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Characterization of Magnetotactic Bacteria (MTBs) Isolated from Different Habitats in Egypt.

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ABSTRACT

The aim of this work is the characterization of some efficient magnetotactic bacterial strains (MTBs) were isolated from different iron contaminated sites. A total of 21 MTBs cultures were from sewage sludge specimen (8 isolates), iron-contaminated agricultural soil (10 isolates) and (3 isolates) from water wells. Twelve bacterial isolates show a magnetic response individually to the applied external magnetic stimulus by the two methods either microscopic or semi solid plate technique. Three distinguished morphologies of MTB (cocci, large slow moving rods and small actively moving rods) out of 21 isolates were dominant. From anaerobiosis selection step only 8 isolates show the ability to grow in sodium sulfide plates. TEM analysis revealed that these bacterial isolates contained numerous mineral crystals similar to magnetosomes. From the previous successive purification steps only one isolates showed the best iron uptake as the iron concentration was completely consumed from the medium during the growth period, as and cell biomass productivity (OD_{600}: 0.229). Based on their morphological and microscopy characteristics as well as 16S rRNA genes sequence analysis, the isolate was identified as *Pseudomonas aeruginosa.*

Keywords: Bacteria, Iron uptake, iron contaminated sites, TEM, 16S rRNA, MTBs

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INTRODUCTION

A number of prokaryotic organisms are known to use the Earth's magnetic field for orientation and navigation, a behavior referred to as magneto reception [1]. Although the mechanisms involved in magneto reception are not well understood, the presence of magnetic mineral crystals has been established in some of these organisms [1]. The most well understood example of magneto reception is found within the prokaryotes and involves a group of motile, aquatic bacteria known as the magnetotactic bacteria (MTB). MTB are morphologically, metabolically, and phylogenetically diverse prokaryotes [2]. MTB are a group of Gram-negative that passively align and actively swim along the geomagnetic field and other fields by rotating their helical flagella [3]. Known cultured and uncultured MTB are phylogenetically associated with the *Alpha*-, *Gamma*- and *Deltaproteobacteria* classes of the phylum *Proteobacteria*, the *Nitrospirae* phylum and the candidate division OP3 of the *Planctomycetes-Verrucomicrobia-Chlamydiae* (PVC) superphylum [4]. MTB are ubiquitous in almost all types of aquatic environments [5] and are cosmopolitan in distribution as they have been found on every continent [6]. MTB are known to mainly inhabit the oxic-anoxic interface (OAI) of aquatic habitats [5] and it is currently thought that the magnetosomes function as a means of making chemotaxis more efficient in locating and maintaining an optimal position for growth and survival at the OAI [7].

Formation of magnetosomes is coupled with uptake of large amounts of extracellular iron in these bacteria. Due to their high remarkable capacity of iron minerals accumulation, it is assumed that MTB have great ecological roles in biogeochemical cycle of iron in natural habitats [8]. In MTB, the magnetoreceptive behavior is called magnetotaxis due to the presence of intracellular membrane-bounded-tens-of-nanometer-sized crystals of a magnetic mineral consisting of either magnetite (Fe_3O_4) or greigite (Fe_3S_4). These structures, termed magnetosomes [9].

In spite of their wide distribution and abundance in aquatic environments [10], most MTB are intractable, and so far only a few of them are isolated in pure culture [11]. Enrichment of ferromagnetic minerals and magnetite is observed in top layer of soil therefore all biogenic and abiogenic process facilitate to produce magnetite [12]. Despite the ubiquitous occurrence of MTB and their high abundance in the sediments of many freshwater and marine habitats, the isolation and cultivation of MTB are difficult due to their fastidious lifestyle [13]. Because physiology and nutritional requirements of MTB are diverse in many cases, isolation and axenic cultivation of MTB in pure culture is very difficult. Therefore, development of a general guideline has been postponed to isolation of these fastidious bacteria [14].

The first magnetotactic bacterium to be isolated and grown in pure culture was *Magnetospirillum* (formerly *Aquaspirillum*; [15] *magnetotacticum* strain MS-1 [16]. Although a *Magnetococcus* and a *Magnetovibrio* have also been isolated, cultured and described. Reports have shown that, apart from magnetotactic bacteria that synthesize magnetic nanoparticles, an aerobic and acid tolerant bacteria, *Leptothrix ochracea* [17] and *Leptospirillum ferriphilum* [18], can also produce magnetic nanoparticles. Another aerobic bacterium called *Acinetobacter* species was found by Bharde *et al*. [19] to synthesize magnetite and gregite, which has protein coatings. The discovery of MTB proved to have a serious impact in a number of diverse research fields including microbiology, geology, mineralogy and biomineralization, crystallography, chemistry, biochemistry, physics, limnology and oceanography, and even astrobiology.

Based on the previously mentioned studies, the aim of this work is the characterization and identification of some efficient MTBs strains was isolated from different ecological habitats.

MATERIALS AND METHODS

Site description and sampling

Three different samples were collected from aquatic and soil habitat for the isolation of magnetotactic bacteria: including sludge specimen from Sewage Treatment Station at El-Monofia (Quesna), iron-contaminated agricultural soil from El-Marg (Qalubia) and water wells from El-Wahaat (Giza).

Enrichment of the selected samples

Sewage samples, 250ml of sample was collected from sewage treatment station in El-Monofia (Quesna) and ground water well El-Wahaat (Giza) in loosely capped bottles, and left undisturbed for 1 month under dim light at room temperature. For the soil sample El-Marg (Qalubia), 1gm was inoculated in 100 ml of MSM medium incubated at room temperature in dark for 7 days (Geological Survey of India. 2006). In order to obtain a dense population of magnetotactic bacteria, enrichment technique was performed. A modified mineral salt medium (MSM) contains (1.0g/l peptone, 100 ul/l of 1 mol ferric citrate, 0.1 gm/1 of yeast extract, , 0.5g/l gm of sodium thioglycolate), which was designed to survive and increase the number of Magnetotactic consortium [20]. The pH was adjusted to 6.8-7.0 then the medium was autoclaved at 121 °C for 20 min [21]. The culture flasks were filled to about 90% of their capacity, sealed tightly and microaerobic conditions arose through the cellular oxygen [22].

Isolation of magnetic cells

Preliminary screening of magnetotactic organisms isolates

The two water samples as well as the soil sample were then filtered through 0.45 micrometer filter membrane to obtain a pure extract. Initially, 2 ml of the extracts individually were distributed in each 20 ml of MSM medium in capped bottles and incubated at 30°C for 0, 3 and 7 days with applied magnetic field. The number of total magnetotactic bacteria (MTB) cells closely tracked by Hemocytometer of the three enriched samples. Cell numbers peaked after the three incubation periods, and then the collected sample (20 mL) was inoculated in 100 mL of enrichment medium and incubated for 7 days. After the incubation period in order to select only the potentially magnetotactic bacterial community, the enriched flasks were subjected to external magnetic field in order to hold and trap the magnetotactic bacterial clumps and then quietly pour off the excess of the samples under sterilized conditions. The aggregated magnetotactic bacterial clumps were then washed several times with distilled deionized water (DDH_2O) and in each time the magnetotactic bacteria were collected by the applied external magnetic field then the rest were spread on (MSM) agar plates under microaerophilic conditions and incubated. After that the resulted individual colonies were slanted individually and stored for further studies.

The obtained cultures were tested for their magnetotactic response by two ways.

1. In the first way hanging drop technique under an optical microscope with the south pole of a bar magnet being placed about 2 cm distant distance from the slide.
2. In the second way magnetic response was tested in terms spreading of their growth on the surface of a semisolid medium containing 0.5% agar and motility response. The growth pattern after incubation was observed for any spreading towards the magnetic pole. The obtained cultures were further separated by streak plate method.
3. Selection of magnetotactic bacteria

After the previous enrichment process and detection of magnetotactic bacteria in the TEM images of the enriched samples, a further experiment was done on solid MSM to select the isolates which capable for growing in anaerobic condition by sterilize swapping the individual previously isolated bacterial culture suspension on MSM agar plates and with sterile screw cab making a hole in the plate center then add about 0. 2g of sodium sulfide pellets per plate in order to obtain anaerobic condition inside the plate [23].

From the previous purification steps, isolates were picked from the anaerobic plates in order to testing their individual potentiality for iron consumption, magnetosomes formation as well as the cell biomass production in 25 ml MSM broth medium in 30ml tightly closed glass bottles which contained 3ul of 1M ferric citrate as iron source and 13mg of sodium thioglycolate as well as 25ul of 1M Dithiothritol (DTT) (both as oxygen scavenge agents) and allow to grow for 24hrs at 30°C.

From the previous successive purification steps, isolates showed the best iron uptake, magnetosomes formation and cell biomass productivity, so those isolates were subjected to further studies.

Determination of Cell Density and Biomass Wet Weight

The presence of magnetosome affects both the optical density and the total wet weight of the biomass. Therefore, in order to obtain cell density values, equivalent aliquots were used to determine both the

OD600 [24], as well as wet weight of the cellular biomass, an equal volume of sample (i.e., bacterial culture possessing magnetosomes) was centrifuged at 10000 rpm for 5 minutes.

After washing with 0.02M H_2SO_4 several times for removing of any surroundings ions [25], the wet weight was determined gravimetrically. From this combined data, a calibration curve of OD600 and biomass wet weight for a culture possessing magnetosomes was constructed.

Analysis of Iron uptake

After the cellular biomass was separated by centrifugation, the liquid supernatant was subjected directly to PerkinElmer atomic absorption spectrometer (AAnalyst 400, USA). In order to determine the consumption by the bacterial cells during growth and the resting iron concentration remained after. These results of iron consumption were coupled by UV-spectrophotometer (UV-2401PC) for the determination of iron [26].

High-Resolution Transmission Electron Microscopy (HR-TEM) and Image Analysis

A. Cells concentrated by centrifugation from the growth medium were washed three times with 0.02 M H_2SO_4. Cells were then resuspended in phosphate buffer saline (PBS) buffer, and the cell suspensions were centrifuged for 5 min at 10000 rpm and the supernatant was discarded. The above process was repeated several times [25]

B. Cells in the pellets were rinsed three times, suspended in distilled water, adsorbed onto a 300-mesh carbon-coated copper grid, and viewed directly by transmission electron microscope (Philips Tecnai F 30) at an accelerating voltage of 300 kV for recording magnetosomes [27]

Identification of selected bacterial isolates

The most efficient magnetite synthesizing bacterial isolates were subjected to a set of morphological, physiological, biochemical tests as well as 16S rRNA gene sequencing for the purpose of identification.

Morphological characterizations

Macroscopic characterization

The isolates were grown on the surface of MSM agar plates and their morphological features such as margins, pigmentation, color and texture were observed.

Microscopic characterizations

The microscopic morphological features of the isolates were analyzed by using a Zeiss light microscope (Zeiss, West Germany, Germany). Gram stain reaction and Motility tests were conducted. All reagents used were obtained from Fisher Scientific (Leicestershire, UK).

Biochemical tests

Biochemical tests were carried out according to the method of Cappuccino and Sherman [28] with 24 hr old cultures.

Molecular characterization based on 16S rRNA gene

Genomic DNA was extracted from strains using the Wizard Genomic DNA kit (Promega, Madison, WI, USA) using the protocol according to the manufacturer instructions.

Oligonucleotide primers with specificity for eubacterial 16S rRNA genes, primers 16Sa (CGCTGGCGGCAGGCTTAACA); 16Sb (CCAGCCGCAGGTTCCCCT) van Berkum and Fuhrmann (2000) was used to amplify the 16S rRNA gene fragments. The following conditions were used for DNA amplification: 35 cycles

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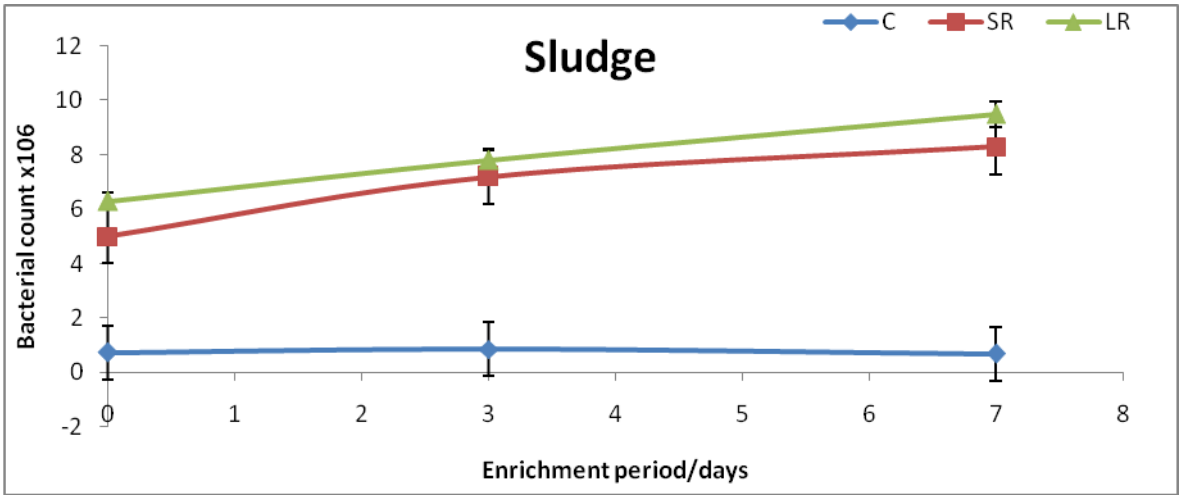
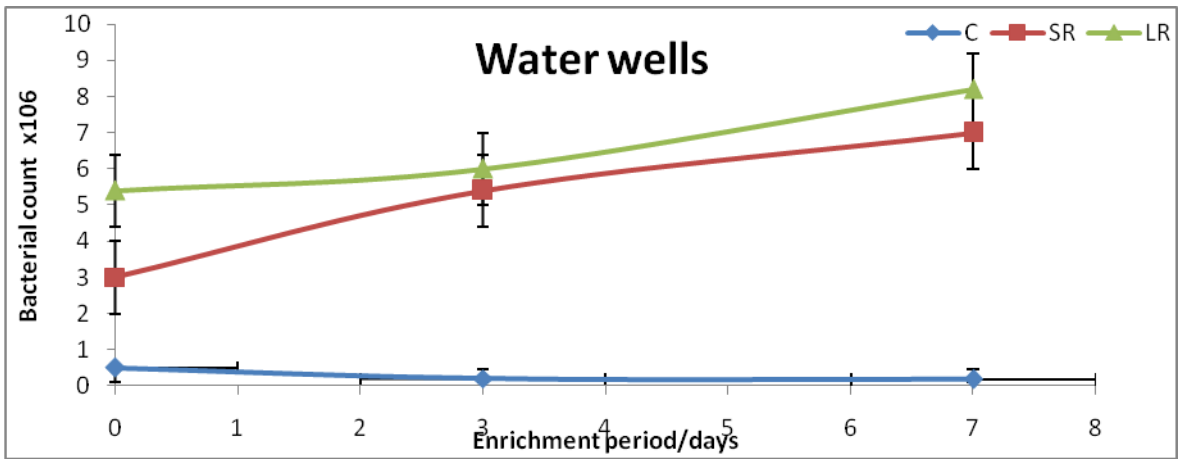
consisting of denaturation at 94 C for 0.5 min, annealing at 55 C for 1 min, and extension at 72 C for 1.5 min followed by a final extension at 72 C for 3 min. Amplified PCR products of the proper size (about 1500 base pair fragment) were confirmed by electrophoresis of 10 uL subsamples through a 1% horizontal agarose gel containing 0.5 ug/mL ethidium bromide. Gels were examined under u.v. light and photographed (van Berkum et al. 1996; van Berkum and Fuhrmann 2000). PCR products were purified using QIAquick Spin columns (Qiagen Inc., Chatsworth, CA). A Perkin Elmer 377 DNA sequencer, in combination with Dye Deoxy Terminator Cycle Sequencing Kit (Perkin Elmer, Foster City, CA) was used for sequencing the purified PCR products as described previously (van Berkum et al. 1996). A search of GenBank with BLAST (Altschul et al. 1997) was used to identify named bacterial species with 16S rRNA gene sequences similar to those of the isolates.

RESULTS

Preliminary screening of MTBs from different habitats by microscopic method analysis

Corresponding with visual observations of increased turbidity and a change in samples opacity, cell numbers reached the highest values after 7 days of incubation (Fig. 1).





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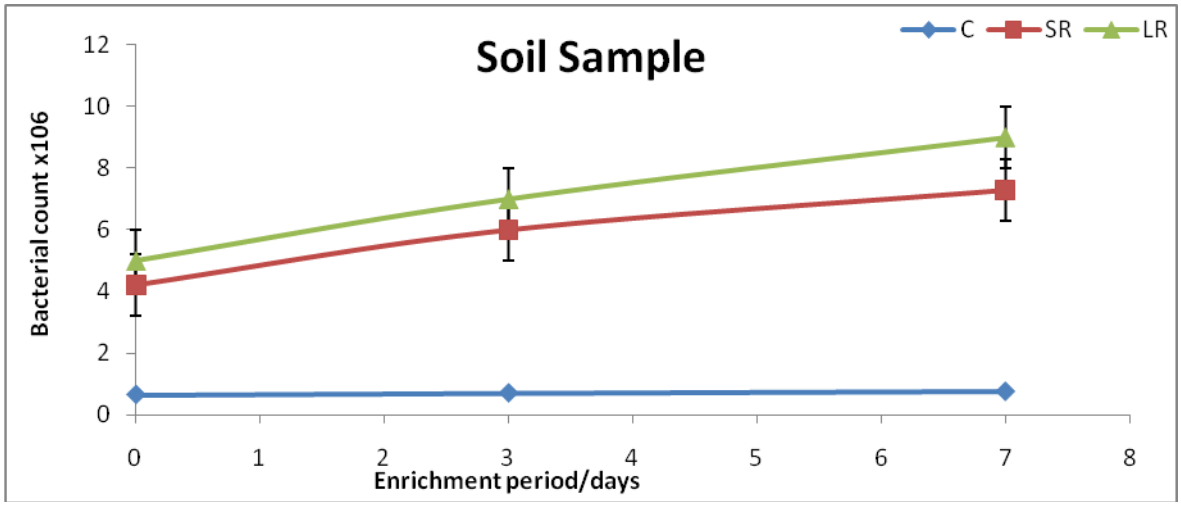

Figure (1): Bacterial counts of the three morphologically distinct (MTBs) in the enriched habitats (cocci (C), actively motile small rods (SR) and slow motile large rods (LR).

From the results obtained the total bacteria counts in the soil habitat is the highest before and after the enrichment (12.02 $x10^6$ and 18.47 $x10^6$), followed by the sludge sample (9.85 $x10^6$ and 17.05 $x10^6$) and the less by the water well (8.9 $X10^6$ and 15.38 $X10^6$) which may be due to the high organic constituents in these habitats which is necessary for (MTB) as electron acceptor during its growth and multiplication while the bacterial community of the water well sample is the less due to the limited supplies of organic nutrients (Table 1). The corresponding results in Table (1) also, indicate that the coccoid shaped bacterial count is the least in the three habitats (0.5 $x10^6$, 0.65 $x10^6$ and 0.72 $x10^6$ respectively) which depleted during the enrichment period intervals that may revealing its hard isolation and controlling as individual bacteria in vitro followed by the small rods bacterial count and finally the large rods bacteria which represents the highest bacterial community in the three samples (Fig. 2).

Table 1: Represents total bacterial count $x10^6$ in the three enriched samples during the enrichment period (0, 3 and 7 days)

Samples	Enrichment period (days)		
	0	3	7
Water well (WW)	8.90±0.012	11.60±0.006	15.38±0.005
Sludge (S)	9.85±0.034	13.70±0.011	17.05±0.004
Soil sample (SS)	12.02±0.048	15.85±0.027	18.47±0.010

SEM (±) Standard error mean according to (Snedecor and Cochran, 1980)

Three readily distinguished morphologies of MTB were dominant: cocci, large slow moving rods and small actively moving rods were observed. Light micrographs and TEM images of the three dominant morphotypes are shown in Fig. 2 Each MTB morphotype showed tactic and motile responses unique to the morphotype: The coccid, small actively motile short rods both of them exhibited a rapid bouncing motion back and forth at the drop edge as well as a third morphologically distinct, large, slow moving magnetotactic rod, approximately 2.38 µM long and 0.80 µM wide (Fig. 2).

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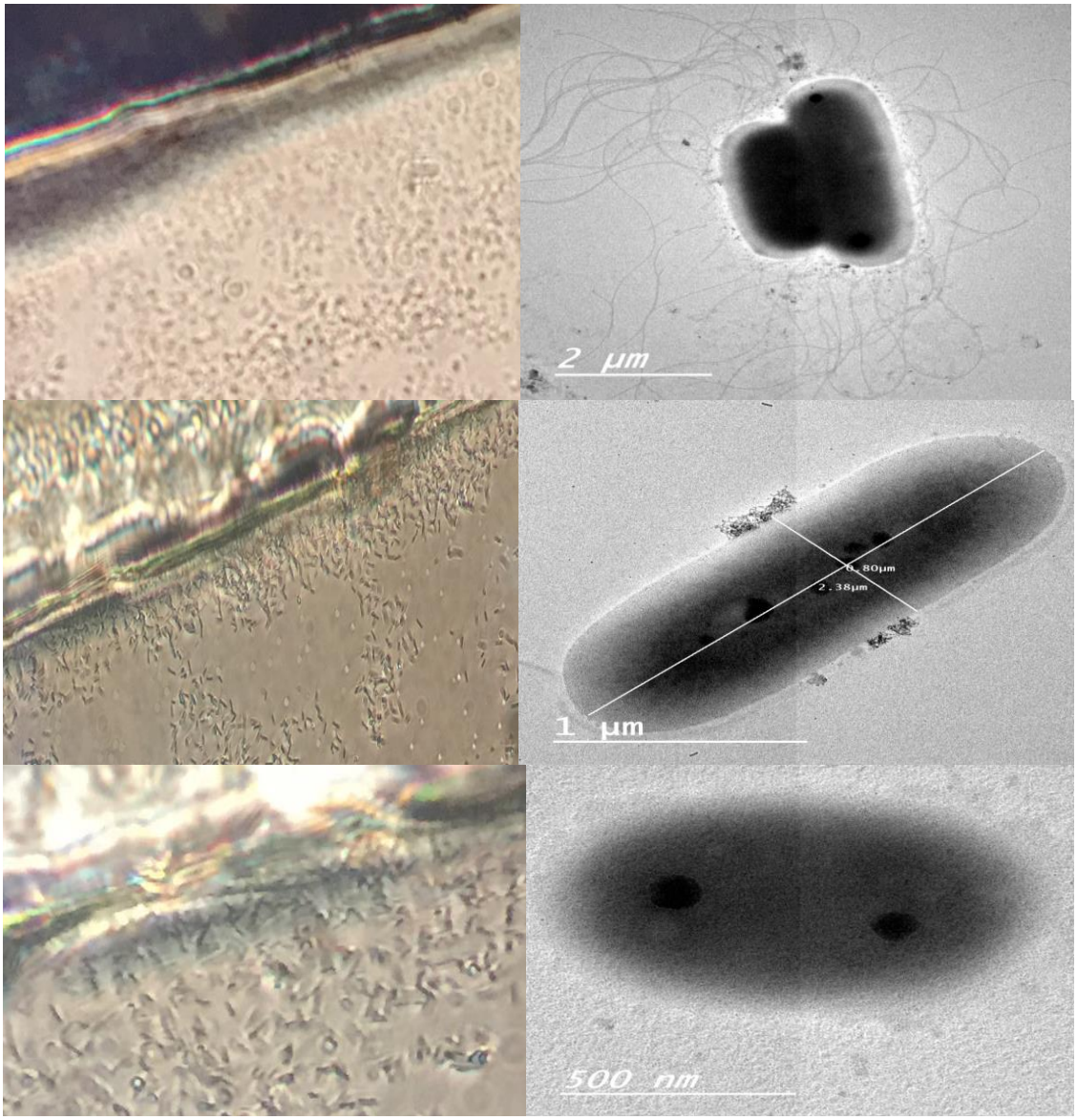

Figure (2): Magnetic response to some morphologically different isolates under light microscope which shows a steady swimming and accumulation toward the external applied magnetic field (right) and TEM imaging of those isolates (left) showing intracellular magnetite crystals as black dots inside the cell.

These organisms swam very slowly to the drop edge where they accumulated without exhibiting the back and forth swimming behavior displayed by the cocci. TEM analysis revealed that the rod contained numerous mineral crystals similar to magnetosomes observed in other organisms. The crystals appear as irregularly shaped black dots in Fig. 3

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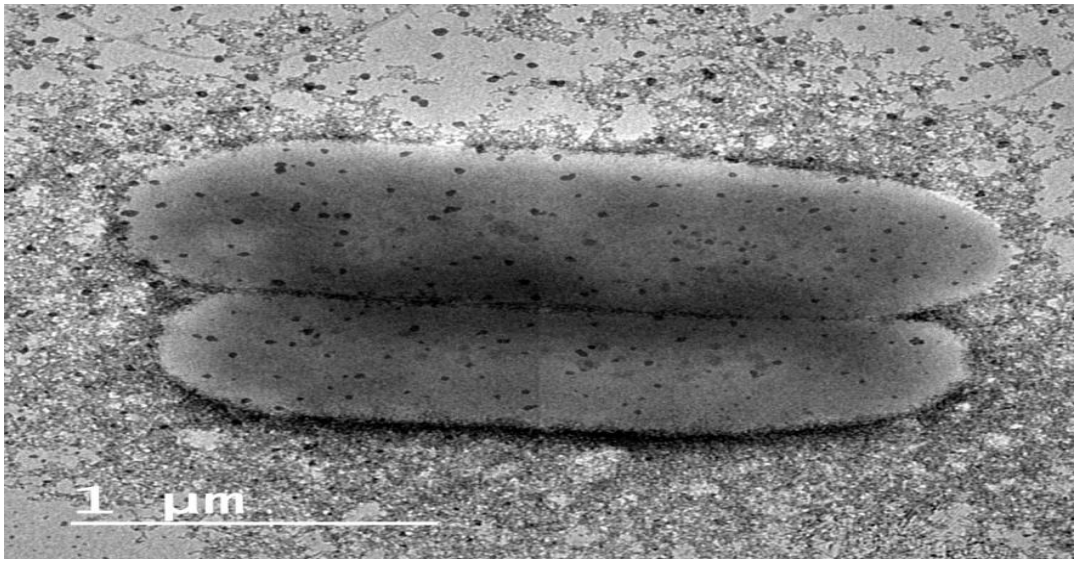

Figure (3): Photo of TEM illustrate numerous mineral crystals similar to magnetosomes in some distinct morphological type of MTB

From the preliminary screening step a total of 21 isolates were raised from the MSM agar plates, a total of 8 isolates were separated from the sludge specimen, 10 isolates from the soil sample and 3 isolates which were slanted individually for determination of both magnetic response ability as well as growing under anaerobic condition as main co-factors for the synthesis of intercellular magnetosomes.

Selection of MTB

From the two magnetic technique that tested we obtained 12 bacterial isolates which show a magnetic response to the applied external magnetic stimulus by the two methods either microscopic or semi solid plate technique (Fig. 4). Which were subjected to further studies to insure the capability of the isolate to grow and reproduce in anaerobic condition.

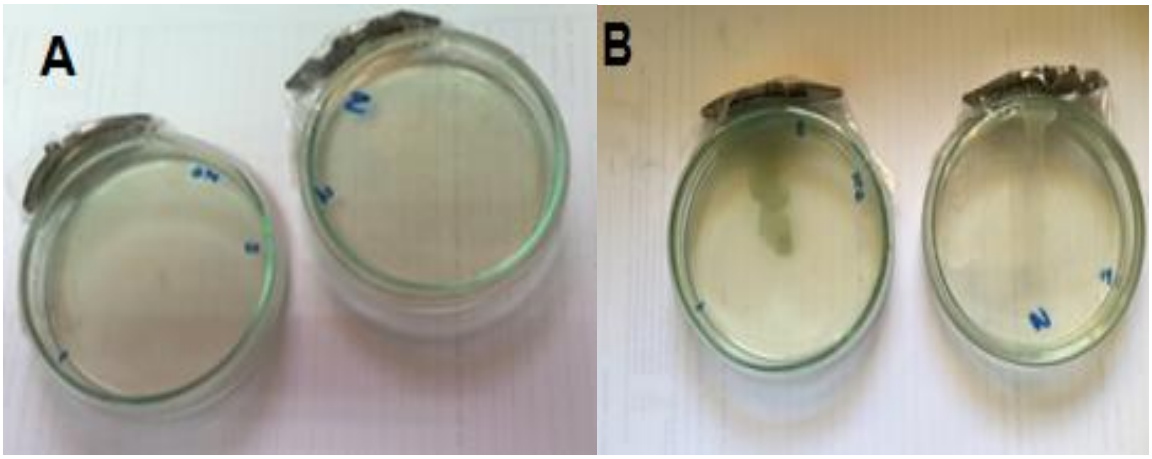

Figure (4): Magnetic response of some morphologically different isolated magnetotactic bacteria (MTB) (green and white) to external magnetic field after the incubation period on semi solid MSM agar medium before (A) and after (B) incubation

Selection of the most efficient MTB

Although not all the biologically synthesized magnetite produced under anaerobic condition but the largest percentage of magnetite production obtained by anaerobic bacteria. In order to obtain the bacterial isolates which has the ability to grow under anaerobic condition as a main factor for forming a uniform equal

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sizes and shapes intercellular magnetosomes, a total of 12 isolates which were selected from the previous magnetism procedure were subjected to anaerobiosis experiment. Sodium sulfide was added as oxygen scavenger chemical, recognizing that after the pellets was diffused in the agar medium a greenish color appear which indicates the arising of anaerobic condition which as soon as the incubation period ended (about 48 hours) it turns back again to the normal medium color as all the sulfide consumed and turned into sulfates salts.

Initially the plate turned greenish in color (Fig. 5). This is due to the fact that Na_2S reduces the ferric citrate as iron source present in the media. The Na_2S thus removes the oxygen by getting oxidized. Fig.5 shows the condition of plate as monitored after 12 hour. As seen from the images it is very well clear that the Na_2S reacts with ferric citrate, giving Fe^{+2} and hence the green color

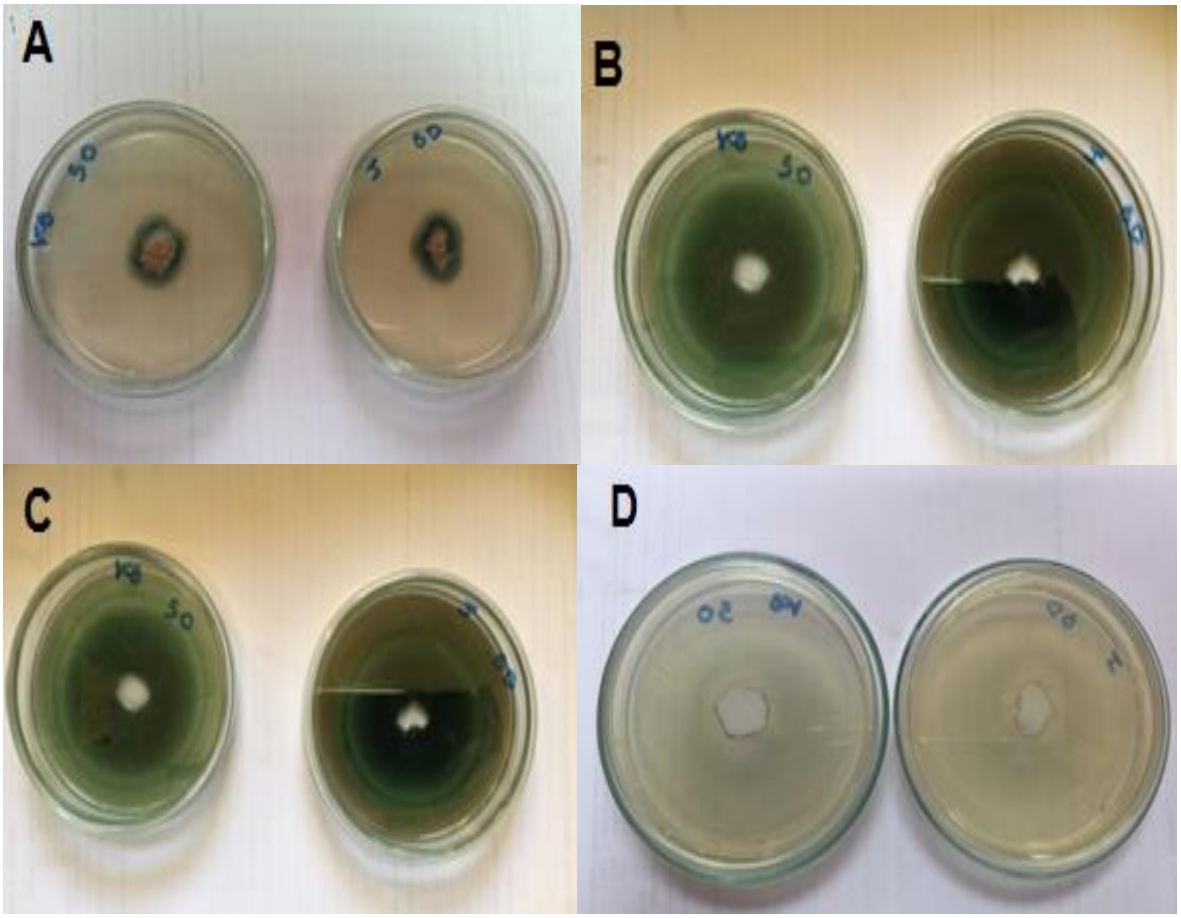

Figure (5): Example of arose of microaerophilic condition on MSM agar plates inoculated with some isolated MTB after sodium disulphide addition (A: condition of plate after plating, B: condition of plate after 12 hours of incubation, C: condition of plate after 24 hours of incubation, D: incubated plates of the two selected strains).

From this anaerobiosis selection step only 8 isolates show the ability to grow slightly (at the plate edges) -not a strict anaerobic condition- in sodium sulfide plates (Fig. 5) which were subjected to further studies according to: iron consumption, cellular biomass and turbidity as well as TEM imaging under those conditions of anaerobiosis and magnetic responses.

Cellular iron uptake was determined by measuring iron depletion from the media spectrophotometerically to detect the iron concentration remaining in the supernatant after 3 days of population growth. Cells took up increasing amounts of iron for increasing initial iron concentrations in the media.

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Fig. 6 represent that the two most efficient strains in iron uptake Kb and J bacterial isolates both at cellular weight and iron consumption capacity as well. The data also supported that not all actively growing cells indicated by their cellular mass turbidity at OD600 have the same potentiality to uptake iron metal as well, which indicated by the strain J* which had a high cellular turbidity ratio while iron consuming potentiality is very low. While some other selected strains have the ability to potentially accumulate a high iron concentration while their cellular turbidity retain reasonably low as 3 bacterial strain which in contrast to their small cellular turbidity (OD 600) 0.095 their iron consumption is considerably high.

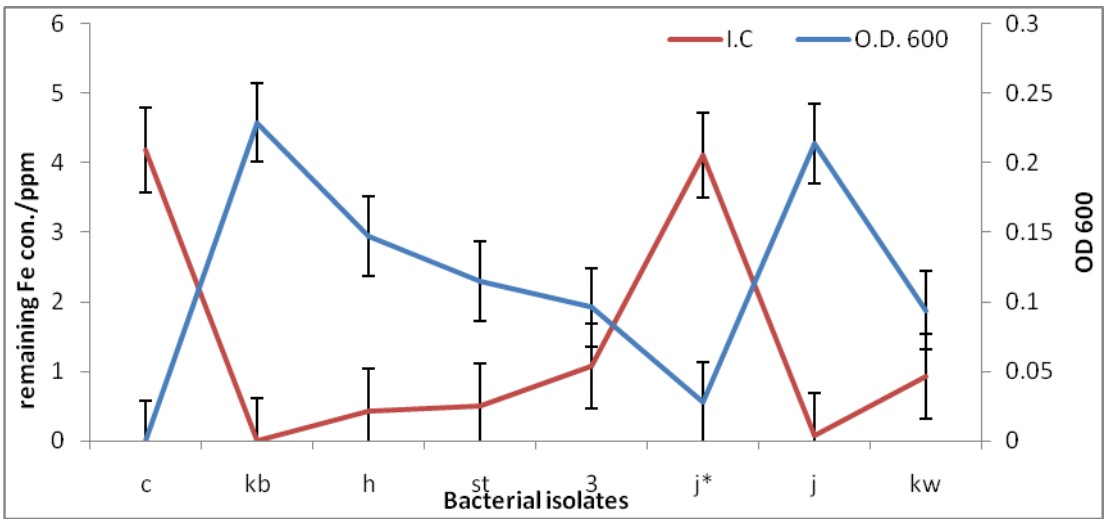

Figure (6): The selection criteria and indicating the most efficient MTBs isolates (green columns for iron concentration, red line for MTBs wet weight, blue line for O.D. 600)

From the previous successive purification steps only 2 isolates showed the best iron uptake, magnetosomes formation and cell biomass productivity one of those isolates (Kb) were subjected to further studies.

Morphological and molecular identification of MTB

Kb is gram-negative rod-shaped bacilli with unipolar motility. It can secret a variety of pigments, including pyocyanin (blue-green), pyoverdine (yellow-green and fluorescent), and pyorubin (red-brown). These can be used to identify the organism. Kb strain is a facultative anaerobe, as it is well adapted to proliferate in conditions of partial or total oxygen depletion. This organism can achieve anaerobic growth with nitrate or nitrite as a terminal electron acceptor (Fig 7).

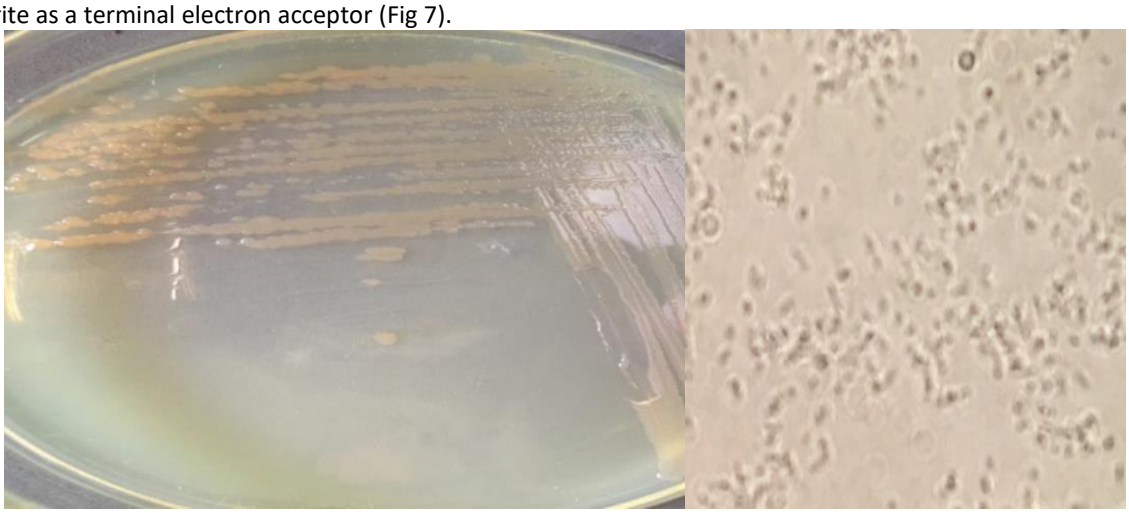

Figure (7): Morphology and colony shape of one of the most efficient isolated MTBs (KB isolate) morphological characteristics on MSM agar plates.

Taxonomically the colony morphology of Kb isolate was small blue green in color when the culture 24hrs which changed to red-brown color after 72hrs in which with irregular colony margin. By light microscope examination the cells were rod and motile gram negative reaction (Fig. 7).

Biochemical tests revealed that (Tables 2) Kb isolate showed that citrate, catalase, and oxidase tests were positive as well as nitrate and nitrite reduction. Gelatin liquefaction and urease test were positive while the starch hydrolysis and Indole production gave negative reaction. For the Sugar fermentation tests showed that Kb isolate were unable to ferment cellobiose, maltose, lactose, sucrose and rhamnose as well as sorbitol. Mean while capable of utilize glucose, fructose and xylose as well as mannitol, glycerol and ethanol.

Analysis of the 16S rRNA sequences confirmed that bacterial isolate (Kb) was closely related to Pseudomonas aeruginosa based on 99% similarity in their 16S rDNA gene sequences. The nucleotide sequence coding for these 16S rRNA genes have been submitted to the GenBank database under the accession number KT962901.

Table (2): Different biochemical tests applied for Kb isolate physiochemical identification

No.	Biochemical test	Reaction	No.	Biochemical test	Reaction
1	Growth at 4c	**-**	**16**	Glucose	**+**
2	Growth at 41c	**+**	**17**	Arabinose	**+**
3	Growth on blood agar	**+**	**18**	Cellobiose	**-**
4	Citrate utilization	**+**	**19**	Ethanol	**+**
5	Indole production	**-**	**20**	glycerol	**+**
6	Starch hydrolysis	**-**	**21**	Fructose	**+**
7	Gelatin liquefaction	**+**	**22**	Mannitol	**+**
8	Casein hydrolysis	**+**	**23**	Rhamnose	**-**
9	oxidase	**+**	**24**	Maltose	**-**
10	Hydrogen sulfide	**-**	**25**	Lactose	**-**
11	Catalase	**+**	**26**	Raffinose	**-**
12	urease	**+**	**27**	Xylose	**+**
13	Nitrate reduction	**+**	**28**	Sorbitol	**-**
14	Lysine decarboxylase	**-**	**29**	sucrose	**-**
15	Nitrite reduction	**+**			

For the physiological tests, Kb isolate uncapable for growth at 4ºC while there was recorded growth at 41ºC and on blood agar plates.

DISCUSSION

MTB occur ubiquitously in a wide range of different habitats [29, 30], and have been even reported from water-logged soils [31]. Despite their ubiquitous occurrence, different environments appear to support the development of specific populations of MTB. For example, in a survey of several freshwater habitats, the population of MTB in a eutrophic pond was found to contain only three different morphological forms dominated by a species of a magnetic coccus occurring in high numbers [32] MTB of this type were usually abundant in habitats with high content of organic nutrients [33, 34]. In contrast, MTB from an oligotrophic lake sediment were phylogenetically and morphologically more diverse with at least 10 morphologically distinct forms of MTB [32]. In certain microhabitats, cells of a single rod-like species of MTB were the dominant fraction of the microbial community and accounted for up to 30% of the biovolume [34].

We observed a marked stratification in the distribution of three different types of MB at the three enriched samples, which we hypothesize, is due to their as yet unknown respiratory requirements.

Major factors determining the distribution of bacteria in a stratified aquatic habitat include the location and width of the chemocline, as well as the proximity and concentrations of electron donors and

acceptors. Motile microbial populations are often finely layered around the chemocline with respect to these properties.

Magnetotaxis may also be the relatively rare consequence of a more common form of prokaryotic iron metabolism. Metal inclusions of various kinds have been found in non-magnetotactic γ-Proteobacteria. [35] Identified membrane-bound crystalline Fe inclusions in *Shewanella putrefaciens* grown with ferrihydrite as the electron acceptor. [36] Identified "magnet-sensitive" particles in *Ectothiorhodospira shaposhnikovii*, which were rich in Fe but did not contain sulfur.

The clear stratification in populations of MTB with respect to the chemocline suggests that each type of MTB is adapted to different chemical and physical gradients within the water column. It is likely that iron and sulfur compounds play a key role in determining the population dynamics of MTB. We have sampled three different ecological samples an attempt to better determine these gradients.

contaminated soil habitat is the highest before and after the enrichment (12.02 $x10^6$ and 18.47 $x10^6$), followed by the sludge sample (9.85 $x10^6$ and 17.05 $x10^6$) and the less by the water well (8.9 $X10^6$ and 15.38 $X10^6$) which may be due to the high organic constituents in these habitats which is necessary for (MTBs) as electron acceptor during its growth and multiplication while the bacterial community of the water well sample is the less due to the limited supplies of organic nutrients.

In other studies samples collected from different seasons contained actively swimming magnetotactic cocci that ranged from 1.2 to 4 µm in diameter. Magnetotactic multicellular organisms have also been occasionally found in this environment [37]. The features of the magnetotactic cocci were those of a typical gram-negative bacterium. In this study TEM analysis revealed that the rod contained numerous mineral crystals similar to magnetosomes observed in other organisms. The crystals appear as irregularly shaped black dots in Fig. 3.

Water bacterial communities of MTB have been studied by several microscopic, cultural. Microscope observations suggested that they were most abundant when stratification was greatest (Fig. 3).

Isolation, Cultivation, and Physiology of MTB

Despite their ubiquitous occurrence and high abundance, cultivation of MTB in the laboratory has proven difficult. Problems in isolation and cultivation of these bacteria arise from their lifestyle, which is adapted to sediments and chemically stratified aquatic habitats. This can be achieved by exploiting their active migration along magnetic field lines in microscopic visualization method [38].

Growth media involving sulfide and redox gradients have proven useful in the isolation of MTB [39]. Only a limited number of MTB has been isolated in pure culture so far and most of the isolates are poorly characterized in terms of growth conditions and physiology. Examples of isolates which can be grown under laboratory conditions include several freshwater species of *Magnetospirillum* [15-16, 40- 41]. Two strains of a marine magnetic vibrio were isolated that are facultative anaerobes and can use either oxygen or nitrous oxide as terminal electron acceptors [42- 43]. The only cultivatable magnetic coccus was grown microaerobically in gradient cultures [44]. Sakaguchi *et al*. [45] isolated an obligate anaerobic, sulfate-reducing magnetotactic bacterium.

Highest numbers of MTB 10^5 -10^6/ml [16; 34] are usually found at the oxic-anoxic transition zone generally located at the sediment-water interface, which is consistent with their microaerophilic to anaerobic lifestyle.

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