A preliminary report on the isolation and identification of *Magnetotactic bacteria* from Iran environment

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Abstract
Several species of *Magnetotactic* bacteria have been discovered recently. These bacteria synthesize intracellular magnetic nanoparticles in specific sizes and shapes and arrange them in chains. These particles called magnetosomes and can be used for drug-delivery, cell-targeting and hyperthermia. *Magnetotactic* bacteria navigate along the magnetic field; this process is known as 'magnetotaxis' which may be very useful in robotic technology. In this research, two species of *magnetotactic* bacteria were isolated; freshwater specimens were collected from Karkheh River and marine specimens were collected from the Caspian Sea. After enrichment, two species of the *Magnetotactic* bacteria were isolated using a specific solid media culturing. The response of the two isolates to the magnetic field was observed by an optical microscope. SEM photos showed that the freshwater and marine bacteria are rod shaped. TEM images showed chains of magnetosomes in the bacterial cells. Also magnetic behavior of the magnetosomes was investigated by alternating-gradient-force magnetometer, indicating that the magnetosomes have superparamagnetic-to-single-domain properties.
Key words**:** *Magnetotactic* bacteria; Isolation; Characterization

INTRODUCTION

Many organisms have been identified that are able to sense the earth's magnetic field. Certain bacteria are also geomagnetically sensitive and are known as *Magnetotactic* bacteria. For the first time, in 1975, Richard Blakemore observed certain mud bacteria whose swimming direction could be manipulated by magnetic field (Schüler *et al*., 1999). The *Magnetotactic* bacteria are Gram negative, motile, aquatic and heterotrophs. They synthesize intracellular enveloped magnetic grains termed magnetosomes (Blakemore *et al*., 1982). *Magnetotactic* bacteria form chains of magnetite (Fe_3O_4) nano-crystals in microaerophilic environments. Each species of *Magnetotactic* bacteria has a special shape of magnetosomes, but almost all of the magnetosomes are on the theoretical transition lines for single-domain-to-multidomain behavior and superparamagnetic-to-single-domain behavior (Thomas-Keprta *et al*., 2000). Membrane of magnetosomes consists of a bilayer containing phospholipids and proteins (Gorby *et al*., 1988). The exact role of these magnetosome-specific proteins has not been elucidated, but it has been suggested that they have specific functions in iron accumulation, nucleation of minerals; redox and pH control (Grünberg *et al*., 2001).

Some commercial applications have been suggested for bacterial magnetosomes such as hyperthermia, magnetic targeting of pharmaceuticals, cell separation and contrast-enhancement agents in magnetic resonance imaging (MRI) (Stephens *et al*., 2006). *Magnetotactic* bacteria navigate along the magnetic field, this process is known as magnetotaxis and may be very useful in robotics. They can move through porous mediums and carry something controllably. Despite the efforts of numerous researchers through improvement of isolation methods, only a few strains of *Magnetotactic* bacteria are available in pure culture including: *Magnetospirillum magnetotacticum, M*.

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gryphiswaldense, M. magnetotacticum AMB-1 and MGT-1, Vibrios MV-1 and MV-2, coccus MC-1, Marine spirillum MV-4, and RS-1 (Li *et al*., 2007).

In this research, attempts were made to identify and isolate freshwater and marine *magnetotactic* bacteria from Iran. For this reason, the isolates were characterized by optical microscope and scanning electron microscope (SEM). Furthermore intracellular magnetosomes have been studied by transmission electron microscope (TEM) and magnetic behavior of the magnetosomes was characterized by alternating-gradient-force magnetometer (AGFM).

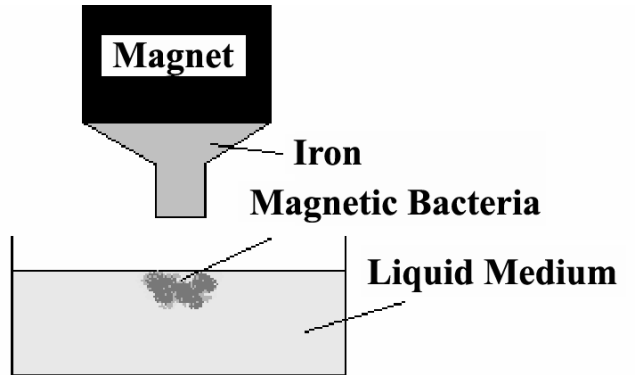

Figure 1. Collection of magnetotactic bacteria using a simple magnet.

MATERIALS AND METHODS

Culture medium: The culture medium contained 10 ml of vitamin solution, 5 ml mineral solution, 2 ml 0.01M ferric quinate, 0.45 ml 0.1% resazurin, 0.68 g KH_2PO_4 , 0.12 g $NaNO_3$, 0.37 g tartaric acid, 0.37 g succinic acid and 0.05 g sodium acetate in 1.0 l of distilled water. The pH of the medium was adjusted to 6.8 with NaOH and autoclaved at 121°C for 15 min. For preparation of solid medium, 12 g/l agar was added to the above liquid medium.

Vitamin solution contained 2 mg biotin, 2 mg folic acid, 10 mg pyridoxine hydrochloride, 5 mg thiamine.HCl, 5 mg riboflavin, 5 mg nicotinic acid, 5 mg calcium D-(+)-pantothenate, 0.1 mg vitamin B12 and 5 mg p-aminobenzoic acid in 1.0 l of distilled water. Mineral solution consisted of (g/l) 1.5 nitrilotriacetic acid, 3 $MgSO_4.7H_2O$, 0.5 $MnSO_4.H_2O$, 1 NaCl , 0.1 $FeSO_4.7H_2O$, 0.1 $CoCl_2.6H_2O$, 0.1 $CaCl_2$, 0.1 $ZnSO_4.7H_2O$, 0.01 $CuSO_4.5H_2O$, 0.01 $AlK(SO_4)_2.12H_2O$, 0.01 H_3BO_3 and 0.01 $Na_2MoO_4.2H_2O$ in distilled water. Ferric quinate (0.01M) contained 0.27 g $FeCl_3$ and 0.19 g quinic acid in 100 ml distilled water (Martins *et al*., 2007).

Isolation method: Samples with mud and water were collected from the Caspian Sea (53° 17' E, 36° 50' N) and Karkheh River (48° 2' E, 32° 50' N). They were then left undisturbed in dim light at room temperature for one month. From these samples, the *magnetotactic* bacteria were separated using a magnet as shown schematically in Figure 1. The water under the magnet, which supposed to contain the *magnetotactic* bacteria, was collected with the help of a pipette and inoculated on solid medium; the oxygen was removed by nitrogen stream and then the plates were sealed. After two days, three colonies from each plate were transferred into liquid medium and incubated at 30ºC for three weeks. Resazurin in the culture medium acts as an oxygen indicator turns pink in the presence of oxygen and colorless in its absence. When the growth medium was colorless, a small magnet was kept near the falcons to gather magnetic particles. In some falcons, it was possible to see magnetic particles. These particles might have been released from dead bacteria. Each of the samples that contained magnetic particles was supposed to have isolated *magnetotactic* bacteria.

Microscopic methods: For thin section preparation, the bacteria were grown in 2.5-liter jars, then centrifuged at 10000 g for 5 min, and washed twice with distilled water. Supernatants were discarded, and the pellet was fixed with 2% glutaraldehyde solution in water for 3 h and then postfixed in 1% osmium tetroxide. Thin sections were cut in an ultramicrotome and uranyl acetate stained samples examined with a Zeiss EM 900 (50KV) transmission electron microscope.
For SEM sample preparation, a few drops of cultured medium were dried on a glass and washed with distilled water without staining. The samples were examined with a VEGA\\TESCAN scanning electron microscope.

Sample preparation for AGFM: Since *magnetotactic* bacteria synthesize magnetosomes in microaerophilic conditions (Lang and Schüler, 2006), the isolated bacteria were incubated at 30ºC in anaerobic jars for one month till it became colorless. Then biomass of the cultured bacteria was centrifuged at 10000 g for 7 min and washed three times with distilled water. The pellet was freeze dried and used for AGFM analysis.

RESULTS

Microscopic analysis: Gram staining results indicated

that the isolated bacteria were Gram negative. SEM images, presented in Figures 2 A,B, showed the fresh-water and marine bacteria are both rod-shaped. Elemental maps of iron are shown in Figures 3 A,B. The concentrated positions of iron and chains of particles containing iron have been shown in these Figures. TEM results, shown in Figures 4 A,B, revealed that the sizes of magnetosomes are about 10 to 20 nm. Higher magnification images showed that these particles were elongated in one direction and therefore they had shape anisotropy properties. Magnetosomes have magnetic anisotropy properties and their magnetic field properties depend on the direction.

Magnetic analysis: Through optical microscopic observations and changing the position of a magnet near the samples, it was revealed that the isolated species are both South-seeking and North-seeking. In addition, AGFM analysis, Figures 5 A,B, on extracted magnetosomes showed superparamagnetic-to-single-domain behavior.

DISCUSSION

Magnetotactic bacteria are able to distinguish between south and north poles of magnet and migrate along the

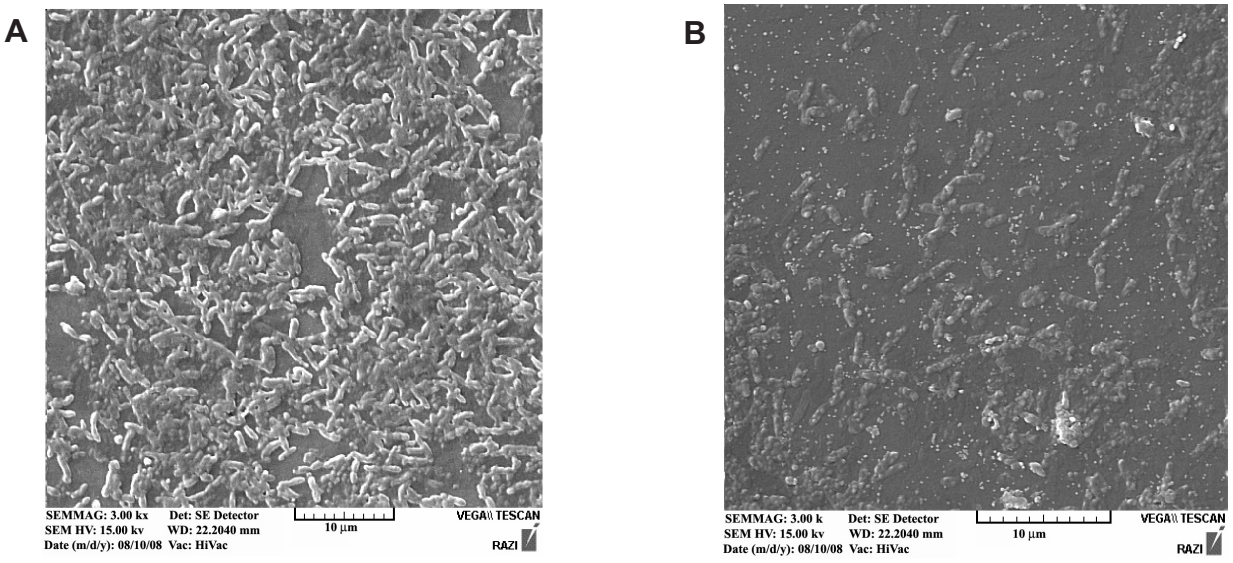

Figure 2. SEM images of magnetotactic bacteria isolated from A: Caspian sea and B: Karkheh river. Both species are rod shaped.

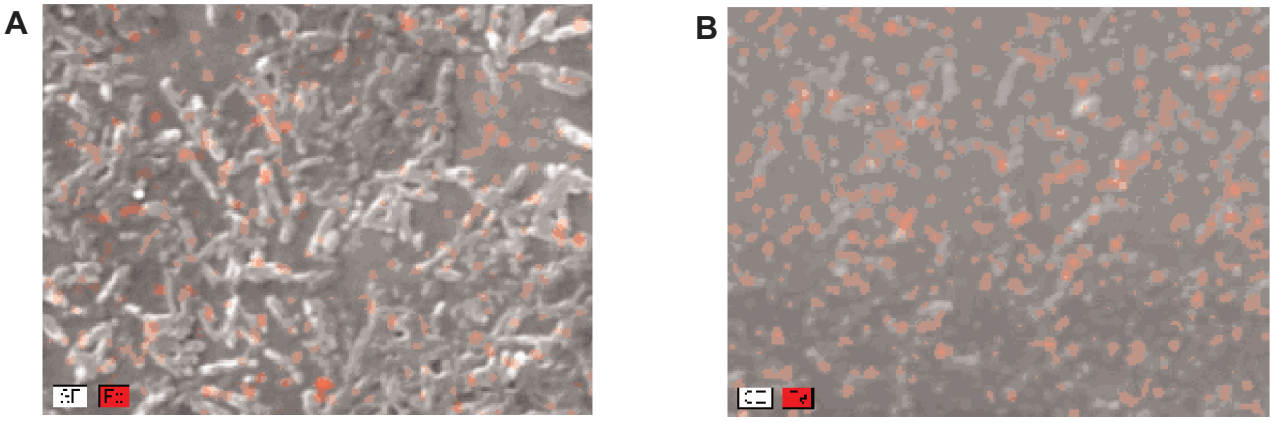

Figure 3. Elemental map of iron in isolated bacteria from A: Caspian sea and B: Karkheh river. Red chains indicate on the formation of magnetoses.

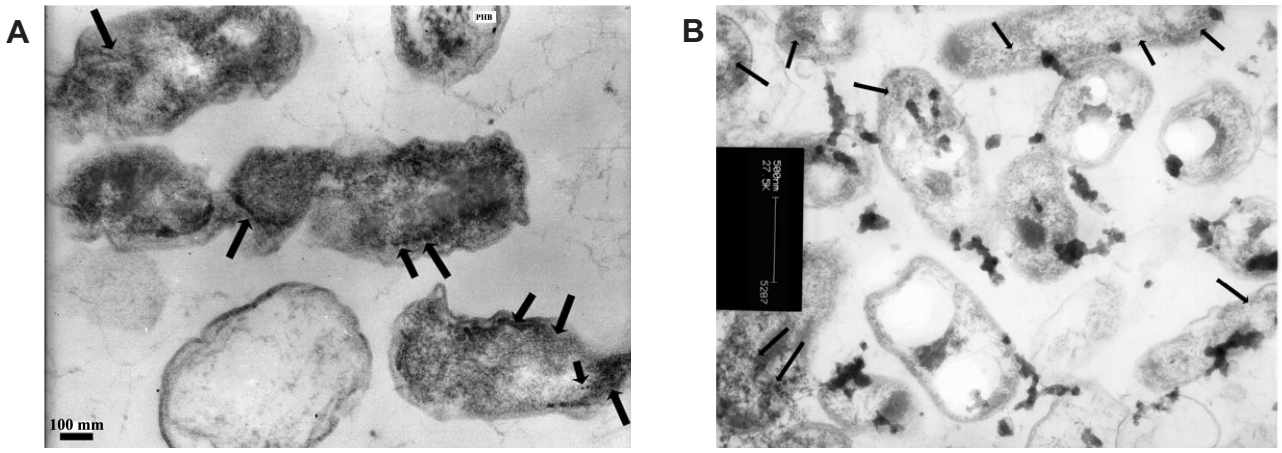

Figure 4. TEM images of magnetotactic bacteria isolated from A: Caspian sea and B: Karkheh river.The arrows point to the magnetosomes.

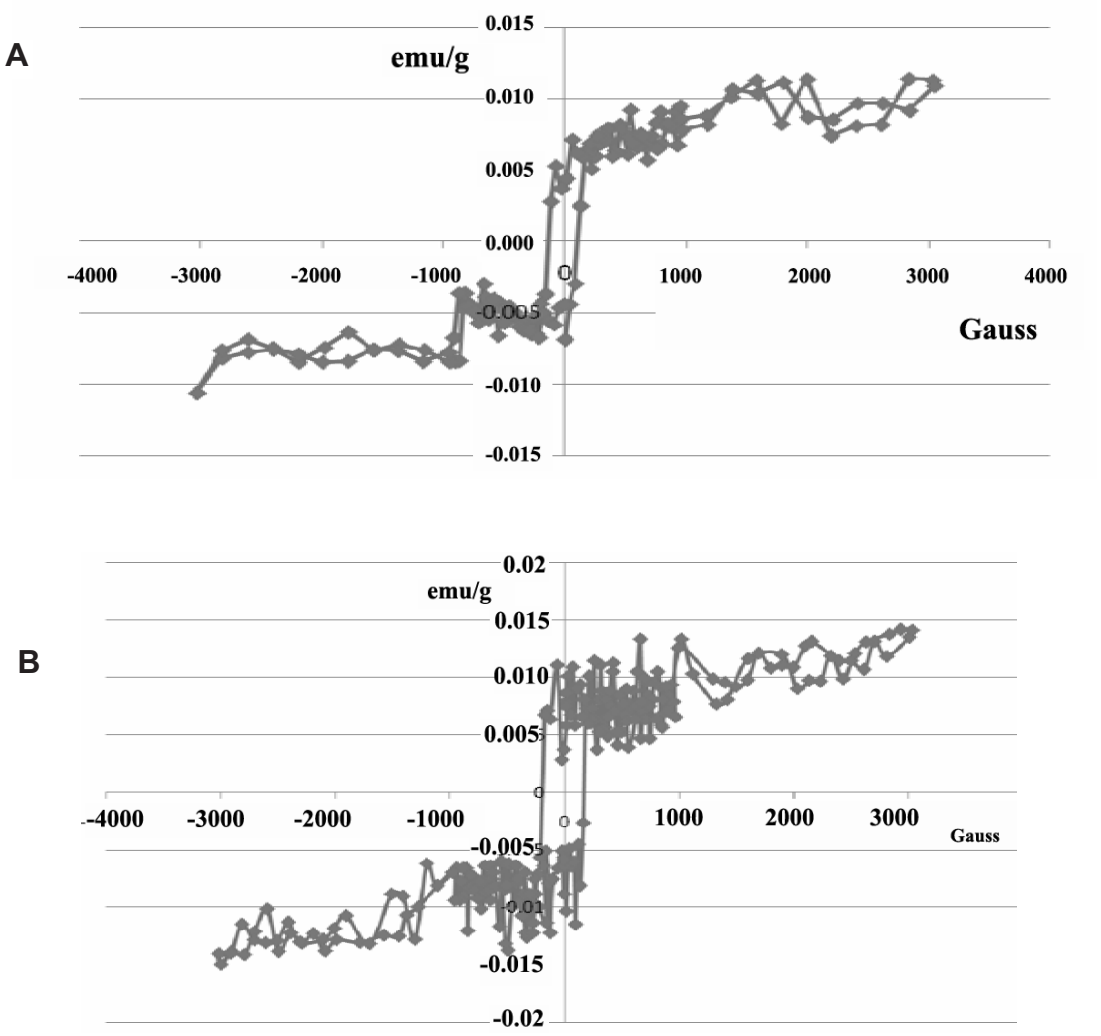

Figure 5. Magnetization curve of magnetosomes extracted from the magnetotactic bacteria isolated from A: Caspian sea and B: Karkheh river.

geomagnetic field; this behavior is known as magnetotaxis. Normally, the polarity of magnetic bacteria living in the northern hemisphere is North-seeking and South-seeking in the Southern hemisphere (Hanzlik *et al*., 2002). In addition, *magnetotactic* bacteria near the geomagnetic equator are both South-seeking and North-seeking (Frankel *et al*., 1981). The polarity of isolated bacteria was investigated and they were attracted to both poles of magnet. So, they can be categorized in the third group.

In elemental map analysis, electron beam had high energy levels, so electrons can penetrate the sample and the characterized area is bigger than the beam diagonal. This phenomenon makes the identification of the exact place and shape of magnetosomes inaccurate. Therefore, it was hard to put an accurate estimate on the dimensions of particles via elemental analysis and the images had a low resolution. Nevertheless the elemental maps of iron showed that iron rich particles were arranged in chains.

TEM images indicated the formation of magnetosomes. Shape and arrangement of these particles are accurately displayed in Figure 4. The shape and chain arrangement of these particles are evidence for synthesis of magnetosomes by the isolated bacteria.

Micron size or larger particles of magnetite at room temperature has a permanent magnetic moment but magnetite nanoparticles below a certain size have no magnetic remanence and show superparamagnetic behavior (Neuberger *et al*., 2007). In magnetic nanoparticles, thermal fluctuations cause the magnetic moment to wander relative to the crystallographic axes (Cullity and Graham, 2009). But Magnetosomes have shape anisotropy properties and the elongated directions of particles are along the easy magnetization direction of the crystals. This superposition of easy axes and elongated direction enhances anisotropy constant and therefore magnetosomes show remanence and superparamagnetic-to-single-domain behavior. Also AGFM analysis show coercivity of the samples is about 120 G and configurationally is in agreement with L. Han *et al*. (2007). These evidences imply that the isolated bacteria are *magnetotactic* and their magnetosomes reveal superparamagnetic-to-single-domain behavior.

Acknowledgments

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