Isolation, Microscopic and Magnetotactic Characterization of Magnetospirillum Moscoviense Ms-24 From Banjosa Lake, Pakistan

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Abstract

At currently, approximately 70 species of magnetotactic bacteria have been identified; thus, there is an urgent need to identify more magnetotactic bacteria from diverse sources with potential applications in industry and biotechnology. To the best of our knowledge, this is the first magnetotactic bacterial strain discovered in Pakistan. The first magnetotactic bacteria, *Magnetospirillum moscoviense* MS-24, was isolated from Banjosa Lake (Rawalakot), Pakistan, in the current investigation. Magnetospirillum moscoviense MS-24 was screened using the Racetrack method and its temperature and pH were optimized. The *Magnetospirillum moscoviense* MS-24 were physically characterised using Atomic Force Microscopy, High-Resolution Scanning Electron Microscopy, and Transmission Electron Microscopy. The current study used microscopy to illustrate the shape of bacteria and to find a very obvious chain of magnetosomes within the bacterial cell. The *Magnetospirillum moscoviense* MS-24 measured about 4 0.04m in length and 600 0.02nm in diameter. The micro fluidic chip experiments were also used to detect magnetotaxis behaviour in bacteria.

Introduction

Magnetotactic bacteria (MTB) belong to a heterogeneous group of microorganisms that can align themselves along the Earth's magnetic field lines and these microorganisms are also known as nano-compass. The characterized movement towards magnet is known as "magnetotaxis, magneto-aerotaxis, magneto-chemotaxis" occurring due to the presence of nanoparticles inside the bacterial cell, known as magnetosomes. These magnetosomes are made up of magnetite or greigite (Lin et al. 2017). Mature crystals of magnetosomes of cultured MTB are up to the size range between 35–120 nm in diameter. However, magnetosomes in some uncultured MTB strains can grow to ~ 180 nm, and even up to 300 nm (Lins et al. 2006; Mitra et al. 2020; Liu et al. 2021). These magnetic nanocrystals have both north and south magnetic dipoles and are in chain forms so that the bacterial cell orients and swims towards or away from the magnetic poles (Liu and Chen 2008; Wang et al. 2020). This behaviour of magnetotactic bacteria makes them self-propelled magnetic compass needle that migrates along Earth's geomagnetic field lines (Schüler 2008; Gandia et al. 2019). The highest number of these bacteria are present just below the oxic-anoxic transition zone (OATZ) (Zhao et al. 2007; Vincenti et al. 2019).

These bacteria play important role in biogeochemical cycles like Fe and S cycles in both fresh and marine water. They are mostly present in the environments and mud sediments that are rich in organic material. They use iron, nitrate and sulphate as electron donors and acceptors (Rismani Yazdi et al. 2018). The environmental parameters like availability of oxygen, iron source, pH and temperature are very important for the growth of magnetotactic bacteria. It is reported that the highest numbers of MTB were present in the oxic-anoxic transition zone (Bazylinski and Lefèvre 2013; Salam et al. 2019).

Most of the magnetotactic bacterial species are from the class Alphaproteobacteria while some of the species are from Deltaproteobacteria and Gammaproteobacteria. Blakemore and co-workers isolated cultivated species of *Magnetospirillum magnetotacticum* strain MS-1, *Magnetospirillum gryphiswaldense*

MSR-1 and *Magnetospirillum magneticum* strain AMB-1 (Flies et al. 2005). However, there are some Magnetotactic cocci strains that are previously affiliated with Alphaproteobacteria class, have been redefined to 'Candidatus Etaproteobacteria' classes (Liu et al. 2021). The Deltaproteobacteria class has been redefined to Desulfobacterota phylum (Koziaeva et al. 2020). MTB strain in 'Candidatus Omnitrophica' phylum was also reported (Kolinko et al. 2012). It is also reported that many species of *Magnetospirillum* genus have morphological and phylogenetical resemblance with the magnetotactic bacteria, but they lack the ability of magnetosomes biomineralization in the absence of iron (Flies et al. 2005; Lin et al. 2017). Yan et al. in 2012 reported that *M. magnetotacticum* and *M. gryphiswaldense* contained empty and partially filled magnetosomes vesicles in iron deficient condition. The Fe availability causes change in magnetosomes crystal size and morphology while the oxygen inhibits the formation of magnetosomes at high O_2 partial pressures (Heyen and Schüler 2003; Faivre and Schu 2008; Zhou et al. 2018). Accordingly, smaller magnetosomes are formed such that the magnetic properties of magnetosomes and MTB indirectly dependent on dissolved oxygen concentration (Lin et al. 2017). There are many uncultured MTB were isolated recently and lots of novel strains of cultured and uncultured MTB affiliated with Alphaproteobacteria class e.g. magnetotactic spirillum strains SH-1, QH-2, CCP-1, XQGS-1 and magnetotactic Deltaproteobacteria strain WYHS-1 have been reported in recent years (Li et al. 2017, 2020; Lin et al. 2017; Du et al. 2019). According to Liu et al, 2020, about 16 magnetotactic cocci from freshwater environments (strains YQC-1, YQC-2, YQC-3, THC-1, MYC-4, MYC-5, BHC-1, MYC-3, MYC-7, DMHC-1, DMHC-2, DMHC-6, DMHC-8, WYHC-1, WYHC-2, and WYHC-3), two from marine environments (strains XJHC-1 and LLTC-1), and one from a brackish environment (strain SHHC-2). Based on FISH-SEM analyses.

The magnetosomes are magnetic nanoparticles that are used in a diverse range of applications not only in technical applications but also in medical applications (targeted drug delivery, hyperthermia, imaging methods) (Yan et al. 2012; Gandia et al. 2019). The process of producing magnetite by the accumulation of iron source from the medium is known as biomineralization in magnetotactic bacteria so that they create chains of magnetic crystals called magnetosomes in the cells (Park et al. 2013; Wang et al. 2020).

In the current study, the water-sediment sample from Banjosa Lake was collected for isolation of magnetotactic bacteria. The magnetosomes within the bacterial cell were characterized by various analytical techniques like Compound Microscopy, High-Resolution Scanning Electron Microscopy, Transmission Electron Microscopy (TEM) and Atomic Force Microscopy. These techniques help to find the bacterial shape, size, and the chain of magnetosomes and the magnetotaxis behavior of bacteria.

Material And Methodology

Sample collection

The water sediments from Banjosa Lake (Rawalakot, Pakistan) (33° 48' 38" N, 73° 48' 59" E) was collected by magnetic enrichment technique in May 2017 (Fig. 1). The temperature of the lake was 25°C and the pH was 7. The sampling jar with magnet was placed in water-sediment (almost 1m deep) for 30

min then carefully tightened the jars without distortion so that the magnetotactic bacteria that were attracted towards the magnet were collected in the jar.

The Technique Of Capillary Racetrack

Enrichment of magnetotactic bacteria was obtained using the capillary racetrack method from the same sediment sample(Blakemore et al. 1979) using the Pasteur pipette shown in Fig. 2. The narrow end of the Pasteur pipette was closed by heating and autoclaved distilled water was filled in the narrow end while the cotton was plugged from the wide end side and then filled the wide end side by water MTB enriched sample by syringe. Then placed the magnet at the side of the narrow end for 30 min. The tip of the capillaries having MTB was broken off after 30 min collecting water sample in the narrow end by using a permanent magnet next to it. The collected water from the narrow end must have magnetotactic bacteria as they moved from the water sample through cotton and collected in the distilled water by the attraction of magnet (Schüler et al. 1999; Lin et al. 2008; Li et al. 2010).

Media Composition

The activated charcoal agar was used for microaerophilic and anaerobic bacteria. The composition of medium was 10 $g.L^{-1}$ of peptone, 5 $g.L^{-1}$ of yeast extract, 1 $g.L^{-1}$ of L-cysteine hydrochloride, 10 $g.L^{-1}$ of activated charcoal, and 15 $g.L^{-1}$ of agar and is adjusted to pH 6.8 with NaOH before autoclaving (Lefevre et al. 2011; Lefevre and Bazylinski 2013; Salam et al. 2019).

The growth culturing medium is a specific and modified iron-containing medium for MTB growth. The composition of the medium was 5 $g.L^{-1}$ of tryptone, 3 $g.L^{-1}$ of beef extract, 5 $g.L^{-1}$ of sodium chloride, and 20 $ml.L^{-1}$ of ferric citrate solution (Chandrajit and Prakash 2011).

Culturing Of Bacterial Strain

The water sample collected from the Capillary Racetrack method was inoculated in the ACA medium that only support the growth of anaerobic bacteria. The ACA plates were incubated in an anaerobic jar for 3 to 4 days. The emerging colonies were streaked on a specific iron-containing growth medium for magnetotactic bacteria that is a modified medium having an iron source to produce magnetosomes in bacterial cells (Lefevre et al. 2011).

Screening And Detection

The screening of magnetotactic bacteria was done by the Hanging drop technique(Schüler 2008) under an optical microscope that showed the movement of magnetotactic bacteria when the magnet was placed near the glass slide shown in Fig. 3. The color of the colonies of magnetotactic bacteria was light

brown and they are Gram-negative. The detection was done by inoculating culture in broth modified iron medium and incubating for 120 h in shaking incubator at 30˚C. After 120 h, the colour of the medium turned from light brown to dark brown indicating the production of iron in the medium (Chandrajit and Prakash 2011).

Molecular Identification Through 16s Rrna Gene Sequencing

The selected strains were then molecularly identified by extracted whole genome using Phenol/chloroform DNA extraction protocol (Leena et al. 2018; Cycil et al. 2020). DNA was partially sequenced for the 16S rRNA gene by Macrogen, Inc., Seoul, South Korea. Sequences were further analysed for similar strains in NCBI using BLAST software (http://blast.ncbi.nlm.nih.gov/Blast.cgi/). Phylogenetic trees were constructed by using MEGA 7 software by neighbour-joining method, which determined the similarity of *Magnetospirillum moscoviense* strain MS-24 with the closely related strains (Masood et al. 2017; Salam et al. 2019).

Microfluidic Chip Formation

Polydimethylsiloxane called PDMS is a polymer widely used for the preparation of microfluidic chips. The microfluidic chip was prepared by mixing PDMS with the cross-linking agent and then this suspension was poured into the micro-structured mould and heated this micro-structured at high temperature to get the chip. The channel depth of the microfluidic chip was 5 µm and lithography and thermal bonding constructed the chip. This chip has straight inlets on both sides of the channels. The prepared chip was then attached with the glass slide by plasma gas. The channel width was 200 µm so that the movement of bacterial could be easy and free (Fig. 4a and b) (Roda et al. 2013; Pichel et al. 2018).

Magnetic Field Setup

The microfluidic chip was positioned on a microscope slide by plasma gas so that no air was present between the glass slide and chip. A schematic set was required for the proper imaging of the movement of the bacterial strain. A permanent magnet (NdFeB magnet) was attached with a stepper motor (Silverpak 17CE, Lin Engineering) on the bottom side of the microfluidic chip. The magnetic field can be adjusted in different directions with a rotation time of 5 s at an acceleration of 745 $rads^{-2}$. The camera (model 1328 1048 at 100 fps, FL3-U3-13S2M-CS, Point Grey) was used to make the movies of the bacterial magnetotaxis. The camera was mounted on a Zeiss Axiotron 2 microscope with a 20 objective (Fig. 4a) (Pichel et al. 2018).

1. The magnetic field lines were applied by using a strong magnet at the centre of the microfluidic chip from the downside of the microfluidic chip slide.
2. A strong magnet attached with a rotating stand in the left and right direction applied the magnetic field lines.

Insertion of Magnetotactic Magnetospirillum moscoviense **MS-24 in microfluidic chip**

Magnetospirillum moscoviense MS-24 was inserted in the microfluidic chip by micropipette. About 0.2 µl of broth inoculated with bacterial culture was inserted in one shallow open end of the channel (Fig. 4b). The air pressure filled the complete channel with the bacterial broth sample. The magnetic field was applied in different directions (Pichel et al. 2018).

Characterization of Magnetospirillum moscoviense **MS-24**

Bacterial cell culture was characterized by various analytical techniques like compound microscopy, atomic force microscopy, high-resolution scanning electron microscopy and transmission electron microscopy.

Compound Microscopy

Under compound microscopy **(LIECA DM6000 M)**, the movement of bacterial strain *Magnetospirillum moscoviense* MS-24 in broth was observed towards the magnet. The bacterial slide was prepared by using the hanging drop method. A drop of bacterial culture in broth media was dropped on a glass coverslip and inverted on a ring so that a drop in hanging position. The image was observed under a compound microscope under the influence of an applied magnetic field (Lefevre et al. 2011).

Transmission Electron Microscopy (TEM)

TEM is used to produce images from a thin specimen by illuminating it with a very high-energy electrons beam and detecting the electrons that are transmitted through the specimen. Transmission electron microscopy **(Philips CM300ST-FEG Transmission Electron Microscope)** operating at 300 kV was used to see the bacterial specimen. The bacterial culture was suspended in distilled water and made a homogenised suspension. About 10 µl of suspension was dropped on the sample holder, air-dried the drop, and washed the sample with formaldehyde to fix it properly and then micrographs were taken (Oestreicher et al. 2012, 2013).

Atomic Force Microscopy (AFM)

AFM is a technique that is based on the measurement of electrostatic and the ability to image surfaces at high resolution and measure forces between surfaces. AFM is also used for three-dimensional imaging. The morphology and size of the magnetotactic bacteria were analysed in AFM **(Bruker ICON Dimension Microscope).** For AFM imaging, bacterial broth culture was suspended in phosphate buffer saline (PBS) having pH 7.4 and placed the suspension onto a glass slide and air-dried and then placed the slide under a microscope. AFM can visualize biological species in their native conditions (Oestreicher et al. 2012).

High-resolution Scanning Electron Microscopy (HR-SEM)

HR-SEM **(FEI Quanta 250 FEG scanning electron microscope)** is operating at 10 kV in a high vacuum was used for the characterization of the size and morphology of bacterial cells and Magnetosomes chains

inside. For high-resolution scanning electron microscopy, an aqueous suspension of magnetotactic bacteria was dropped onto pieces of Si wafers (5x5 mm) with a native oxide layer, subsequently washed with sterile distilled water and blow-dried with N_2 gas (Ji et al. 2017).

Results

Screening and detection

The movement of magnetotactic bacteria towards the magnet revealed magnetotaxis behaviour and help in its screening by using a compound microscope (Leica DM6000 M). The capillary racetrack method helped in the separation of magnetotactic, non-magnetotactic bacteria, the cotton inhibited the movement of non-magnetotactic bacteria to move, and only magnetotactic bacteria showed a movement towards the magnet. The magnetotactic bacteria accumulated in the autoclaved distilled water at one end of the Pasteur pipette shown in Fig. 2. The hanging drop technique also showed the movement of magnetotactic bacteria towards the magnet shown in Fig. 4. The colonies of magnetotactic bacteria appeared light brown on a Specific iron medium shown in Fig. 4.

Molecular Identification Through 16s Rrna Gene Sequencing

According to the phylogenetic tree, the magnetotactic bacteria showed its resemblance to Alphaproteobacteria. The magnetotactic bacterial strain MS-24 showed similarity with *Magnetospirillum moscoviense* (Fig. 5). The accession number obtained for strain Magnetospirillum moscoviense **MS-24 was MK367808.1** from the NCBI database (https://www.ncbi.nlm.nih.gov/nuccore/MK367808.1).

Insertion Of Magnetotactic Bacterial Strain In Chip

In the current study, the detection of *Magnetospirillum moscoviense* MS-24 was observed under applied magnetic field lines. The background image of the bacterial movement was constructed by an average of 30 frames spread with videos. Gaussian low pass filter was used for making the high-frequency noise low. The magnetic field was applied in the central line of the microfluidic chip to observe their movement at a central point (Fig. 6). They only showed movement at one point.

The magnetic field line in the next experiment was applied in circular rotation so that the South Pole in one rotation and the North Pole in another rotation were pointed to the bacterial cells present in a microfluidic chip. The bacterial movement was in direction of left and right in the applied magnetic field (Fig. 7).

Characterization Of Magnetotactic Bacteria

Compound Microscopy

The compound microscope showed the movement of *Magnetospirillum moscoviense* MS-24 towards the magnet. This confirmed the magnetotaxis movement of bacteria towards the magnet. Figures 6 and 7 showed the movement towards and away from the magnet as the magnet is moving circularly, so the south and north poles of the magnets were changed in every turn.

Transmission Electron Microscopy

The magnetotactic bacterial strain *Magnetospirillum moscoviense* MS-24 showed its spiral shape and magnetosomes in Transmission Electron Microscopy and this also confirmed the presence of magnetosomes in the cell. The length of the strain MS-24 was 4µm while the width was around 600nm (Fig. 8).

Atomic Force Microscopy

The Atomic Force Microscopy also showed the bacterial spiral shape of magnetotactic bacterial strain *Magnetospirillum moscoviense* MS-24. The atomic force microscopy also showed the three-dimensional structure of the sample so in Fig. 9 the image taken was a three-dimensional structure of the bacterial cell. The presence of magnetosomes in the bacterial cell was also clearly observed.

High-resolution Scanning Electron Microscopy

The best image of magnetotactic bacterial strain *Magnetospirillum moscoviense* MS-24 was observed under high-Resolution Scanning Electron Microscopy that also showed bacterial cell shape and also the magnetosomes chain very clearly. The size of the bacterial cell was approximately 4µm in length and 600nm in width. The magnetosomes are present in a chain form and a single magnetosomes chain was found in a bacterial cell. The flagella of the bacteria were observed (Fig. 10).

Discussion

Magnetotactic bacteria are found worldwide in fresh and marine water or brackish and hyper-saline. The magnetotactic bacterial isolate MS-24 was isolated from a freshwater source from Banjosa Lake (Rawalakot, Pakistan) (Fig. 1). Most of the magnetotactic bacterial strains were present in oxic anoxic interface of water or chemically stratified water (Lefèvre et al. 2014). Banjosa Lake is an artificially created lake and it is located at an altitude of 1,981 m. According to Christopher T. Lefevre et al., 2011), the oxygen concentration in the water directly affects the presence of MTB. The race track method helps to collect the magnetotactic bacterial strain that showed a movement towards the magnet used on one end of the capillary tube (Khalil & Misra, 2014) (Fig. 2). The bacterial cells present in the distilled water was easily collected in Eppendorf for further analysis. In the current study, ACA was used to grow the anaerobic bacteria as ACA decomposed the toxic free oxygen radicals and peroxides and inhibit the growth of many microaerophiles in the agar plate. The water collected from capillary was then spread on charcoal agar plates and incubated in anaerobic jars so that the only anaerobic and microaerophilic bacterial colonies grew on agar plates (Dubbels et al. 2004; Dongre and Alok Goel 2011). The bacterial

colonies that appeared on ACA plates were collected and streaked on a specific iron-containing medium. The magnetotactic bacterial colonies that appeared on iron specific media were preserved on a specific iron broth medium for further analysis (Fig. 4).

The magnetotactic bacteria *Magnetospirillum moscoviense* MS-24 showed movement under a compound microscope in the hanging drop technique. According to (Khalil and Misra 2014), the hanging drop technique help in the detection of magnetotactic bacteria. The magnet should be oriented towards the drop and bacterial strains showed the movement according to the movement of the magnet. The movement of bacterial strain was very clear in the compound microscope.

The magnetotactic bacterial strain *Magnetospirillum moscoviense* MS-24 movement was observed under an applied magnetic field to check if they can use in any application. The microfluidic chip was used to see the movement of bacteria. According to Pichel et al. 2018, the rotation of *Magnetospirillum gryphiswaldense* was also observed in the microfluidic chip and the resulting average rate of rotation of *M. gryphiswaldense* was 0.74 ± 0.03 rad/mTs. The bacteria took a U-turn with the applied magnetic field but the velocity of bacteria slowed down with the turn.

In the current study, MTB was inserted in a microfluidic chip for the observation of bacterial movement according to applied magnetic field lines. The applied magnetic field lines were in different directions in the experiment so that the confirmation of bacterial direction with magnetic field lines was properly checked (Fig. 3). The purpose of this experiment was to confirm the magnetotaxis behaviour of MTB and their application in hyperthermia and drug delivery system. Figure 6 shows bacterial movement at one place in circular motion as the magnet was attached under the microfluidic chip. This bacterial movement was also used in hyperthermia as they vibrate very quickly and as a result, start increasing the temperature of that part. In the case of cancer cell therapy, the cancer cell can be killed by using this method. In Fig. 7, the bacterial cell movement was observed in different directions. This behaviour of bacterial cells can be used in pathogen separation and drug delivery systems. In pathogen separation, antibodies were attached with MTB and made them Nano-robots. These Nano-robots helped in the separation of the pathogen as they get attached to the antibodies and are separated through a magnetic field line.

The confirmation of isolated magnetotactic bacterial isolate *Magnetospirillum moscoviense* MS-24 was done by using transmission electron microscopy, atomic force microscopy and high-resolution scanning electron microscopy. The compound microscope analysis showed the movement of magnetotactic bacterial strain MS-24 towards the magnet, that is its magnetotaxis property. The best imaging was observed by TEM and HR-SEM analysis. Figures 8 and 10 showed the magnetotactic bacterial isolate *Magnetospirillum moscoviense* MS-24 clear image of spiral-shaped and having magnetosomes chain in the cell. Faivre & Schu, 2008) also showed the same magnetotactic bacterial strains of spiral-shaped and having magnetosomes in their cells. Figure 9 showed atomic force microscopy of *Magnetospirillum moscoviense* MS-24. The current study aimed to characterize the shape and size of magnetotactic bacterial strain in an aqueous environment using AFM. According to this analysis, the bacterial cell size is

4μm in length and 600nm in width. According to Yamamoto *et al*. in 2010, it is a great challenge to obtain high-resolution images for a whole organelle that contains various molecular particles and has a complex 3D structure. The magnetosomes structure inside the bacterial cell is also visible in the image taken by AFM (Fig. 9). The HR-SEM showed a very clear image of magnetotactic bacterial isolate *Magnetospirillum moscoviense* MS-24 (Fig. 10). In HR-SEM, the image of *Magnetospirillum moscoviense* MS-24 revealed a proper shape and size of a bacterial cell. The magnetosomes chain was also cleared in this image. The size of the bacterial cell is 4 ± 0.01 μm in length while the width of the bacterial cell is around 600 ± 0.02 nm. The magnetosomes chain was also very clearly shown in Fig. 10.

The conclusion of the current study includes the main investigation of isolation and screening of the new magnetotactic bacterial strain from Pakistan. It was the first magnetotactic bacterial strain isolated from Pakistan. The isolate *Magnetospirillum moscoviense* MS-24 was confirmed by using different techniques like the racetrack method and hanging drop technique. The isolated strain MS-24 showed a close similarity with the magnetotactic bacterial strain *Magnetospirillum moscoviense.* The compound microscopy revealed its magnetotaxis behaviour according to the movement of the magnet. The other microscopic techniques like Atomic Force, Transmission and High-Resolution Scanning Electron Microscopy showed the best images of *Magnetospirillum moscoviense* MS-24 with its proper spirillum shape and size of bacteria about 4μm length and about 600nm width with polar flagella. The HR-SEM also showed a very clear image of the chain of magnetosomes.

Declarations

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Conflicts of Interest

All the authors reported no conflict of interest.

Author Contribution Statement

Conceptualization: Maria Abdul Salam; Data curation: Maria Abdul Salam; Experimental design: Maria Abdul Salam and Fariha Hasan; Formal analysis: Maria Abdul Salam and Nuriye Korkmaz (HR-SEM analysis); Writing – original draft: Maria Abdul Salam; Review & editing: Fariha Hasan, Nuriye Korkmaz and Leena Mavis Cycil. Fariha Hasan provided experimental research guidelines.

Supplementary information Video S1, S2 and S3

Declarations Ethics approval This article does not contain any studies with human or animal subjects performed by any of the authors.

Consent to participate Not applicable.

Consent for publication Not applicable.

References

1. Bazylinski DA, Lefèvre CT (2013) Magnetotactic bacteria from extreme environments. Life 3:295–307. https://doi.org/10.3390/life3020295
2. Blakemore RP, Maratea D, Wolfe RS (1979) Isolation and pure culture of a freshwater magnetic spirillum in chemically defined medium. Journal of Bacteriology 140:720–729. https://doi.org/10.1128/jb.140.2.720-729.1979
3. Chandrajit S, Prakash G (2011) Preliminary isolation report of aerobic magnetotactic bacteria in a modified nutrient medium. Recent Research in Science and Technology 3(11): 71-75 http://recent-science.com/
4. Cycil LM, DasSarma S, Pecher W, et al (2020) Metagenomic Insights Into the Diversity of Halophilic Microorganisms Indigenous to the Karak Salt Mine, Pakistan. Frontiers in Microbiology 11:. https://doi.org/10.3389/fmicb.2020.01567
5. Dongre HN, Alok Goel AG (2011) Mimicking Microaerophilic/Anaerobic Environment for Culture of Anaerobic Bacteria Using Oxygen Scavengers in Agar Well Technique. Indian Journal of Applied Research 3:518–519. https://doi.org/10.15373/2249555x/may2013/166
6. Dubbels BL, DiSpirito AA, Morton JD, et al (2004) Evidence for a copper-dependent iron transport system in the marine, magnetotactic bacterium strain MV-1. Microbiology 150:2931–2945. https://doi.org/10.1099/mic.0.27233-0
7. Du H, Zhang W, Zhang W, et al (2019) Magnetosome Gene Duplication as an Important Driver in the Evolution of Magnetotaxis in the Alphaproteobacteria. mSystems 4;5. https://doi.org/10.1128/mSystems.00315-19
8. Faivre D, Schu D (2008) Magnetotactic Bacteria. Encyclopedia of Neuroscience 2229–2229. https://doi.org/10.1007/978-3-540-29678-2_3292
9. Flies CB, Peplies J, Schüler D (2005) Combined approach for characterization of uncultivated magnetotactic bacteria from various aquatic environments. Applied and Environmental Microbiology 71:2723–2731. https://doi.org/10.1128/AEM.71.5.2723-2731.2005
10. Gandia D, Gandarias L, Rodrigo I, et al (2019) Unlocking the Potential of Magnetotactic Bacteria as Magnetic Hyperthermia Agents. Small 15:1–12. https://doi.org/10.1002/smll.201902626
11. Heyen U, Schüler D (2003) Growth and magnetosome formation by microaerophilic *Magnetospirillum strains* in an oxygen-controlled fermentor. Appl Microbiol Biotechnol 61:536–544. https://doi.org/10.1007/s00253-002-1219-x
12. Ji B, Zhang S da, Zhang WJ, et al (2017) The chimeric nature of the genomes of marine magnetotactic coccoid-ovoid bacteria defines a novel group of Proteobacteria. Environmental Microbiology 19:1103–1119. https://doi.org/10.1111/1462-2920.13637

13. Khalil ISM, Misra S (2014) Control characteristics of magnetotactic bacteria: *Magnetospirillum magnetotacticum strain* MS-1 and *magnetospirillum magneticum strain* AMB-1. IEEE Transactions on Magnetics 50:1–11. https://doi.org/10.1109/TMAG.2013.2287495
14. Kolinko S, Jogler C, Katzmann E, et al (2012) Single-cell analysis reveals a novel uncultivated magnetotactic bacterium within the candidate division OP3. Environmental Microbiology 14:1709–1721. https://doi.org/10.1111/j.1462-2920.2011.02609.x
15. Koziaeva V v., Alekseeva LM, Uzun MM, et al (2020) Biodiversity of Magnetotactic Bacteria in the Freshwater Lake Beloe Bordukovskoe, Russia. Microbiology (Russian Federation) 89:348–358. https://doi.org/10.1134/S002626172003008X
16. Leena MC, Aamer AS, Abdul H, Fariha H (2018) Physiological, biochemical and phylogenetic characterization of extremely halophilic bacteria isolated from Khewra mine, Pakistan. Applied Ecology and Environmental Research 16:1243–1256. https://doi.org/10.15666/aeer/1602_12431256
17. Lefevre CT, Bazylinski DA (2013) Ecology, Diversity, and Evolution of Magnetotactic Bacteria. Microbiology and Molecular Biology Reviews 77:497–526. https://doi.org/10.1128/mmbr.00021-13
18. Lefèvre CT, Bennet M, Landau L, et al (2014) Diversity of magneto-aerotactic behaviors and oxygen sensing mechanisms in cultured magnetotactic bacteria. Biophysical Journal 107:527–538. https://doi.org/10.1016/j.bpj.2014.05.043
19. Lefevre CT, Frankel RB, Abreu F, et al (2011) Culture-independent characterization of a novel, uncultivated magnetotactic member of the Nitrospirae phylum. Environmental Microbiology 13:538–549. https://doi.org/10.1111/j.1462-2920.2010.02361.x
20. Li J, Ge X, Zhang X, et al (2010) Recover vigorous cells of *Magnetospirillum magneticum AMB-1* by capillary magnetic separation. Chinese Journal of Oceanology and Limnology 28:826–831. https://doi.org/10.1007/s00343-010-9068-4
21. Li J, Liu P, Wang J, et al (2020) Magnetotaxis as an Adaptation to Enable Bacterial Shuttling of Microbial Sulfur and Sulfur Cycling Across Aquatic Oxic-Anoxic Interfaces. Journal of Geophysical Research: Biogeosciences 125:. https://doi.org/10.1029/2020JG006012
22. Li J, Zhang H, Menguy N, et al (2017) Single-cell resolution of uncultured magnetotactic bacteria via fluorescence-coupled electron microscopy. Applied and Environmental Microbiology 83:. https://doi.org/10.1128/AEM.00409-17
23. Lins U, Mccartney MR, Farina M (2006) Crystal habits and magnetic microstructures of magnetosomes in coccoid magnetotactic bacteria. 78:463–474
24. Lin W, Pan Y, Bazylinski DA (2017) Diversity and ecology of and biomineralization by magnetotactic bacteria. Environmental Microbiology Reports 9:345–356. https://doi.org/10.1111/1758-2229.12550
25. Lin W, Tian L, Li J, Pan Y (2008) Does capillary racetrack-based enrichment reflect the diversity of uncultivated magnetotactic cocci in environmental samples? FEMS Microbiology Letters 279:202–206. https://doi.org/10.1111/j.1574-6968.2007.01029.x

26. Liu P, Liu Y, Zhao X, et al (2021) Diverse phylogeny and morphology of magnetite biomineralized by magnetotactic cocci. Environmental Microbiology 23:1115–1129. https://doi.org/10.1111/1462-2920.15254
27. Liu Y, Chen Q (2008) Synthesis of magnetosome chain-like structures. Nanotechnology 19:1–6. https://doi.org/10.1088/0957-4484/19/47/475603
28. Masood F, Abdul-Salam M, Yasin T, Hameed A (2017) Effect of glucose and olive oil as potential carbon sources on production of PHAs copolymer and tercopolymer by Bacillus cereus FA11. 3 Biotech 7:. https://doi.org/10.1007/s13205-017-0712-y
29. Mitra R, Xu T, Xiang H, Han J (2020) Current developments on polyhydroxyalkanoates synthesis by using halophiles as a promising cell factory. Microbial Cell Factories 19
30. Oestreicher Z, Lower SK, Lin W, Lower BH (2012) Collection, isolation and enrichment of naturally occurring magnetotactic bacteria from the environment. Journal of Visualized Experiments 1–6. https://doi.org/10.3791/50123
31. Oestreicher Z, Lower SK, Rees E, et al (2013) Magnetotactic bacteria from Pavilion Lake, British Columbia. Frontiers in Microbiology 4:1–6. https://doi.org/10.3389/fmicb.2013.00406
32. Park SJ, Park SH, Cho S, et al (2013) New paradigm for tumor theranostic methodology using bacteria-based microrobot. Scientific Reports 3:1–8. https://doi.org/10.1038/srep03394
33. Pichel MP, Hageman TAG, Khalil ISM, et al (2018) Magnetic response of *Magnetospirillum gryphiswaldense* observed inside a microfluidic channel. Journal of Magnetism and Magnetic Materials 460:340–353. https://doi.org/10.1016/j.jmmm.2018.04.004
34. Rismani Yazdi S, Nosrati R, Stevens CA, et al (2018) Magnetotaxis Enables Magnetotactic Bacteria to Navigate in Flow. Small 14:1–10. https://doi.org/10.1002/smll.201702982
35. Roda A, Cevenini L, Borg S, et al (2013) Bioengineered bioluminescent magnetotactic bacteria as a powerful tool for chip-based whole-cell biosensors. Lab on a Chip 13:4881–4889. https://doi.org/10.1039/c3lc50868d
36. Salam MA, Khan A, Rafiq M, et al (2019a) Isolation of magnetotactic bacteria from environmental samples and optimization and characterization of extracted magnetosomes. Applied Ecology and Environmental Research 17:5355–5367. https://doi.org/10.15666/aeer/1703_53555367
37. Salam MA, Khan A, Rafiq M, et al (2019b) Isolation of magnetotactic bacteria from environmental samples and optimization and characterization of extracted magnetosomes. Applied Ecology and Environmental Research 17:5355–5367. https://doi.org/10.15666/aeer/1703_53555367
38. Schüler D (2008) Genetics and cell biology of magnetosome formation in magnetotactic bacteria. FEMS Microbiology Reviews 32:654–672. https://doi.org/10.1111/j.1574-6976.2008.00116.x
39. Schüler D, Spring S, Bazylinski DA (1999) Improved technique for the isolation of magnetotactic spirilla from a freshwater sediment and their phylogenetic characterization. Systematic and Applied Microbiology 22:466–471. https://doi.org/10.1016/S0723-2020(99)80056-3
40. Sparks NHC, Mann S, Bazylinski DA, et al (1990) Structure and morphology of magnetite anaerobically-produced by a marine magnetotactic bacterium and a dissimilatory iron-reducing

bacterium. Earth and Planetary Science Letters 98:14–22. https://doi.org/10.1016/0012-821X(90)90084-B

41. Spring S, Amann R, Ludwig W, et al (1992) Phylogenetic Diversity and Identification of Nonculturable Magnetotactic Bacteria. Systematic and Applied Microbiology 15:116–122. https://doi.org/10.1016/S0723-2020(11)80147-5
42. Vincenti B, Ramos G, Cordero ML, et al (2019) Magnetotactic bacteria in a droplet self-assemble into a rotary motor. Nature Communications 10:7–9. https://doi.org/10.1038/s41467-019-13031-6
43. Wang X, Li Y, Zhao J, et al (2020) Magnetotactic bacteria: Characteristics and environmental applications. Frontiers of Environmental Science and Engineering 14:. https://doi.org/10.1007/s11783-020-1235-z
44. Yan L, Zhang S, Chen P, et al (2012) Magnetotactic bacteria, magnetosomes and their application. Microbiological Research 167:507–519. https://doi.org/10.1016/j.micres.2012.04.002
45. Zhao L, Wu D, Wu LF, Song T (2007) A simple and accurate method for quantification of magnetosomes in magnetotactic bacteria by common spectrophotometer. Journal of Biochemical and Biophysical Methods 70:377–383. https://doi.org/10.1016/j.jbbm.2006.08.010
46. Zhou X, Chen C, Cao C, et al (2018) Enhancing reaction rate in a Pickering emulsion system with natural magnetotactic bacteria as nanoscale magnetic stirring bars. Chemical Science 9:2575–2580. https://doi.org/10.1039/c7sc05164f

Figures

Figure 1

Arial view of Banjosa Lake, Pakistan (https://www.enroutepakistan.com/location/banjosa).

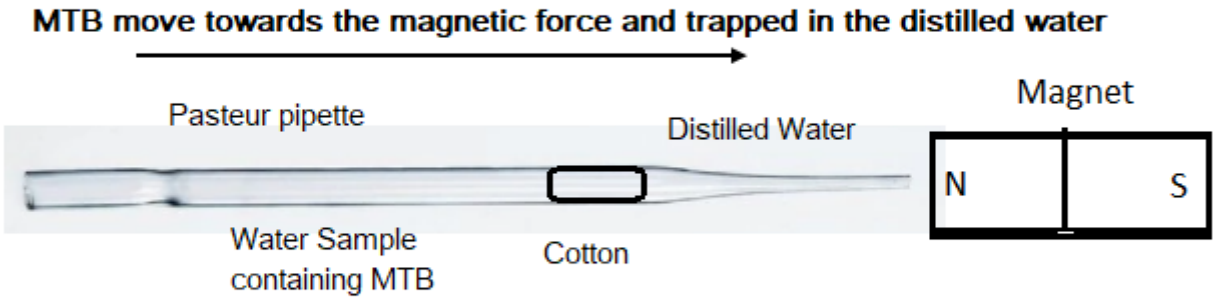

Figure 2

Schematic diagram of the Pasteur pipette and magnetic separation method (Racetrack method) of magnetotactic bacteria.

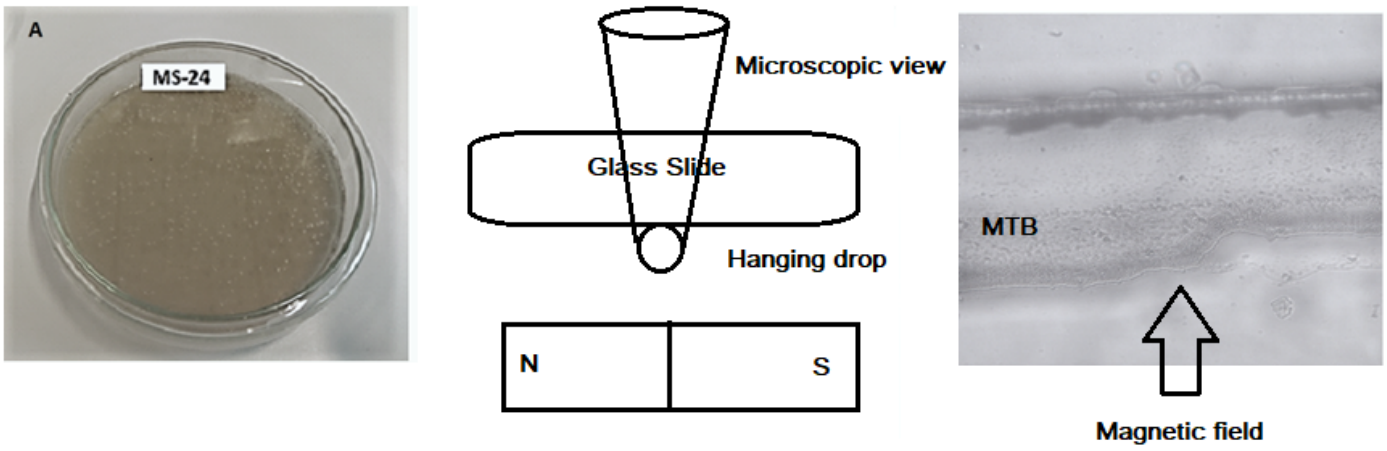

Figure 3

Magnetotactic bacterial strain Magnetospirillum moscoviense MS-24 colonies specific iron-containing medium agar plate. Compound microscopy with 100X magnification imaging of magnetotactic bacteria showed the movement of Magnetospirillum moscoviense MS-24 towards the magnet (Hanging drop technique) (scale bar 200 µm).

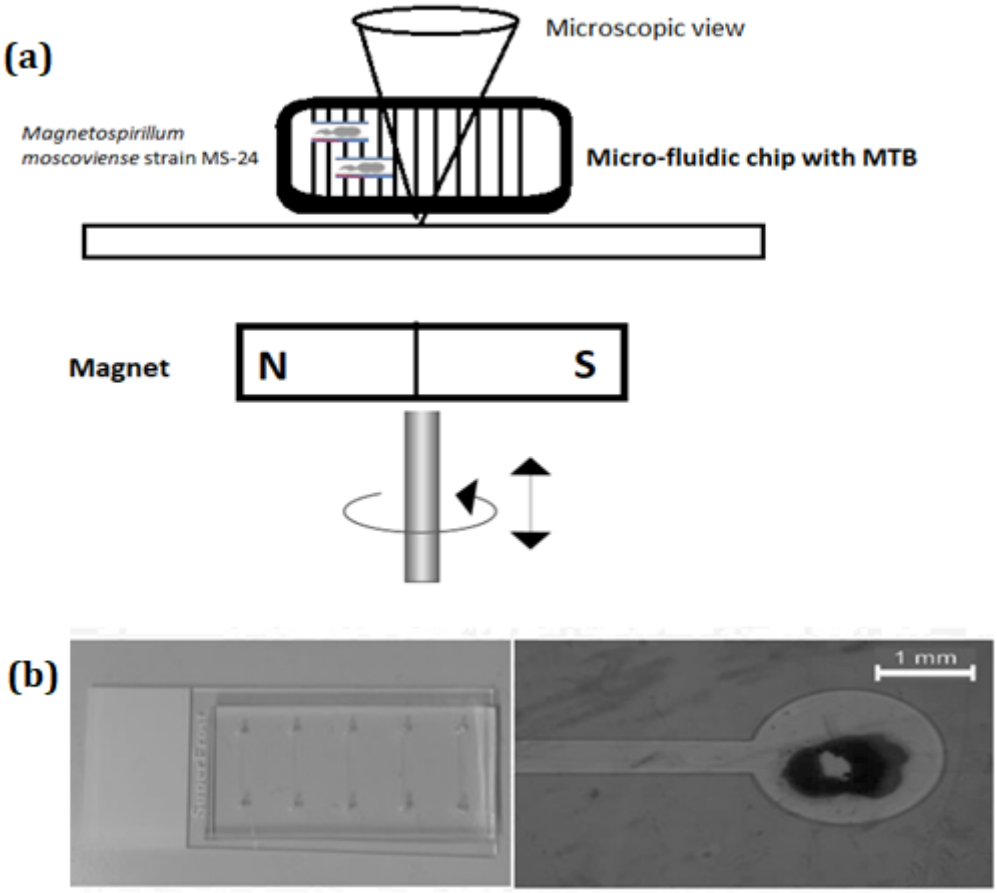

Figure 4

(a) Schematic diagram showed the movement of MTB against applied magnetic field. (b) Design of Polydimethylsiloxane microfluidic chip. The narrow channel of Polydimethylsiloxane microfluidic chip with a channel depth of 5 μm and channel width of 200 μm.

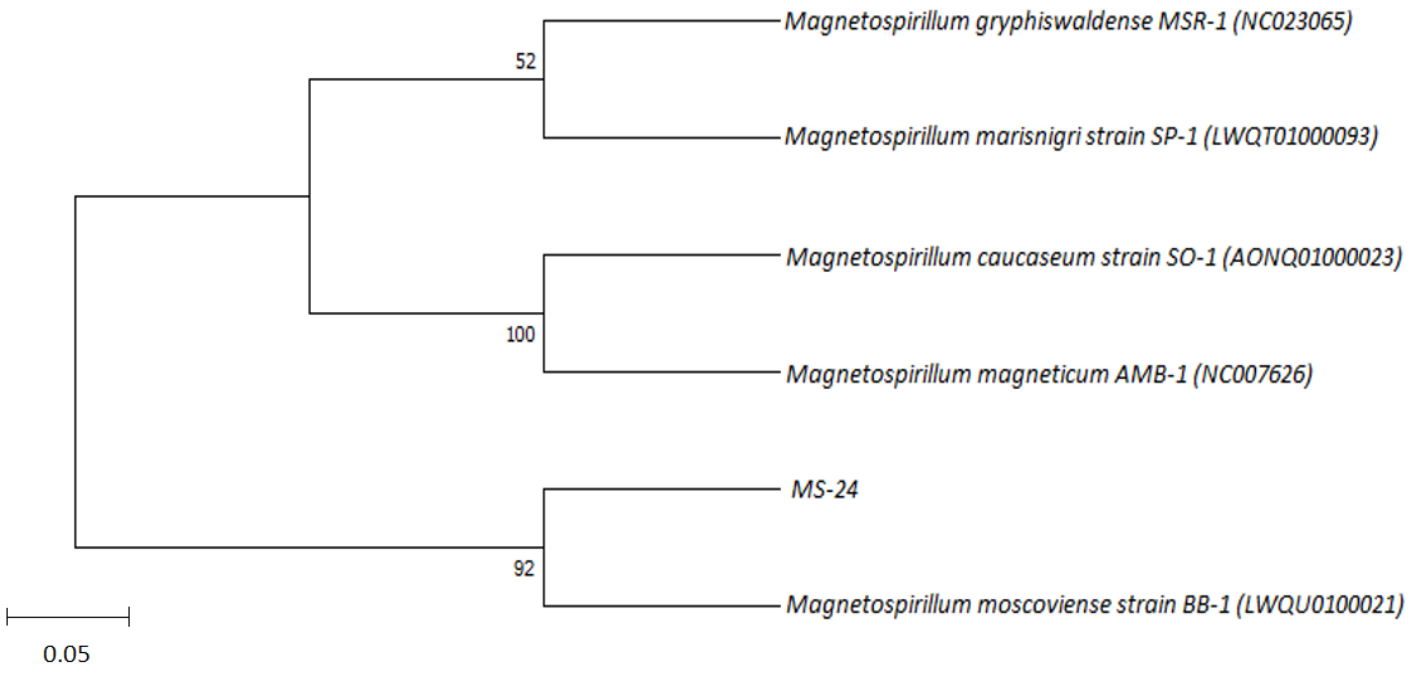

Figure 5

Phylogenetic tree of isolated magnetotactic bacteria Magnetospirillum moscoviense MS-24.

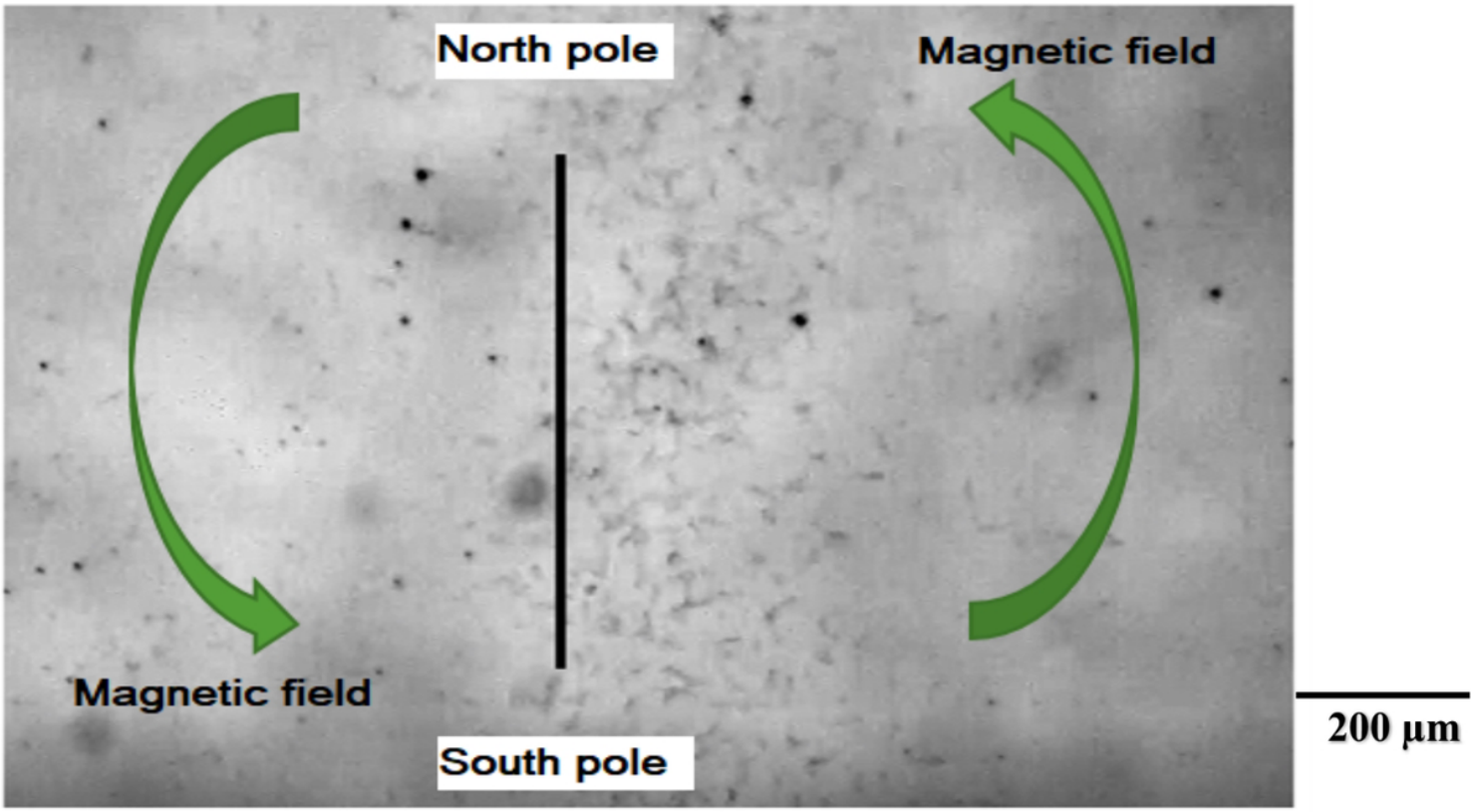

Figure 6

The magnetic field lines were applied at the centre of the microfluidic chip to observe Magnetospirillum moscoviense MS-24 movement at a central point.

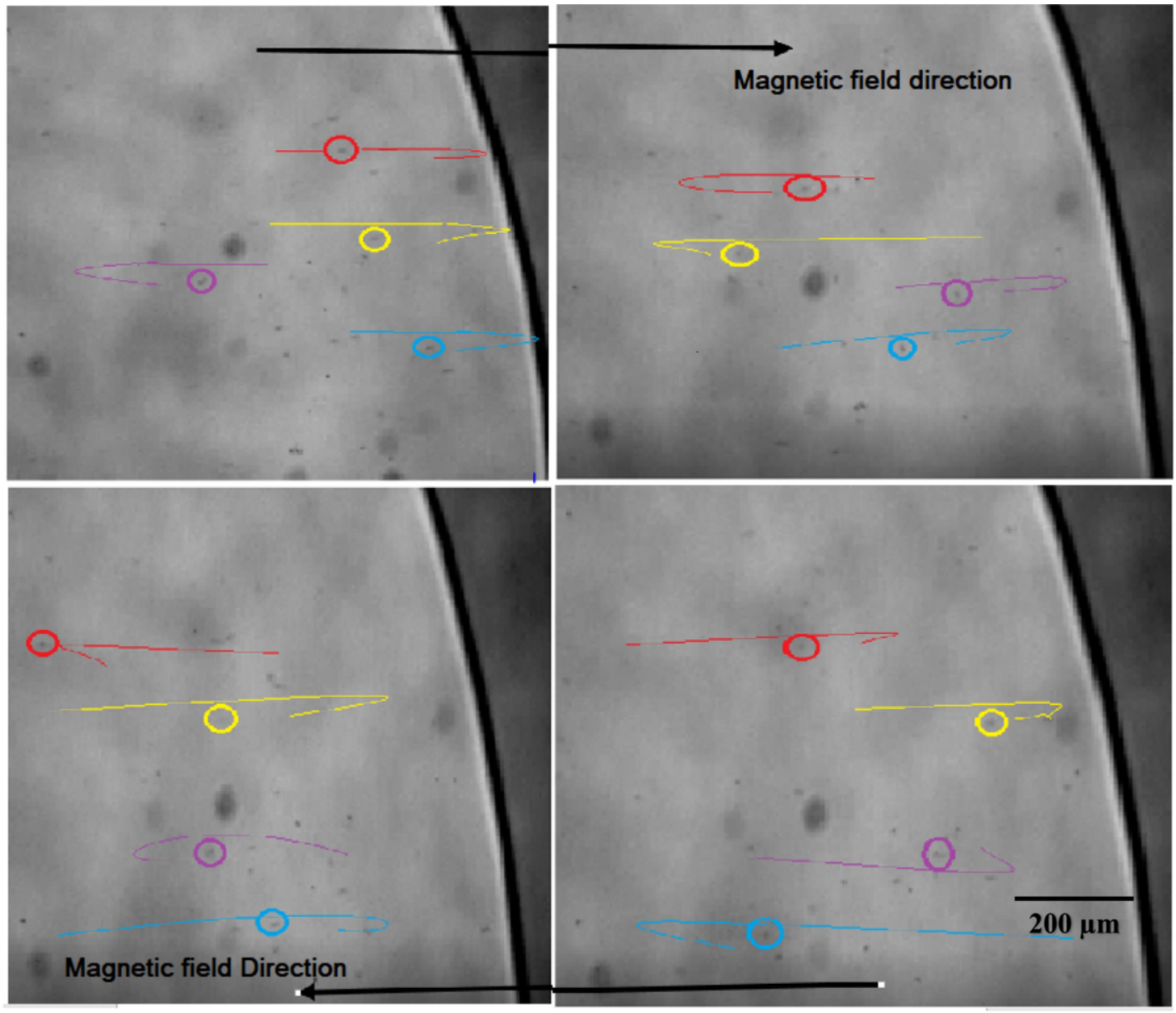

Figure 7

The magnetic field lines were applied in rotating left and right direction to observe *Magnetospirillum moscoviense* MS-24 movement.

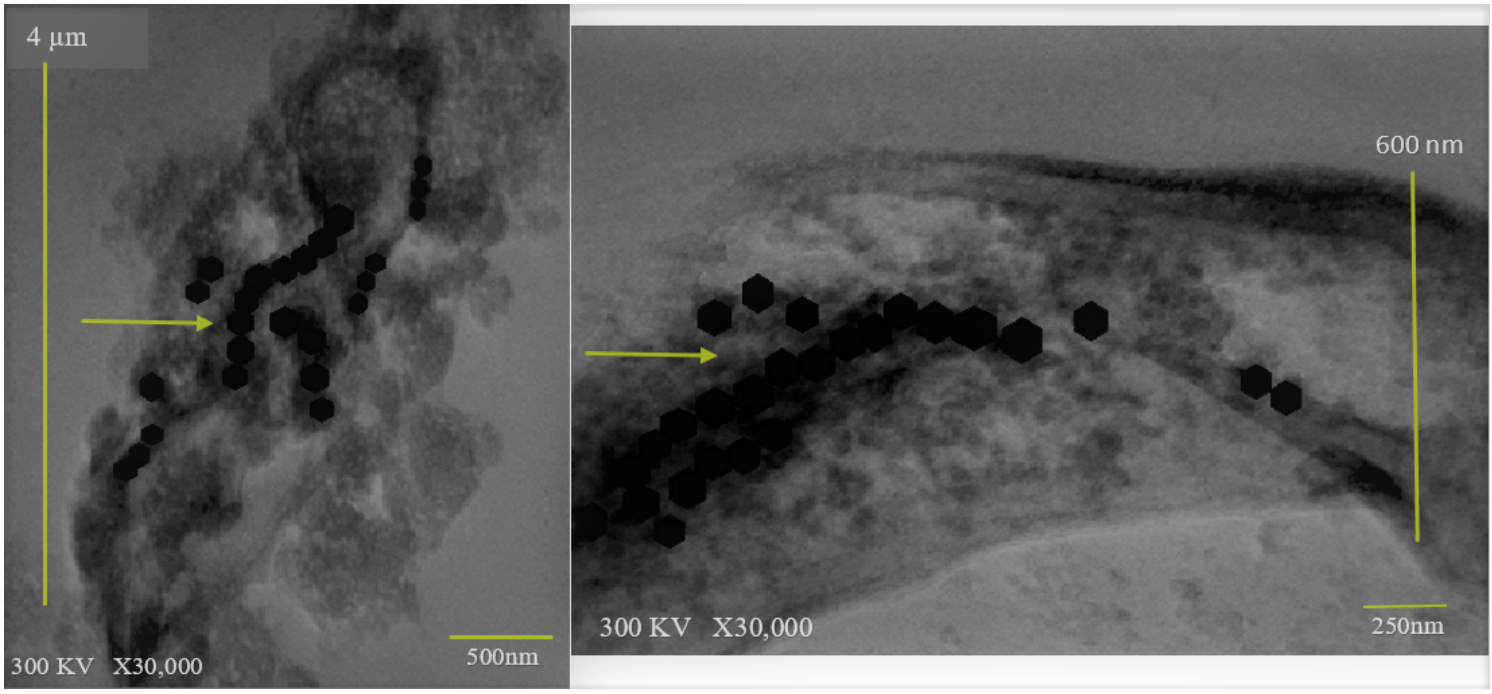

Figure 8

Transmission electron microscopy of *Magnetospirillum moscoviense* MS-24 showed it's spiral-shaped with magnetosomes (Arrow represented the magnetosomes chain).

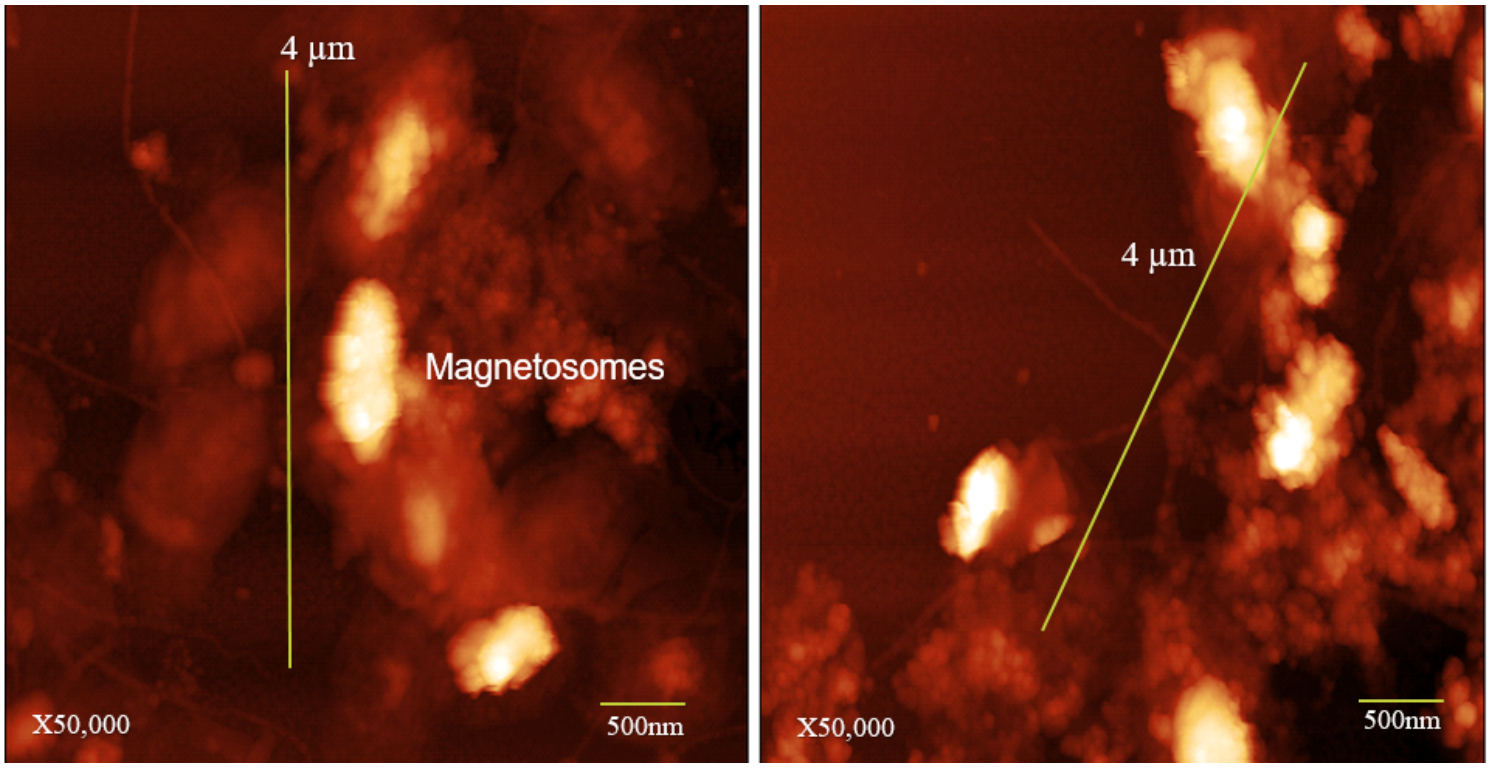

Figure 9

Atomic force microscopy of bacterial culture of *Magnetospirillum moscoviense* MS-24 showed it's spiral-shaped with magnetosomes.

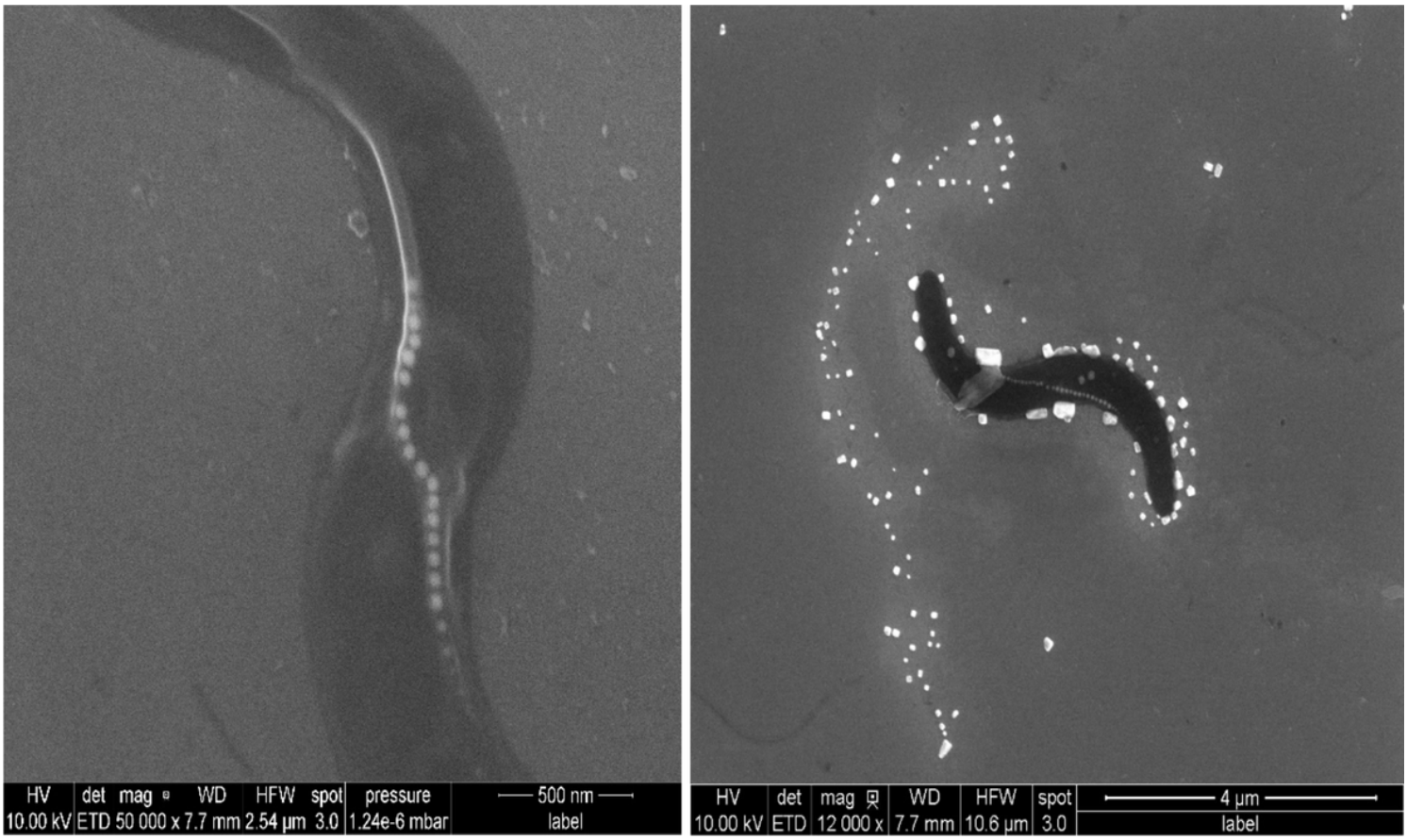

Figure 10

High resonance scanning electron microscopy of *Magnetospirillum moscoviense* MS-24 showed a clear image of magnetosomes chain in a bacterial cell and the bacterial flagella.

Supplementary Files

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