chain assembly	MamJ	48.5
	MamK	39.2
	MamY	40.9

The biosynthesis of magnetosomes is governed by a specialized genomic region, often referred to as the magnetosome gene island, which is composed of guanine-cytosine (GC) rich region containing transfer RNA (tRNA) genes, pseudogenes, mobile genetic elements (e.g., transposases, integrases), and transposons. A few important operons that are located throughout the magnetosome island (e.g., MamAB, MamXY, Mms6, and FeoAB1) regulate specific aspects of magnetosomes, including iron uptake, crystal nucleation, and crystal maturation. The operons involved in magnetosome biosynthesis are coordinated to ensure magnetosomes form in a tight regulated system producing magnetosomes with similar size, morphology and directionality. The biosynthetic process occurs in a series of steps including the biosynthesis of vesicles, uptake of iron, growth of the crystal, and assembly of a chain (see Figure 13). Therefore, the configurational and structural properties demonstrated by magnetosomes can be attributed, in part, to the genes that are associated with magnetosome biosynthesis, resulting in unique physicochemical properties that have made magnetosomes both biologically relevant and technologically relevant (Ren et *al*., 2023).

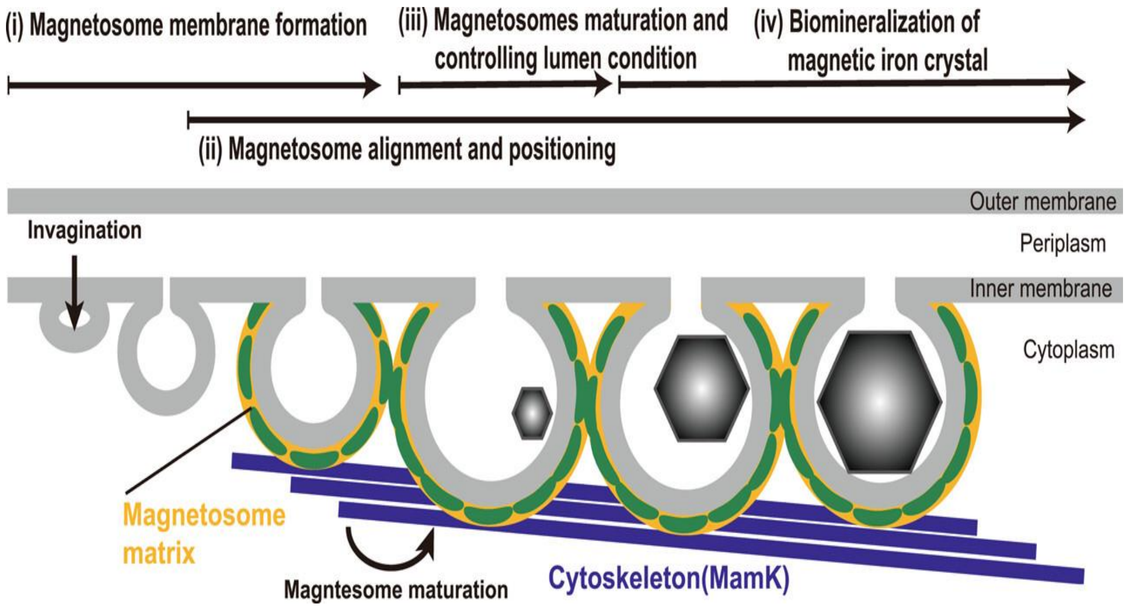

Figure 9: Schematic representation for a model of the magnetosome formation process and magnetosome structures (Murat et *al*., 2010).

7. MTB Applications

Magnetotactic bacteria (MTB) are water dwelling bacteria that utilize magnetosomes nominally sized lipid-bounded greigite or magnetite nanocrystals to move the magnetic field of the Earth. MTB has superior magnetic properties than typical synthetic nanoparticles due to the magnetosomes (Faivre and Schüler, 2008). Their efficient magnetic navigability has attracted attention to medical (example targeted treatment, Hyperthermia and MRI), environmental (bioremediation), and nanotechnology applications (data storage, biosensors) due to their biocompatibility and strong, homogeneous magnetic properties (Alphandéry, 2014; Byrne et *al*., 2011; Pan et *al*., 2008).

7.1. Medical applications

MTB and their magnetosomes have high potential in biomedical research, where they are used extensively in drug delivery systems (targeted drug delivery and as nanocarriers), imaging and diagnostics, and pharmaceuticals (Wang Q et *al*., 2024). MTB can be engineered to deliver anticancer drugs to the sites of tumors, enhancing treatment efficacy and reducing the side effects (Zhang J., 2024)(Figure10) Magnetosomes are a natural drug nanocarrier for therapy molecules like antibodies and siRNA, enhancing stability and targeted delivery(Alsharedeh R.,2024).

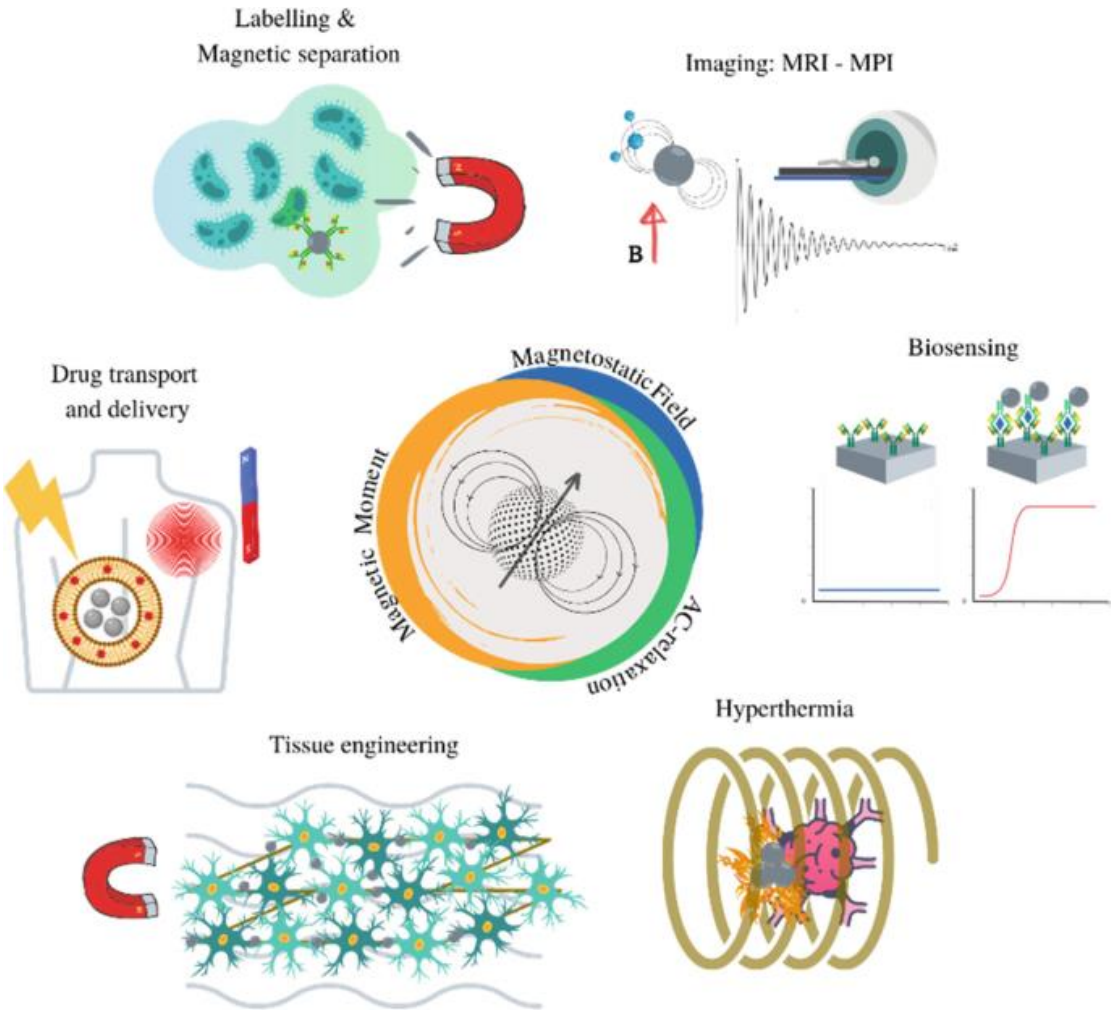

Figure10: Multiple uses of bacterial magnetosomes and magnetite in the biomedical field.(Alphandéry et *al*.,2021)

7.1.1 .Vectors for targeted drug delivery

The use of magnetotactic bacteria (MTB) and magnetosomes is an exciting new method for targeted drug delivery, particularly for cancer therapies. The ability of MTB to be manipulated in the presence of an external magnetic field allows for precise targeting to the tumor site. This would allow the MTB to deliver drug(s) much closer to the therapeutic concentration threshold needed for the cancer to be treated, while minimizing damage to healthy tissue; they can be modified to deliver drugs in either nanoparticles or liposomes that are capable of releasing drugs once in the field of a magnetic field. When the MTB releases the drug at the site of the tumor, the location is controlled by the MTB that have been guided there using the externally applied magnetic field. This limits systemic toxicity to the body. The biohybrid aspect of the MTB allows for greater multidimensional combinations of drugs to be used for more synergistic drug delivery. Researchers have confirmed that magnetosomes can be loaded with a cytotoxic drug and injected targeting the non-healthy tissue, resulting in treatment with no significant side effects to healthy tissue (Kotakadi et *al*., 2022).

7.1.2. Hyperthermia

Magnetosomes have significant potential for cancer therapy using magnetic hyperthermia. It’s a technique that heats tumors by applying an external alternating magnetic field. The therapeutic range for hyperthermia is often 42–45°C, and can last up to 30 minutes at once. This is effective for inducing cell death of tumor cells, or being able to sensitize tumors to be treated with another type of therapy (Alphandéry, E. et *al*., 2011; LeFèvre et *al*., 2017). Tumor temperatures above 47°C can induce direct cell death, while mild hyperthermia (41–47°C) (Figure 11) can increase vascular permeability, thereby, changing the tumor microenvironment and increasing the effectiveness of performed therapies (Lui.X et *al*., 2020). Magnetosomes can create a much more precise and controlled intracellular heating because they have superior magnetic properties and high coercivity; magnetosome heating will allow for even heating at the intracellular level and has less collateral damage to healthy tissue when compared to classical hyperthermic methods of heating tissue externally and in homogeneously (Molcan M et *al*., 2022; Alphandéry, E. et *al*., 2011). Clinical and laboratory tests have shown that maintaining tumor temperatures in the recommended range during multiple therapies can result in regressing tumors or even complete disappearing of tumors with fewer side effects than radiotherapy and chemotherapy (Zargar FA et *al*., 2024; Hao X et *al*., 2024; Zeng et *al*., 2023).

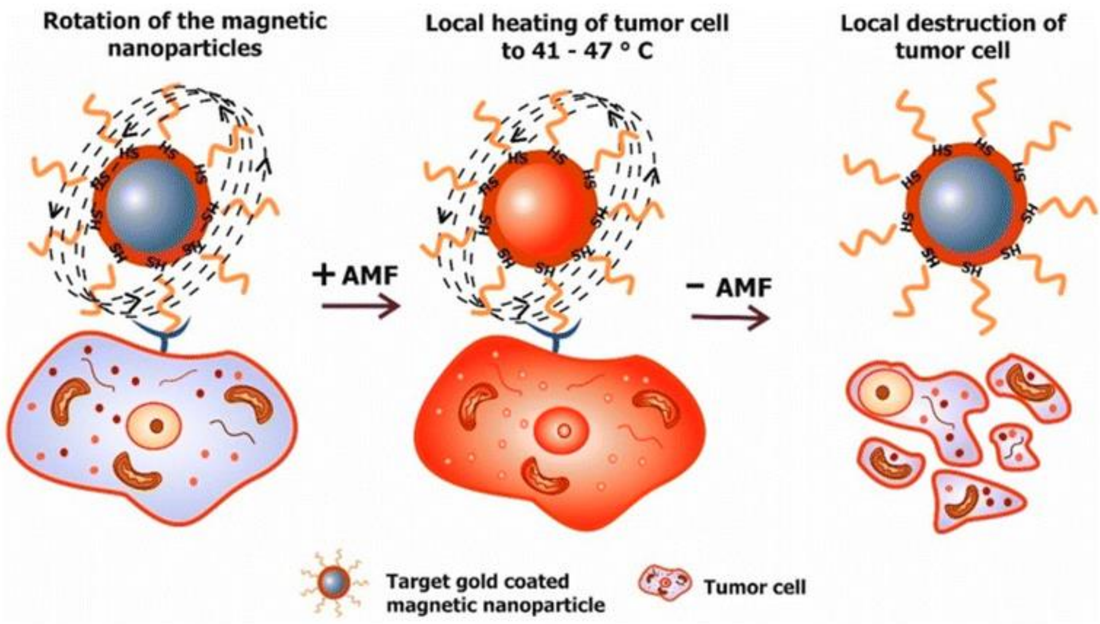

Figure 11: Working principle of magnetic hyperthermia. Targeted MNPs delivered to a tumour site are exposed to an external magnetic field. Afterward, the increase of the tumour temperature to 41–47 °C is responsible for cell death (Belyanina.I et *al*.,2017).

7.1.3. Magnetic resonance imaging

MRI conveys high resolution 3D images with excellent visualization of soft tissue due to the labelling of diseased tissue against healthy tissue/differences in both proton density and/or relaxation times (Gae.H et *al*., 2020) (Figure 12). It is important to understand how MRI contrast agents work, the purpose of them, and the categories which they belong to; MRI uses different types of contrast agents from chemical agents to nanoparticles which are classified as elastic extracellular fluid agents to blood pool agents/blood-bound local agents; contrast agents can also be developed which will specifically target certain organs/tissues, and sometimes the pulmonary vasculature (Wahsner.J et *al*., 2018). Iron oxide nanoparticles, specifically bacterial magnetosomes, have been highlighted as one of the most studied types of MRI contrast agent because of their unique magnetic properties and excellent biocompatibility. Bacterial magnetosomes reduce T2 relaxation time, making T2-weighted MRI images darker. Their uniform size, shape, and crystalline structure often in chains give them superior magnetic properties compared to chemically synthesized iron oxide nanoparticles. As a result, they produce higher MRI contrast, and magnetic bacteria have become promising candidates for use as MRI contrast agents (Wang Y et *al*., 2019; Zijian Zhou et *al*., 2019).

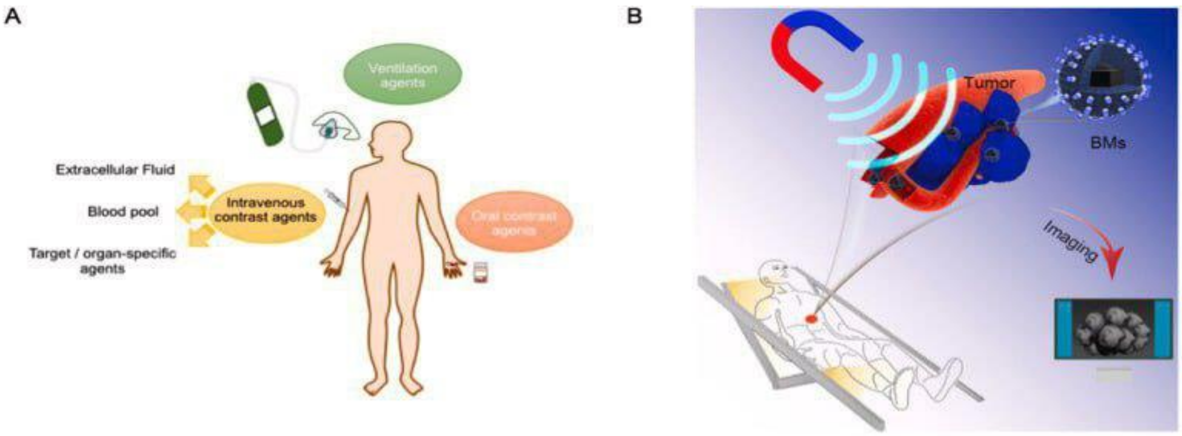

Figure 12: Application of magnetosomes in MRI. (A) Route of administration of MRI contrast agents(B) Schematicillustration of bacterial magnetosomes (BMs) used as contrast agents for MRI (Wahsner.J et al.,2018).

7.1.4. Magnetic particle imaging

Magnetic Particle Imaging (MPI) is one application of superparamagnetic nanoparticles in molecular imaging that offers positive contrast with zero background noise or radiation and infinite tissue penetration. It is utilized in multimodal imaging, cell tracking, drug delivery, and tumor imaging(Zheng.B et *al.*, 2015, Fidler.F et *al.*, 2016, Yu.E et *al.*, 2016, Haegele.J et *al.*, 2012, Zheng.B et al. 2017). Superparamagnetic iron oxide nanoparticles also enable magnetothermal tumor therapy with less normal tissue damage (Tay et al., 2018) and long circulation and high-contrast 3D imaging in mice (Song et al., 2019). Natural magnetosomes have advantages over synthetic tracers due to higher purity and magnetic qualities.

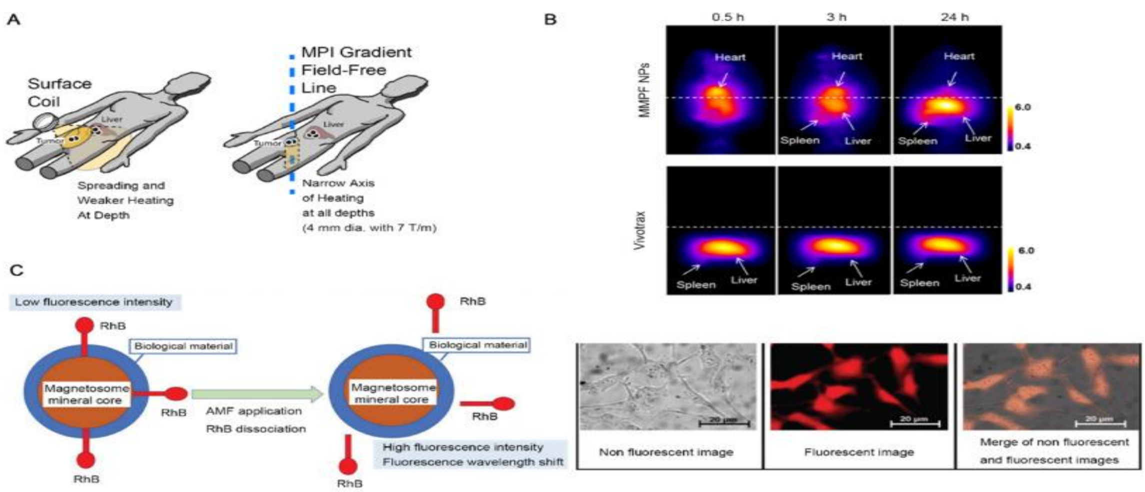

Figure 13: Application of magnetosomes in MPI and fluorescence imaging (Tay.W et *al.*,2018).

- **The future of MTB in biomedical applications:**

Magnetotactic bacteria (MTB) and magnetosomes hold great promise in medicine due to their unique magnetic properties and biocompatibility. MTB are being developed for drug targeting, imaging contrast enhancement, and cancer therapy by magnetic hyperthermia, providing treatments with increased accuracy and fewer side effects. New advancements in the engineering of MTB, such as using MTB as microrobots, will revolutionize targeted drug delivery and treatment accuracy. Genetic research on magnetosome development is unlocking the door to customize these particles for application in humans and on an industrial level. In spite of production and regulatory challenges, rapid progress suggests that MTB based technology has the potential to offer highly effective biomedical therapy and diagnosis tools in the near future.

7.2. Environmental Applications

Magnetotactic bacteria (MTB), due to their unique ability to biomineralize magnetic nanoparticles known as magnetosomes, have emerged as useful agents for various environmental applications, particularly bioremediation, wastewater treatment, and environmental monitoring. Magnetotactic bacteria are capable of aligning along geomagnetic fields, a feature that not only renders them fascinating in the context of microbiology but also highly useful in engineered environmental systems (Figure 14).

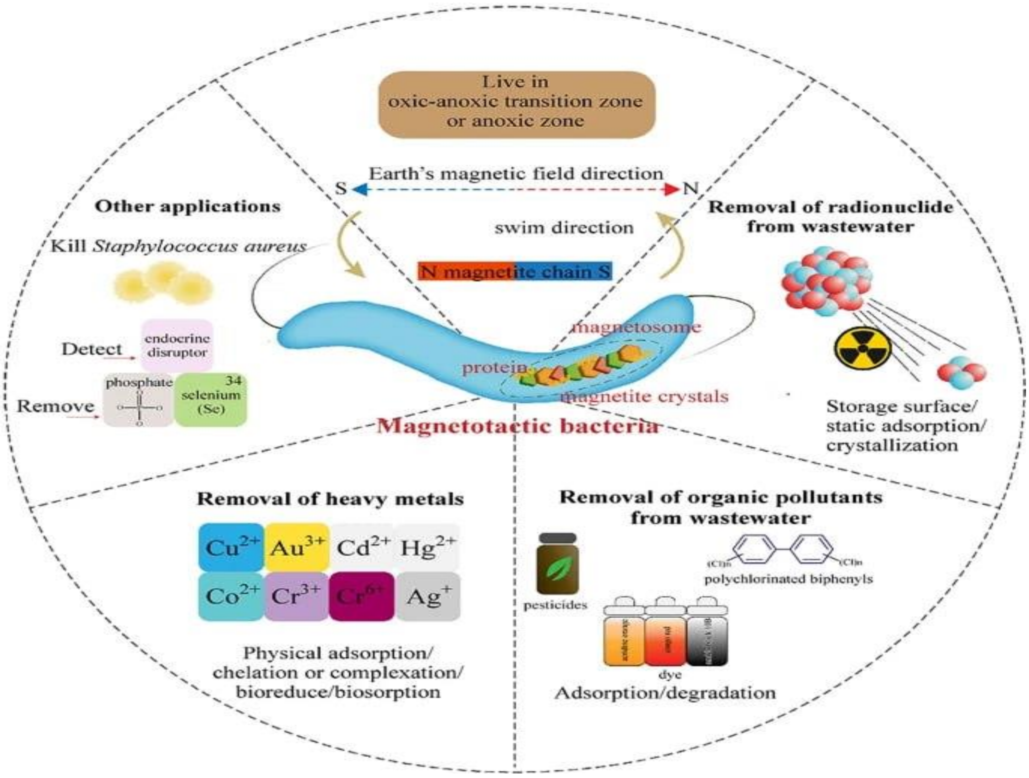

Figure 14: Environmental Applications of magnetotactic bacteria (Xinjie wang, 2020).

7.2.1. Bioremediation

Magnetotactic bacteria (MTB) are valuable players in ecosystem remediation thanks to their unique ability to immobilize, transform, or accumulate contaminants through two main mechanisms: bioaccumulation and biomineralization. Magnetosomes, magnetic nanoparticles, associated with these mechanisms, function as "nanomagnets" to trap pollutants and facilitate their recovery.

- **a.Bioaccumulation by Magnetotactic Bacteria**

Magnetotactic bacteria (MTB) implement similar mechanisms to bioaccumulate pollutants, combining active transport, magnetosomal adsorption, and intracellular chelation. Indeed, their ability to internalize heavy metals (cadmium (Cd^{2+}), lead (Pb^{2+}), cobalt (Co^{2+}), zinc(**Zn^{2+}**),) or organic pollutants (hydrocarbons, pesticides) is based on specialized membrane transporters (e.g., ZIP proteins for zinc Arakaki et *al*., 2008) and on the electrostatic affinity of magnetosomes, which are equipped with hydroxyl (-OH) and carboxylate ($-COO^-$) groups capable of adsorbing almost all cations (Wang et *al*., 2021). Let us mention here *Magnetospirillumgryphiswaldense*, which can accumulate up to 12% of its dry weight in lead (50 ppm), which differentiates it from some non-magnetotactic bacteria but especially from other magnetotactic bacteria that do not have this trapping capacity on their magnetosomes (Li et *al*., 2020).

The fields of application for bioremediation are diverse:

- Selective decontamination: BMTs have removed 90% of soluble uranium (U^{6+}) guided by a magnetic field, preferring it to insoluble U^{4+}, stored in vesicles associated with magnetosomes (Beazley et *al*., 2023).
- Metal recovery: Magnetosomes loaded with rare earth or precious metals, particularly silver, are recoverable and reusable, as an alternative to mining (Sivan et *al*., 2023).
- Pesticide treatment: Magnetococcusmarinusbioaccumulates DDT via heat shock proteins (Hsp70), up to 25 ppm in contaminated soils (Gomez et *al*., 2022).

- **b.Biomineralization by Magnetotactic Bacteria**

Magnetotactic bacteria (MTB) play a key role in controlled biomineralization by synthesizing, under strict physiological conditions, magnetic nanoparticles (magnetosomes) of magnetite (Fe_3O_4) or greigite (Fe_3S_4). This mechanism is genetically and biochemically controlled by specific genes (mam, mms) regulating, among other things, the formation of membrane vesicles, iron uptake, and mineral crystallization (Bazylinski and Frankel, 2004). Magnetosomes, organized in chains, provide bacteria with orientation to the Earth's magnetic field, allowing them better motility in stratified environments. These biomineralizing capabilities present innovative perspectives in biotechnologies and the environment.

The fields of application of Biomineralization are diverse:

- Decantation of elements contaminated by heavy metals in BMT: they reduce pollutants: for example, hexavalent chromium Cr^{6+} is reduced to non-toxic Cr^{3+} by reducing enzymes (Chen et *al.* 2020) in water, efficient in the order of 95%.
- Arsenic treatment: Magnetospirillumgryphiswaldense transforms arsenic As^{3+} into iron arsenate $FeAsO_4$, a stable compound, 85% of the pollutant is eliminated (Wang et *al.*, 2022).
- **c. Magnetorecovery by magnetotactic bacteria**

The magnetorecovery technique using the magnetic properties of magnetotactic bacteria (abbreviated as MTB) aims to separate and recover contaminants or precious metals from complex media (water, soil, sludge) using their magnetosomes. Magnetosomes, which are very small nanoparticles of magnetite (Fe_3O_4) or greigite (Fe_3S_4) forming intracellular chains, give MTB a magnetizing capacity, which can be exploited by guiding them with an external magnetic field. The magnetorecovery mechanism is based on three steps: (1) adsorption of pollutants (heavy metals, radionuclides) on the surface of magnetosomes via electrostatic or chemical bonds, (2) magnetic capture of charged BMTs generated using a magnet or a magnetized matrix, and (3) recovery of contaminants by desorption or chemical treatment (Bazylinskiand Frankel, 2004; Sivan et *al.*, 2023).

The fields of application of magnetorecovery are diverse:

- Effluent remediation: BMTs can reduce up to 95% of hexavalent chromium (Cr^{6+}) in effluents treated with Cr^{3+} and then adsorb it on magnetosomes (Chen et *al.*, 2020). This is an economical solution compared to recipes based on conventional chemicals.

- Critical metal recycling: *Magnetospirillummagnetum* strains contribute to the extraction of rare earth elements (e.g., neodymium, dysprosium) from electronic waste with an efficiency of 80% towards a circular economy (Sivan et *al.*, 2023).
- Oil spill treatment: BMTs functionalized via hydrophobic ligands trap aromatic hydrocarbons (e.g., benzene) in seawater, with an adsorption capacity of 45 mg/g and rapid magnetic recovery (Martinez-Boubeta et *al.*, 2021).
- **d. Challenges and Opportunities for Innovation**

Magnetotactic bacteria (MTBs) possess the ability to extract metals free from dangerous chemicals which positions them as ideal candidates for metal recycling and bioremediation (Schüler, 2008). The main obstacles for this process include toxic effects of metals and competition between iron and other metals together with high production expenses (Wang et *al.*, 2022). Research developments including Cad A pump overexpression (Chen et *al.*, 2023) together with chelators and synthetic biology techniques and magnetosome functionalization approaches (Uebe and Schüler, 2016) enhance their potential.

7.2.2. Wastewater Treatment with Magnetotactic Bacteria

MTB is a strong and environmentally friendly method in wastewater treatment. Magnetosomes are magnetic magnetite (Fe_3O_4) nanoparticles, which characterize them, that are stored or concentrated by the bacteria under various physiological conditions, allowing them to adsorb various pollutants (heavy metals, organic substances, residues of drugs) on one of their surfaces via bioaccumulation, electrostatic adsorption, and enzymatic degradation mechanisms. For example, strains that are engineered can eliminate 70-80% of tetracycline from pilot reactors, preventing the potential to develop resistance (Gomez et *al.*, 2022).

One of the greatest advantages is the possibility of recovering BMTs using magnetic field separation, hence avoiding costly centrifugation or filtration steps, in an exceptionally clean (no harsh chemicals utilized) and cost-effective (40% less energy consuming than conventional methods, Sivan et al., 2023) process. Nonetheless, there is still difficulty and cost of production on a biomass scale, at least partly due to the remaining high iron requirements and partly due to the microaerophilic growth conditions required (Schüler, 2008), and partly due to the danger of release of pollutants at various pH levels, for which long-term stability tests are necessary.

Prospects include the genetic engineering of strains capable of thriving in extreme environments (acidity, salinity) and the integration of BMT into hybrid biofilms that would incorporate nanoparticles, making it possible to simultaneously target metals and micropollutants. Pilot projects conducted in wastewater treatment plants are already testing their potential in a circular economy whose objective is to recover critical metals (rare earths), while depolluting (Wang et *al.*, 2022).

7.2.3. Environmental Monitoring with Magnetotactic Bacteria

Magnetotactic bacteria (MTB) serve as bioindicators to monitor redox conditions in aquatic ecosystems. Their presence is correlated with oxygen and iron gradients, two compounds that characterize oxic-anoxic interfaces in sediments or water columns. For example, in the Seine estuary, *Magnetospirillum*populations peak at specific depths that can be interpreted as redox transition zones, redox conditions conducive to microbial activity and essential for the iron cycle (Lefèvre et *al.*, 2014). This ability to reflect biogeochemical dynamics makes them valuable tools for monitoring the health of natural or degraded environments.

Bacterial magnetosomes (BKs) serve as extremely sensitive biosensors which use motility inhibition and magnetosome synthesis reduction caused by heavy metals to detect toxicity (Li et *al.*, 2017). The expression of reporter genes enables genetically engineered BKs to react to hydrocarbons and pesticides and other contaminants for real-time monitoring of contaminated water and soil.

Magnetosomes functionalized through specific ligand attachment enable the magnetic separation of pollutants including lead together with microplastics and PFAS; chitosan magnetosomes demonstrate maximum 90% lead desorption from wastewater (Sun et *al.*, 2020). The combination of magnetosomes with enzymes enables the breakdown of persistent organic substances including textile dyes which supports environmentally friendly remediation approaches.

The technology remains promising yet faces difficulties from strict cultivation requirements and GMO ethical issues. Researchers study the combination of magnetosomes with electronic sensors and artificial biofilms and pilot programs test their wastewater treatment and metal recovery applications to maintain BKs as leading tools for green monitoring and pollution control (Wang et *al.*, 2023).

7.3. Nanotechnology and Materials Science Applications of Magnetotactic Bacteria

Magnetotactic bacteria (MTB) and their magnetosomes have created a new paradigm in nanotechnology and materials science. MTB, under ambient conditions, are able to bioreduce, biosynthesize, and biomineralize single domain magnetic nanoparticles that are highly homogenous. These naturally produced nanostructures possess properties that are otherwise very difficult to produce by traditional synthetic methods, such as monodispersity, controlled morphology, high crystallinity, and surface functionality (Faivre and Schuler, 2008).

7.3.1. Nanotechnology and Materials Science

Magnetosomes in MTB can be found in chains, often induced by a cytoskeletal filament called MamK (Komeili, 2007). The natural formation of chains is very useful for magnetic nanowire and nanoribbon formation, which are critical components for spintronic device manufacturing, as well as high density magnetic memory and magnetic sensors.

By taking advantage of the biological self-assembly of MTB, researchers can make ordered magnetic architectural designs with magnetosomes without using complicated and expensive lithography techniques. Additionally, the magnetic dipolar interactions between the magnetosomes may help stabilize and uniform the resulting nanostructure. This can be advantageous for nanoscale electronics (Faivre and Schuler, 2008).

7.3.2 Biosensing and Actuation Devices

Magnetosomes can be functionalized with specific biomolecules (e.g., antibodies, aptamers), rendering them excellent choices for the development of biosensing devices that respond to magnetic fields (Lefèvre andBazylinski, 2013). These biosensing devices can detect many biological or chemical targets with high sensitivity and selectivity. Magnetosomes can also be manipulated by applying an external magnetic field, which enables magnetosomes to serve as microactuators in lab-on-a-chip devices, which require precise movement of particles (Alphandéry, 2014).

7.3.3. Nanofabrication Templates

The small size and highly homogeneous surface chemistry of magnetosomes favor their use as templates for nanofabrication. Indeed, by coating magnetosomes with metallic or semiconducting materials, scientists can obtain core-shell nanostructures whose optical, electrical, or magnetic properties can be tuned (Yan et *al.*, 2012).

Furthermore, MTB and magnetosomes are currently being investigated for their integration into bottom-up nanofabrication strategies, which aim to exploit biological systems to precisely and reproducibly assemble materials in the nanoscale range. The latter approach represents a departure from more conventional top-down fabrication methods and is in line with the principles of green chemistry and sustainable nanotechnology (Bazylinski and Frankel, 2004).

7.3.4. Advantages over synthetic nanoparticles

Compared to synthetic magnetic nanoparticles, magnetosomes possess (table 4):

- Better magnetic properties: higher magnetization saturations and lower coercivities.
- High monodispersity: narrow size distribution, improving performance reproducibility.
- A natural membrane coating: simplicity of bioconjugation and implied biocompatibility (Faivre and Schuler, 2008).

MTB and its biomineralization processes therefore constitute a veritable biological toolbox for the production of advanced nanomaterials incorporating naturally optimized functionalities, which presents clear advantages for future applications in nanoengineering.

Chapter II

Materiel and Methods

1. Sample Collection

1.1.Criteria for Site Selection

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-anoxictransition zones (OATZ) of aquatic sediments, particularly freshwater environments, estuaries, and shallow marine environments (Figure 15), the components that comprise these specific locations allow for low oxygen levels and support the cycling of iron and sulfur important for magnetosome formation (Lefèvre and Bazylinski, 2013). 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. We prioritized low-turbulence sites to help maintain the redox gradient and preserve the microbial microhabitat. Additionally, we considered site accessibility, the potential to take repeated samples, and the limited presence of human influence aided additionally in the decision-making process during site selection (Lin et *al.*, 2008). Furthermore, Magnetotactic bacteria are typically found in temperate conditions, with optimal growth at 20 to 30 °C (Guggler and Schuler, 2009).

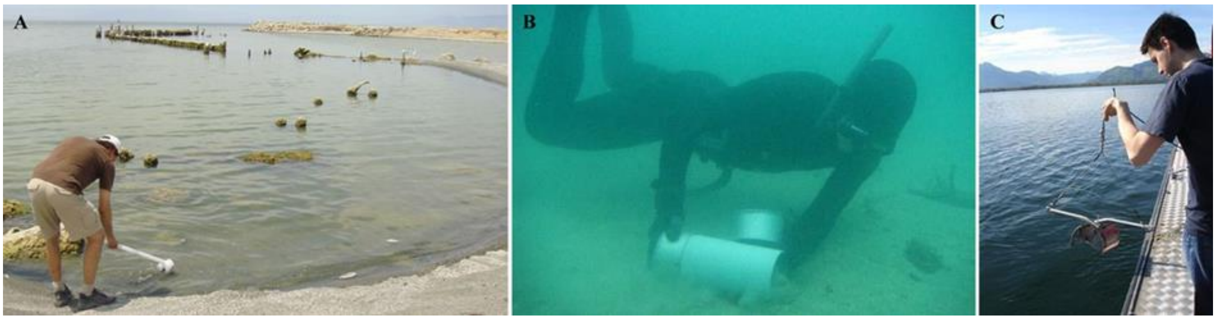

Figure 15: Sampling for magnetotactic bacteria using different strategies: from the shore of the Salton Sea with a scooper (A), underwater in the Mediterranean Sea by free diving (B), and with a bottom sampler in Lake Chiemsee, Bavaria (C). (Panel C courtesy of S. Kolinko and D. Schüler, reproduced with permission) (Lefèvre and Bazylinski 2013).

1.2.Description of Sampling

In order to isolate, characterized, identifying and detecting the diversity of magnetotactic bacteria, samples from two different lakes: DJEBEL EL OUAHCH Lake, EL MRIDJ Lake (Figure 16, 17).

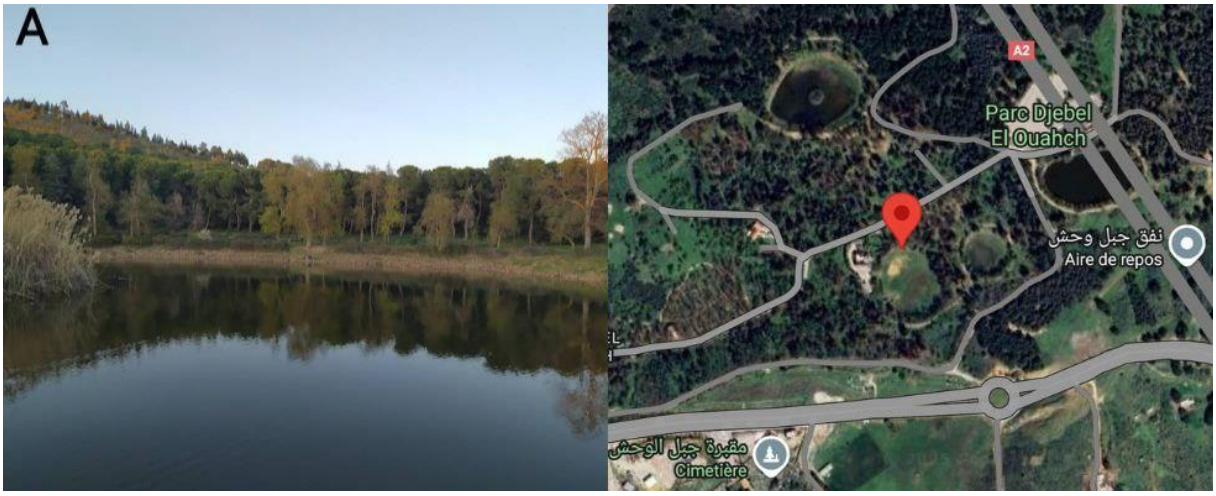

Figure 16: A) DJEBEL EL OUAHCH Lake

Figure 17: B) EL MRIDJ Lake

According to Li et *al* (2017), Wei lin et *al*., 2012 method, at each site composite samples of surface water and surficial sediments were collected manually from depth 20–30 cm with an emphasis on the oxic-anoxic transition zone (OATZ) where MTB are thought to be concentrated in the water column because of their preference for microaerobic habitats (Bazylinski and Frankel, 2004). Sterile autoclaved glass bottles were used to take samples where it is fully filling with 30–40% of sediments and 60–70% of water on top (Figure 18). Air bubbles were prevented. To limit the addition of oxygen into the samples caps are closed as quickly as possible without oxygenating the sample. Moreover, the samples were not subjected to any kind of strong agitation because this would run more risk of exposure to oxygen. Once entering the laboratory, bottles were placed with their caps slightly sealed so that just a little gas exchange and pressure release could occur while preventing water from evaporating too much and in a cool dark (approximately 4°C) location to allow for the natural stratification of microorganisms and the development of a redox gradient .This arrangement enabled a situation conducive for the occurrence of an oxic-anoxic transition zone (OATZ) for the activation and movement of magnetotactic bacteria. The samples were not disturbed for approximately 24 hours to provide enough time for MTB to congregate at the sediment-water interface and increase their visibility for microscopic observation (Abreu et *al*., 2011; Lefèvre and Bazylinsk i2013).

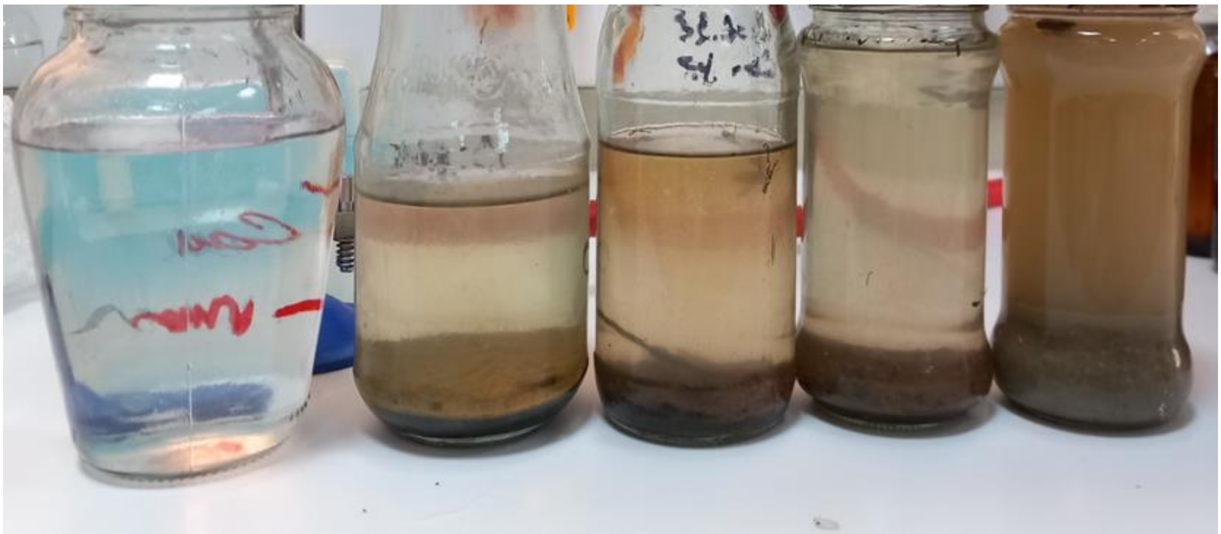

Figure 18: Sediment and water samples collected from the two different locations.

Depending on the type of habitat, whether freshwater or marine, magnetotactic bacteria (MTB) can survive for periods ranging from weeks to years, even without any nutrient supplementation(Lefèvre and Bazylinski 2013) . The majority of our study focused on bacteria from sediments due to the fact that the sediment samples could still be observed and magnetically sorted up to 5 years after their sampling and storage in the laboratory (Monteil et *al*., 2020).

2. Detection of MTB in microscope

2.1. Enrichment 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. So before placing the sample under the microscope, it is first concentrated by setting two magnetic rods were placed on either side of the container with one rod's north pole and the other rod's south pole facing the sample container, creating a directional magnetic field across the sample(Figure 19). This encourages the MTB to align and cluster along the magnetic field lines, which increases the local density, allowing for easier visualization of the MTB in the microscope. After the MTB were allowed to migrate for a sufficient time (generally from 1h to 3 h) if MTB are abundant, a grayish, whitish, or brownish spot composed mainly of these cells appears inside the flask wall, on the side exposed to the magnet. Afterthat, a small volume was removed from the magnetic region of the sample using a sterile pipette and placed on a microscope slide. This method greatly enhanced the visualization of MTB motility and morphology in the optical microscope (Blakemore 1975; Abreu et *al*., 2011).

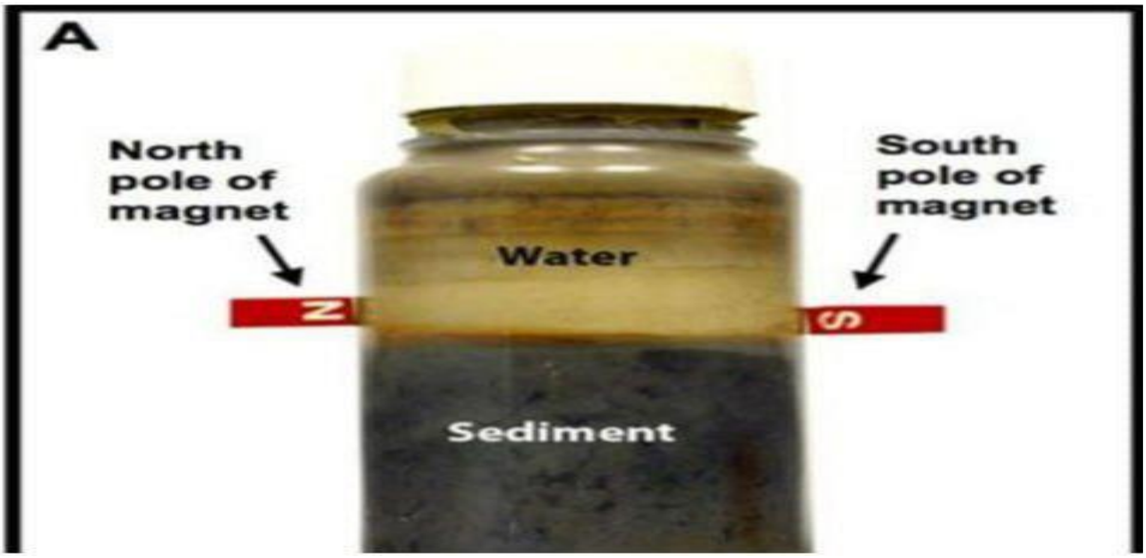

Figure 19: Sediment and water sample collected from the Olentangy River in Columbus, Ohio (USA). The bottle contains approximately one-half sediment and one-half water. The south end of a magnet is placed approximately 1 cm above the sediment for up to several hours (Wei lin et *al*., 2012).

2.2.Microscope slide preparation

Then according to Wei lin et *al*., 2012 , Lefèvre and Bazylinski (2013), the hanging drop detection technique was used to prepare for microscopic observation which involves placing with micropipette a few drops of the magnetically enriched sample (water/sediment) on A clean microscope slide (lame), prepared with a small circular ring of silicone or rubber cement to form a shallow well, served as a barrier for containing the liquid sample, and reducing the drying rate (evaporation) of the sample during the observation (Figure 20).A clean coverslip was placed carefully on the top avoiding air bubbles and ideally covering the entire silicone (wet) sample area. The wet mount technique controlled movement of the sample and allowed for the continued motility of magnetotactic bacteria by limiting oxygen availability prior to the sample that being observed with optical microscopy. Finally, a small stir bar magnet is placed on the microscope stage, close to the drop, so that its axis is parallel to the plane of the slide and passes through the center of the drop. To observe MTB collected in the Northern Hemisphere, the south pole of the magnet must be oriented toward the drop, generating a local magnetic field of at least a few gauss. The cells then respond by migrating toward the edge of the drop closest to the magnet, where they become observable under the microscope. By reversing the magnet's polarity, the bacteria also change direction, confirming their magnetotaxis.

The identification of MTB done via the optical microscope with 400x to 1000x magnification.

Figure 20: Slides contain the magnetically enriched samples collected from two different locations.

3. Purification of MTB

The purification of magnetotactic bacteria (MTB) from environmental sources is important for enrichment and to eliminate the influence of non-magnetic bacteria. After initial microscopic screening for possible MTB, samples from two freshwater lakes were cultured in Two media were made to allow for purification and selective growth of magnetotactic bacteria (MTB), standard liquid medium and semi-solid *Magnetospirillum* growth medium

(MSGM) (Heyen, U and Schüler, D. 2003), as both can support the aerobic and chemolithoautotrophic nature of MTB (Lefèvre et *al*., 2011).

3.1.Media preparation

Magnetotactic bacteria were grown in MSGM (*Magnetospirillum*Growth Medium), formulated according to (Heyen and Schüler, 2003) specifically for the cultivation of *Magnetospirillumgryphiswaldense*, and standard medium initially developed by Blakemore et *al*. (1979). The composition of standard and MSGM mediums is as follows (table 4):

Table4: The composition of standard and MSGM mediums.

Medium/ composition	**Carbon source**	**Nitrogen source**	**Source of sulfur**	**Buffer**	**Source of iron**	**Trace elements**	**vitamin's**	**Reducers**
standard liquid nutrient media	Sodium lactate	yeast extract	sodium sulfate	potassium phosphate	Ferrous chloride	Trace elements Solution	Vitamin's solution	Cysteine-HCl
MSGM (*Magnetospirillum* Growth Medium)	Sodium lactate	Ammonium chloride	Magnesium sulfate heptahydrate	Potassium phosphate monobasic	Iron(III) sulfate heptahydrate	Trace elements Solution	Vitamin's solution	sodium thioglycolate

This non-specific medium promoted the initial microbial growth in a completely aerobic condition. Meanwhile, semi-solid MSGM (0.10% agar) was used to establish the redox gradient which is important for MTB localization in the oxic-anoxic transition zone. This formula (in 1 litre of distilled water) is provide here based upon the previously described methods (Schüler, 2008; Heyen and Schüler, 2003). The media are sterilized by autoclaving (120°C, 20 min), except for the heat-sensitive components (vitamins, sodium thioglycolate), which are sterilized by filtration (0.22 µm) and then added under high laminar flow to the media.

Figure 21: Standard liquid nutrient media

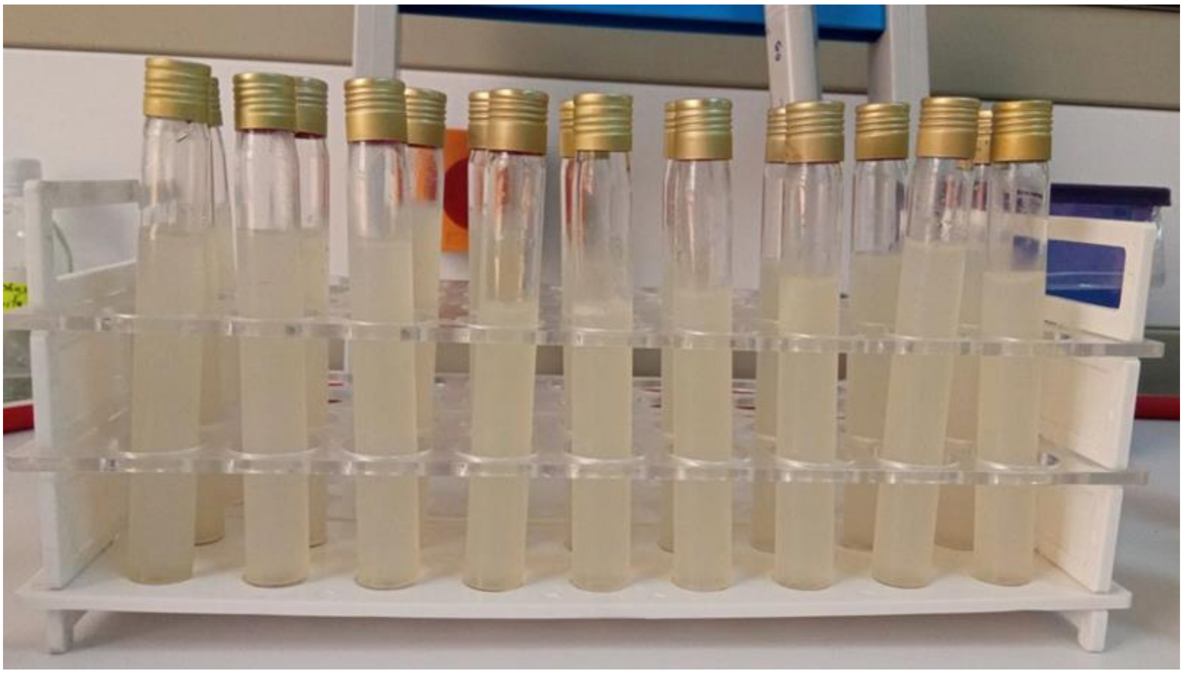

Figure 22: Semi-solid *Magnetospirillum*growth medium (MSGM)

3.2.Inoculation of Culture Media with Environmental Samples

Once the standard liquid medium and semi-solid *Magnetospirillum*Growth Medium (MSGM) had been prepared and sterilized the environmental samples were added to each normal culture or growth media type in order to test the possible growth and enrichment of the magnetotactic bacteria (MTB). Small portions of sediment-containing water sample were aseptically transferred into sterile test tubes containing either standard medium or MSGM and were subsequently inoculated. Inoculation of the semi-solid culture was done by gently layering the environmental sample onto the surface of the semi-solid MSGM, or by injection just below the surface (transferring 1 ml of the sample volume) in order for the MTB to colonize near the oxic-anoxic transition zone (OATZ), thetype of zone where MTB are known to thrive. Inoculation of the standard liquid culture was done by simply adding the environmental sample and gently mixing.

All samples were inoculated and incubated in the dark at a controlled temperature (28 - 30 °C), conditions that are ideal for breeding MTB and minimizing oxygen (0.3–1% O_2).exposure (Heyen and Schüler, 2003). The incubator (cuve) was kept at these conditions for a number of days (7-15 days) (Blakemore, 1982).

3.3.Visualization and observation

Bacterial growth is visualized by optical microscopy and by detection of the magnetic response using a magnetic bar (Bazylinski et *al*., 1995). Using the same process of (MTB) detection , (Figure 23).

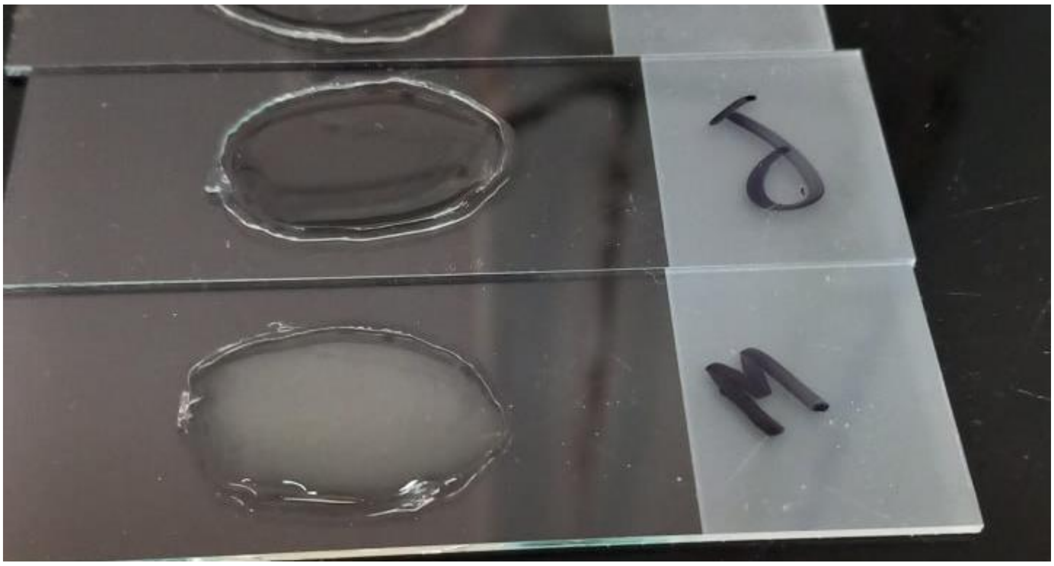

Figure 23: An optical microscope slides contain the magnetically enriched samples after inoculation in selective media collected from two different locations.

4. Gram Staining of Cultured Sample

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. A loopfull of culture was aseptically removed and spread on a clean glass microscope slide, to form a thin smear. After air-drying and heat-fixing, the glass microscope slides were flooded with, in proper order, crystal violet, iodine solution, alcohol (as a de-colorizer), andfuchsin , as part of the Gram staining protocol (Cappuccino and Welsh, 2017). After heat fixation and application of Gram staining reagents, the glass microscope slides (Figure23)were observed under an optical microscope (magnification ×100 with oil immersion).

Table 5: Gram Staining Protocol.

step	**Reagent**	**time**	**Key action**
1-Primary stain	Crystal violet	1 minute	All bacteria turn puple.
2- Rinse	Distilled water	5 seconds	Remove excess stain.
3-Mordant	Lugol (iode)	1 minute	Fixes dye in Gram-positive.
4-Rinse	Distilled water	5 seconds	Wach off iodine.
5-Bleaching	Alcohol/Acetone	10-20 seconds	Gram-negative lose color, Gram-positive resist.
6-Rinse	Distilled water	5 seconds	Stop decolorization.
7-Counterstain	Fuchsine or Safranin	30-1 minute	Gram-negative turn pink.
8-Rinse	Distilled water	5 seconds	Remove excess stain.
9-Drying	Absorbent paper/Air	/	Ready for microscopy.

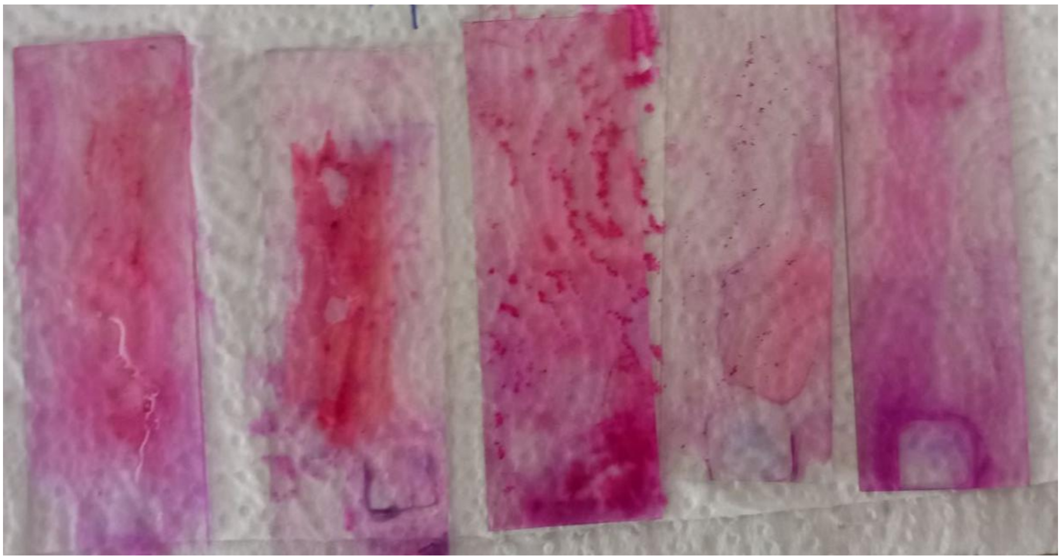

Figure 24: An optical microscope slides contain gram staining cultured samples of MTB.

Chapter III
Results and Discussions

1. Enrichment and detection of MTB

The observation of magnetotactic bacteria (MTB) from the samples that have collected from Lake Djebel El Ouahch and Lake Mridj led to the demonstration of their specific behavior under the action of an applied magnetic field. In the case where no field is applied, the MTBs exhibit a random mobility close to that observed for other motile microorganisms (Figure 25). On the other hand, under the action of a uniform magnetic field, the MTBs orient themselves and then move linearly along the field lines(Figure 26), thus confirming their magnetotactic origin and as such, demonstrating the possible presence of magnetotactic bacteria orchestrating orientation and swimming along the lines of the magnetic field. Movement in this direction demonstrates magnetotactic behavior, where bacteria generate intracellular chains of magnetosomes that are membrane-bound nanocrystals of magnetite (Fe_3O_4) or greigite (Fe_3S_4) allowing them to align to geomagnetic fields or applied magnetic field. This directional behavior is particularly evident in hanging drop preparations where the bacteria migrate towards the air/water interface when the field is directed upwards, while allowing their good separation from other microorganisms contained in the sample. Moreover, in confined environments such as drops, collective self-organization phenomena, such as the formation of vortex-like organizational structures, demonstrate the hydrodynamic interaction between cells. These results obtained by simple optical microscopy with a magnification of 40× and controlled magnetic field, beyond confirming the magnetotacticcharacter of bacteria.

Figure 25: Microscopic observation of water and interface sediment samples from DJEBEL EL OUAHCH Lake under an optical microscope (× 40) in the absence of a magnetic field.

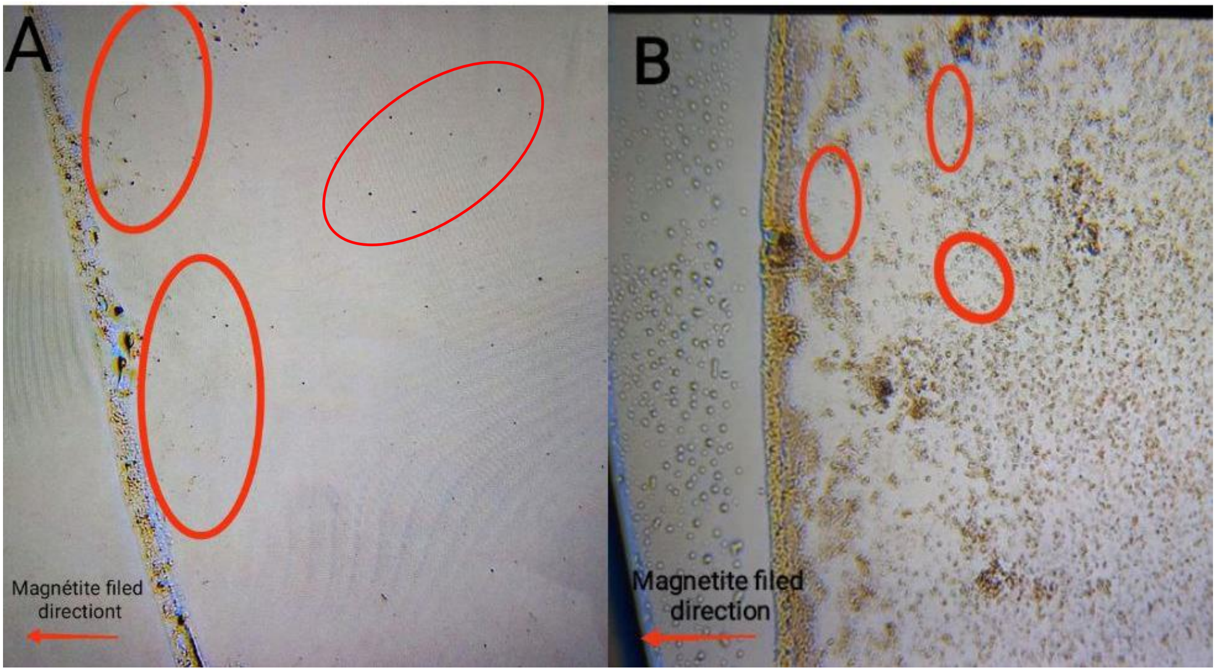

Figure 26:A) Microscopic observation of water and interface sediment samples from DJEBEL EL OUAHCH Lake under an optical microscope (× 40) in the presenceof a magnetic field. B) Microscopic observation of water and sediment samples from MRIDJ Lake under an optical microscope (× 40) in the presence of a magnetic field.

These initial results are consistent with what Faivre and Schüler (2008) also observed in similar experiments leading to a rapid and linear placement of the MTB within the field lines. Lefèvre and Bazylinski (2013) 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 swimming dynamics. To complete, a set of collective self-organization phenomena observed in confined systems, such as drops or microfluidic channels (in particular the formation of vortex structures), is consistent with the work of Waisbord et *al.* (2016) 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) 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).

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; Lefèvre and Bazylinski, 2013). In this preliminary study diversity and magnetosome morphology were not determined, and identification would require additional phylogenetic and electron microscopic studies for proper classification (Pósfai et *al*., 2013; Lin et *al*., 2014).

Therefore, the results presented here offer, beyond the confirmation of the magnetotactic identity of the bacterial strains studied, possibilities for more detailed studies on their collective behavior and on their potential applications in bio-inspired systems or in microbiological engineering, in particular for the themes of targeted transport or biological micro-robotics.

2. Purification of MTB

2.1. Macroscopic results of purification on selective media

a) After seven (7) days

Samples collected from Lake Djebel El Ouahch and Lake Mridj were inoculated onto two types of selective media: semi-solid MSGM, intended to promote the specific growth of magnetotactic bacteria (MTB), and a standard liquid medium as a control for undirected culture. After incubation in the dark at room temperature of 28-30°C a black layer was observed in the tubes containing the semi-solid MSGM, generally forming at the interface zone between dissolved oxygen and the anaerobic environment, indicating preferential growth of microaerophilic bacteria. In contrast, the bottles containing the standard liquid medium showed a more diffuse and non-localized turbidity due to a cloudy white layer within the culture.

In the tubes containing the semi-solid MSGM inoculated with samples from Lake El Meridj, a clear central band of bacterial growth formed, providing strong evidence to substantiate the occurrence and enrichment of MTB. The central band exhibited consistent development at the oxygen–anoxic transition zone (OATZ) a microaerobic zone where MTB are best suited to their metabolism and magneto-aerotaxis behaviors. Which mean that the semi-solid state of MSGM medium provides suitable conditions for a vertical oxygen gradient to develop, allowing MTB to migrate actively and to aggregate in this region (Figure 27 (A)).Unlike the tubes, containing the semi-solid MSGM inoculated with samples from Djebel El OuahchLake that showed just a three tubes had growth and the rest were all clear, indicating either there was no enrichment or that enrichment failed. Also, the partial enrichment success may be indicative of heterogeneity in the abundance of MTB throughout the sediment portions collected, as magnetotactic bacteria tend to be highly localized in microenvironments with iron and appropriate redox conditions, this inconsistency is likely natural variability in the sediment composition, the variation is likely due to either non-sterile sediment particles, or competing microbial species in some aliquots also there is a possibility due to the duration, meaning that some species of these bacteria need a long time to grow, so the tubes are clear and free of any growth. Another factor could be sensitivity to exposure to oxygen during sampling or inoculation, MTB can be rapidly inactivated due to oxidative stress. The difference in growth also elucidates the importance of having a robust sampling protocol, and subsequent anaerobic handling (Figure 27 (B)).

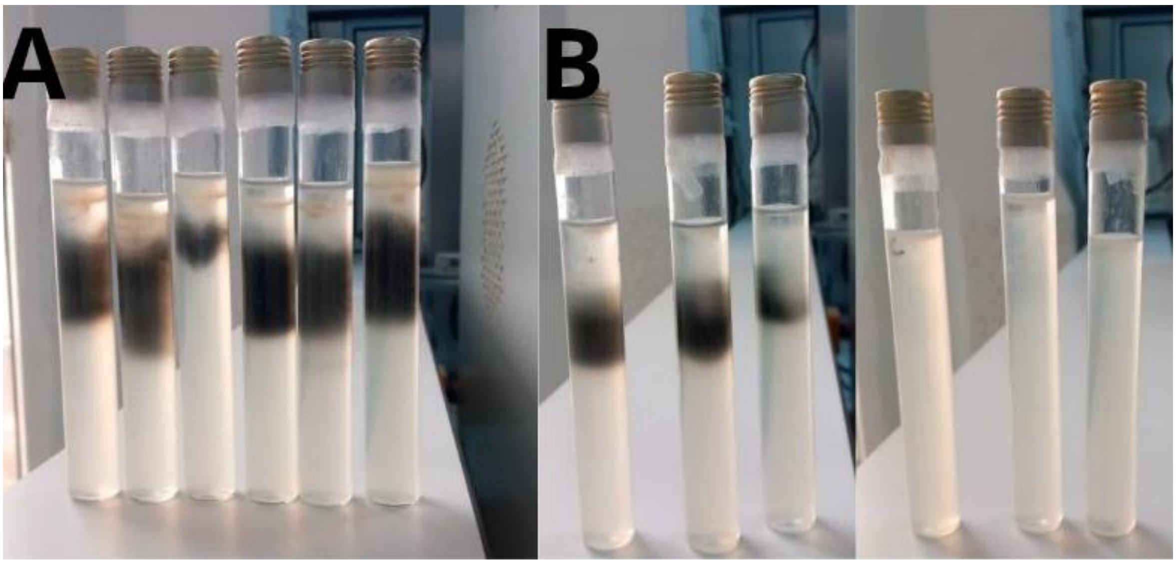

Figure 27: A) Semi-solid *Magnetospirillum* growth medium (MSGM) for MRIDJ Lake after incubation for 7 days showing a band of MTB growing at the oxic-anoxic interface (black layer). B) Semi-solid *Magnetospirillum* growth medium (MSGM) for DJEBEL EL OUAHCH

Lake after incubation for 7 days showing a band of MTB growing at the oxic-anoxic interface (black layer) in just three tubes.

As for the bottles containing the standard liquid medium, the white turbidity seen usually indicates suspended bacterial biomass in the medium and is suggestive of aerobic or facultatively anaerobic microbial growth, which may be common in environmental samples that are cultivated from mixed microbial populations. This microbial population is likely from the endogenous bacteria that were in the sediment or water sample and are not magnetotacticbecause MTB are microaerophilic or anaerobic, and generally require specific conditions like a redox gradient (such as in semi-solid MSGM), they typically do not fare very well in liquid media that are uniformly oxygenated (Figure 28).

Figure 28:A) Standard liquid nutrient media for DJEBEL EL OUAHCH Lake after incubation for 7 days showing non-localized turbidity of MTB. B) Standard liquid nutrient media for Mridj Lake after incubation for 7 days showing non-localized turbidity of MTB.

In the end, the consistency seen across the tubes show successful enrichment and supports the use of MSGM as a selective medium for MTB, and importantly this results stands in contrast to more variable or contaminated results in standard liquid media and supports the importance of controlled redox environments for MTB cultivation and purification and also the potential consistent growth seen in samples from Lake El Meridj, this result may suggest that Djebel El Ouahchhas either a smaller overall MTB density or more environmental heterogeneity. This finding supports earlier studies that have found MTB to be very sensitive to both physicochemical gradients and metal availability in their ecological niche (Lin et *al*., 2014;

Zhang et *al*., 2017). Thus, differences in growth among replicates could reflect both ecological limitations also the methodological limitations of isolating MTB from environmental samples.

b) After fifteen (15) days

The inter-site differences become even more evident after 15 days of incubation, where the black layer developed to include the entire semi-solid MSGM tube in the cultures from Mridj Lake unlike the cultures from Lake Djebel El Ouahch the black layer remains confined in the interface zone between dissolved oxygen and the anaerobic environment with the appearance of bacterial growth in the tubes that were previously clear. As for the bottles of liquid standard medium, the turbidity decreased and a black clump has appeared.

For the MSGM the bacteria appear to have expanded their reach across the OATZ zone after 15 days, supporting the selective advantage in the growth of MTB for this medium, as they use their magnetosomes to navigate to microaerophilic niches. This increase was observed in all tubes of Mridj Lake (Figure 29 (B)), especially in the reused one's which indicates several possibilities such as: differing initial concentrations of MTB in each sample, or a potential growth of facultative anaerobic aerobic MTB species. As for the Djebel El Ouahch samples, bacterial growth was observed in the tubes that were previously clear, which confirms the previous possibility that they neededa longer period of time to grow (Figure 29 (A)).

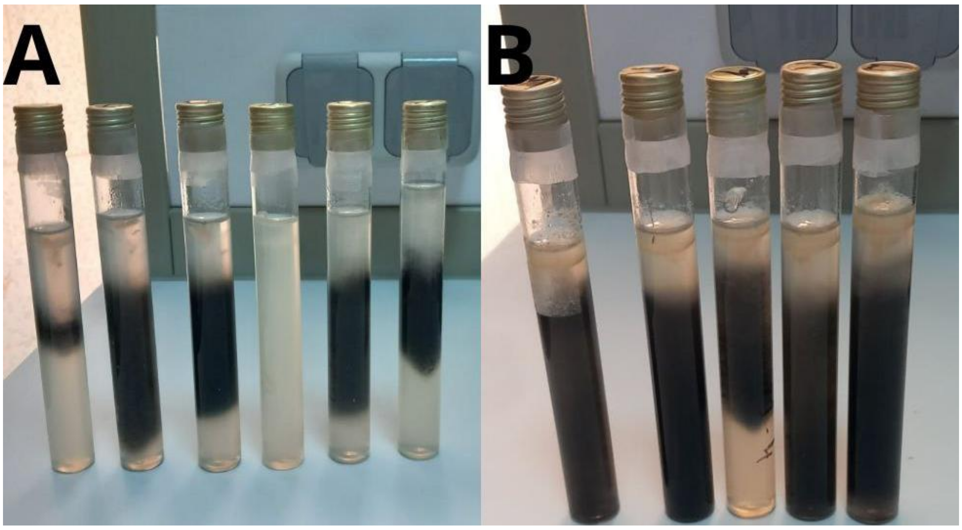

Figure 29:A) Semi-solid *Magnetospirillum*growth medium (MSGM) for DJEBEL EL OUAHCH Lake after incubation for 15 days showing a band of MTB growing include the entiretubes(black layer). B) Semi-solid *Magnetospirillum* growth medium (MSGM) for

MRIDJ Lake after incubation for 15 days showing a band of MTB growing at the oxic-anoxic interface (black layer).

For the bottles of liquid standard medium the appearance of black flocs in the upper part of the medium (Figure 30) rather indicates contamination and it evident that is a fungal contamination. This finding is inconsistent with the observed features of magnetotactic bacteria growth and provides strong evidence that there was some lapse in sterile technique. The most reasonable explanation for such contamination is that the glass bottles were not properly sterilized, either before preparing the medium or during inoculation of the sample. Fungal spores are hardy and can survive under sterilization conditions by way of autoclave, so caution must always be taken in microbial studies to ensure the process is thoroughly sterilized after 15 days, leading to difficulty in controlling turbidity, namely the death of most MTB.

In nutrient-rich liquid media without selective inhibitors or physical barriers, fungi can swiftly dominate culture conditions; this was likely the case in this instance, which would have inhibited selective growth characteristics of magnetotactic bacteria (Cappuccino and Welsh, 2017). Furthermore, the possible occurrence of opportunistic contamination is much more favorable to the conditions of higher concentration of nutrients in liquid media where redox gradients cannot have their control as is the case for MTB enrichment.

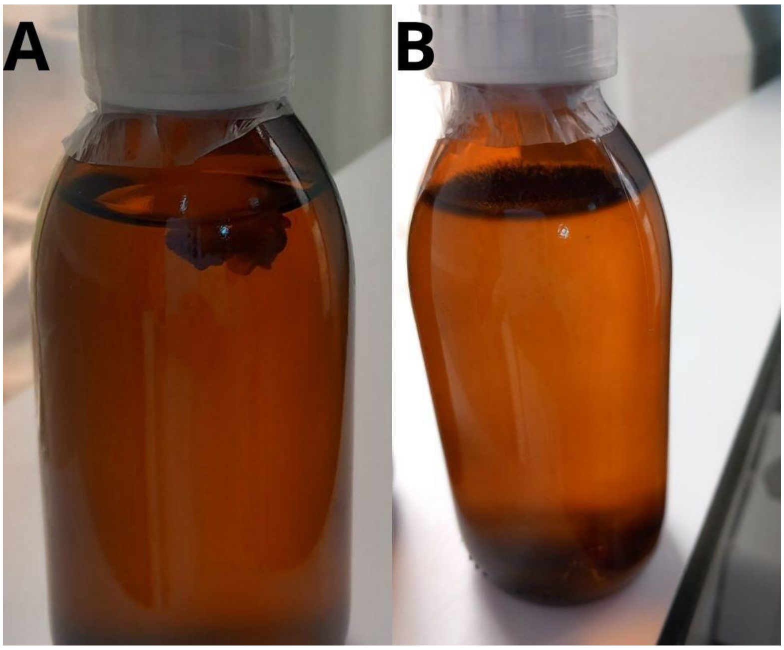

Figure 30: A) Standard liquid nutrient media for DJEBEL EL OUAHCH Lake after incubation for 15 days showing non-localized less turbid of MTB with the appearance of

black clumps. B) Standard liquid nutrient media for MRIDJ Lake after incubation for 15 days showing non-localized less turbid of MTB with the appearance of black clumps.

The observed differences in magnetotactic bacteria (MTB) growth dynamics between Mridj Lake and Lake Djebel El Ouahch align with prior studies highlighting the influence of environmental conditions and medium composition on MTB enrichment. The expansion of the black layer (indicative of magnetosome formation) throughout the semi-solid MSGM tubes from Mridj Lake corresponds to findings by Lefèvre et *al.* (2012), who demonstrated that MSGM selectively promotes MTB growth by simulating the oxic-anoxic transition zone (OATZ), a critical niche for microaerophilic MTB. The delayed growth in Lake Djebel El Ouahch cultures, where growth became visible only after prolonged incubation, mirrors observations by Lin et *al.* (2018), who noted that MTB from oligotrophic environments often exhibit slower metabolic rates, requiring extended adaptation periods in vitro. This suggests that the initial microbial load or species-specific traits (e.g., facultative anaerobic capabilities) may influence enrichment efficiency, as proposed by UebeandSchüler (2016).

The fungal contamination in liquid standard medium, characterized by black flocs and turbidity loss, is consistent with challenges reported in non-selective liquid cultures. Cappuccino and Welsh (2017) emphasize that nutrient-rich liquid media lacking selective inhibitors (e.g., cyclosporine or antifungal agents) are prone to fungal overgrowth, which disrupts redox gradients critical for MTB proliferation. Similarly, Bazylinski et *al.* (2013) noted that improper sterilization of glassware a likely factor here could introduce resilient fungal spores, outcompeting MTB under aerobic conditions.

2.2.Microscopic results of observation of purified cultures

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 exhibited a coccus 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 31). In the absence of a magnetic field, the observed movements were random. This motility confirmed that the incubation and enrichment of magnetotactic bacteria were successful.

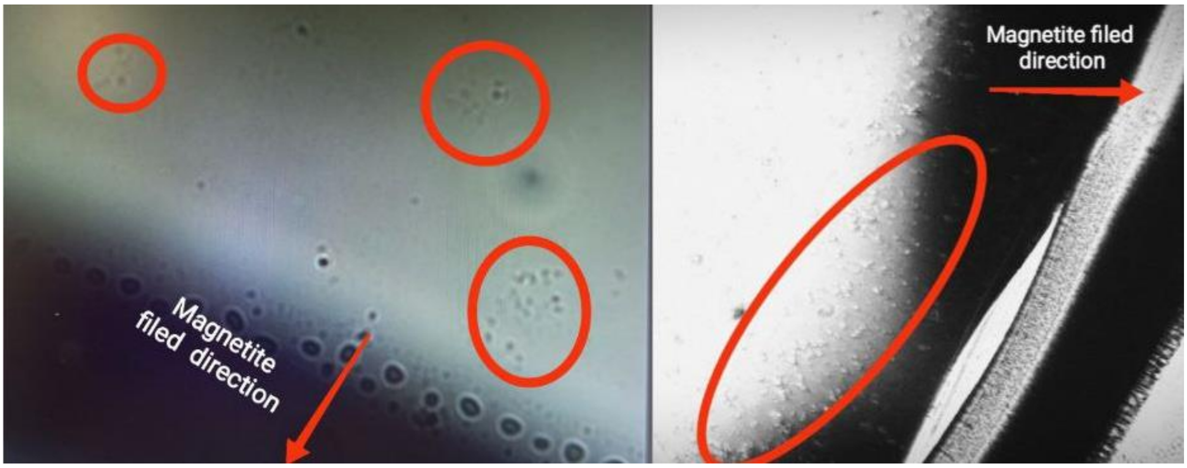

Figure 31: Microscopic observation of enriched cultures from the semi-solid MSGM medium, from MRIDJ Lake under an optical microscope (× 40) in the presence of a magnetic field.

Microscopy of the resulting liquid medium after 15 days' growth (figure 32) showed bacterial motility, but the movement was not towards the magnetic rod as would be observed for magnetotactic bacteria (MTB). Rather, the motility of the bacteria was active and non-directional, where they were moving very well but essentially from the same source, with no magnetotaxis in evidence. The observed organisms were not MTB but rather motile, non-magnetotactic bacteria that developed under the rich nutrition and unstructured conditions of the liquid medium. The lack of an oxic–anoxic transition zone (OATZ.) in a standard liquid medium would not provide the environmental signals required for MTB to orient and migrate along the field lines of the magnetic field (LefèvreandBazylinski, 2013). Furthermore, the previously discussed fungal contamination may have modified the chemicals and physical properties for the medium such that it was less challenging for these organisms to exhibit magnetotactic behavior or support selective MTB growth.

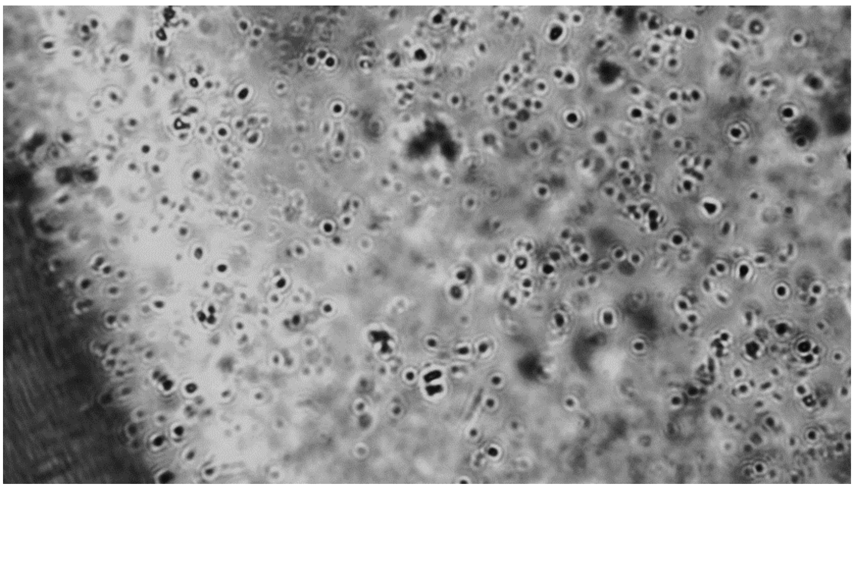

Figure 32: Microscopic observation of enriched cultures from the semi-solid MSGM medium, from DJEBEL EL OUAHCH Lake under an optical microscope (× 40) in the presence of a magnetic field.

These results are consistent with the work of Blakemore (1975), who was the first to demonstrate the oriented swimming behavior of MTB in the presence of a simulated Earth's magnetic field. Furthermore, more recent work by Lefèvre et *al*. (2011) also confirmed that bacteria with coccoid morphology, such as *Magnetococcus marinus 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), 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), 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 (Schüler, 2002).

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.

3. Gram staining of purified cultures

After the purification phase of the magnetotactic bacteria, the resulting cultures were subjected to Gram staining to determine the nature of their cell walls. This staining was performed on purified cultures of magnetotactic bacteria from samples collected from Lakes Djebel El Ouahch and Mridj, grown on semi-solid MSGM medium (Figure 33) and standard liquid medium (Figure34) . The results revealed that the bacterial cells stained pink to light red, clearly indicating a Gram-negative reaction. This result is consistent with the well-established cell structure of MTB, which possess a bacterial wall composed of a thin layer of peptidoglycan surrounded by a lipopolysaccharide outer membrane, as reported by Bazylinski and Frankel (2004). This Gram-negative signature is a key marker for distinguishing MTB from other environmental bacteria, often Gram-positive.

Microscopic morphology shows small, dispersed, coccoid or rod-shaped cells found singularly or grouped in small clusters. These descriptions are also consistent with other genera such as *Magnetospirillum*,*Magnetococcus* and other related MTB which were reported in microaerobic aquatic environments, whose morphological and structural characteristics have been well documented by Lefèvre and Bazylinski (2013).

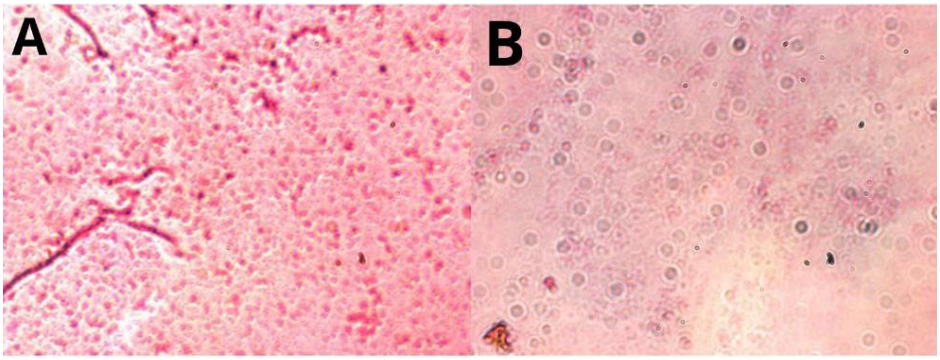

Figure 33: A) Microscopic observation of gram staining of purified cultures from the semi-solid MSGM medium, from DJEBEL EL OUAHCH Lake under an optical microscope (× 1000). B) Microscopic observation of gram staining of purified cultures from the semi-solid MSGM medium, from MRIDJ Lake under an optical microscope (× 1000).

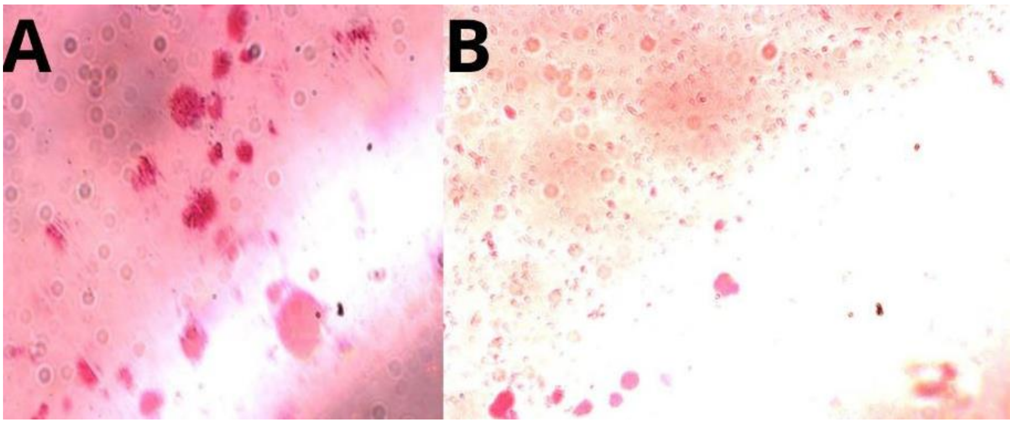

Figure 34: A) Microscopic observation of gram staining of purified cultures from standard liquid nutrient media, from DJEBEL EL OUAHCH Lake under an optical microscope (× 1000). B) Microscopic observation of gram staining of purified cultures from the standard liquid nutrient media, from MRIDJ Lake under an optical microscope (× 1000).

These results are consistent with studies in the past, where MTB isolated from similar aquatic ecosystems, such as freshwater lakes, sediments, and estuaries, were also established to be Gram-negative thru this method (Pósfai et *al*., 2006).

Conclusion

At the outset of this conclusion, it is important to emphasize that this type of applied study on magnetotactic bacteria and their characterizations is being conducted for the first time in Algeria. To the best of our knowledge, no previous research has addressed this specific topic, making it an original and pioneering study in the country.

This study successfully demonstrated the presence of magnetotactic bacteria (MTB) in sediments of two Algerian lake ecosystems, Djebel El Ouahch and El Meridj, using an integrated experimental approach that included magnetic enrichment, culturing on half-solid and liquid MSGM media, and optical microscopy with an onsite magnetic field application. The half-solid MSGM medium was the most effective medium, not only because it allowed for the establishment of the oxic-anoxic transition zone (OATZ), an important zone needed for MTB to grow, but bacterial growth was consistently observed in that transition zone. All microscopic observations gave evidence to the characteristic magnetotactic swimming behavior of MTB (towards a magnetic source) and were cytologically confirmed to be consistent with carriers of the functionally diverse group of magnetotactic bacteria known to be gramnegative, coccoid, and consistent with what was observed in the literature. Mtb growth in the liquid medium was limited, and was easily subject to contamination, which highlights the needg for a consistently rigorous sterilization protocol, particularly when studying these sensitive organisms.

The practical and microscopic observations strongly support that magnetotactic bacteria (MTB) were present during these experiments, with an estimated confidence level of at least 85%. These results perform the important function of highlighting some of the special physicochemical conditions of the Algerian lakes that sustain the vast and amazing ecology of MTB and ultimately promote excellent insights into the ecology and behavior of such magnetotactic bacteria.However, to confirm we can accept these observations and evaluate the genetic diversity of the isolated strains, more experiments at the molecular level will be required, including genetic sequencing. Undertaking molecular level studies would add validation to the identification of MTB and extend our research into their possible application in biotechnology or nanotechnology. The results further confirmed and add to foundational studies related to redox gradients and magnetotaxis to help in identifying magnetotactic bacteria.Also, the observed collective self-organization of MTB in the presence of magnetic fields opened new possibilities for studying microbial interactions at the microscopic level.

In terms of future prospects, the study’s should focus on more sophisticated methods of identification and characterization. Examples of genetic and molecular methods, including

16S rRNA or whole genome sequencing, are appropriate means to establish the taxonomic significance of the isolated strains and to determine their phylogenetic relationships. Moreover, future studies could optimize the media and environmental conditions to improve the growth and amount of production of MTB, which will help to increase their use and accessibility for applied studies. Considering their verified ability to biosynthesize and produce magnetosomes, future studies on the capability for targeted drug, and bioremediation, and for MRI imaging and development, need to be pursued. Expanding the sampling of the broad number of marine and sedimentary systems, can help with the understanding the ecological diversity and distribution of MTB in Algeria and beyond.

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Titled: Highlighting and characterization of magnetotactic bacteria from a lake habitats

Study submitted in partial fulfillment of the requirements for the Master's Degree

Field: Natural and Life Sciences
Sector: Biological Sciences /Biotechnologies/Ecology and Environment
Specialty: Molecular Biology of Microorganisms

Abstract:

Magnetotactic bacteria (MTB) are widespread in aquatic environments. They possess magnetosomes, which are intracellular organelles composed of iron crystals, allowing them to align with and move along magnetic field lines. MTBs have several iron and sulfur biogeochemical applications, particularly in medical diagnostics, such as targeted drug delivery and magnetic hyperthermia .In the environment; they play a crucial role in the biogeochemical cycle and in the bioremediation of heavy metals and organic pollutants, as well as in nanotechnology. This study presents a theoretical review and an experimental study of MTB in aquatic environments, based on environmental samples from two lakes in the city of Constantine. Microscopic observation and purification using standard and semi-solid MSGM liquid media confirmed the presence of MTBs in the samples taken from both lakes, though molecular identification is still required.

Keywords: Magnetotactic bacteria, MTB, magnetosomes, magnetotaxis, lake.

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