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Design and application of the method for isolating magnetotactic bacteria

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Abstract A simple apparatus was designed to effectively isolate magnetotactic bacteria from soils or sediments based on their magnetotaxis. Through a series of processes including sample incubation, MTB harvesting, isolation，purification and identification, several strains of bacteria were isolated from the samples successfully. By Transmission Electron Microscopy (TEM) and Energy-Dispersive X-ray Analysis (EDXA), these bacteria were certificated to be magnetotactic bacteria. The phylogenetic relationship between the isolated magnetic strains and some known magnetotactic bacteria was inferred by the construction of phylogenetic tree based on 16SrDNA sequences. This apparatus has been proven to have the advantages of being inexpensive, simple to assemble, easy to perform and highly efficient to isolate novel magnetotactic bacteria. The research indicated that the combined approach of harvesting MTB by home-made apparatus and the method of plate colony isolation could purify and isolate magnetotactic bacteria effectively.

Key words magnetotactic bacteria; home-made apparatus; isolation; identification

1 Introduction

Magnetotactic bacteria are a general designation to a group of microorganisms which orient and migrate along geomagnetic field lines. Magnetotactic bacteria are of morphological and habitat diversity. The cells of various morphological types including cocci, short or long rods, vibrios, spirilla and multicellular aggregates (Bazylinski et al., 1994; Rodgers et al., 1990), have been found from world-wide diverse aquatic environments (including marine, river, pond, etc.), surface of marine sediments, and soil. The magnetotaxis of magnetotactic bacteria is based on specific intracellular magnetic structures, magnetosomes, which comprise nano-sized, membrane-bound crystals of magnetic iron minerals. Magnetosomes consist mainly of magnetite (Fe_3O_4) or greigite (Fe_3S_4) (Bazylinski et al., 1988; Bazylinski and Garratt-Reed, 1993). Additionally, FeS_2 and Fe_7S_8 have also been reported recently (Mann et al., 1990; Farina et al., 1990). Research on magnetotactic bacteria is of great significance, for example: as a model organism of biomagnetism (Bazylinski and Frankel, 1995) to investigate the complex biomineralization in advanced organisms (Dunn, 1995), as circumstantial evidence for ancient extraterrestrial life from a Martian meteorite (Kathie et al., 2002). Research on magnetotactic bacteria is of significance for environmental markers (Lida and Akai, 1996), for example, it has been widely concerned in geology, i.e., the contributions of magnetotactic bacteria to the stable remanence of sediments and to the magnetic susceptibility of Chinese loess-paleosol sequences (Kirschvink and Chang, 1984; Peng Xianzhi et al., 2002). Additionally, research on the correlation between magnetotactic bacteria and iron in the environment is an issue involved in biogeochemistry, which is helpful to shed light on the mechanism of tolerance of some microbes and metal-tolerant plants to heavy-metal pollution (Yang Yuangen et al., 2004; Sadaf Naseem et al., 2005). Besides the significant application of magnetotactic bacteria, magnetosomes also could widely be applied in many fields such as: material science, biology, pharmacy, electronics, optics, magnetism, and electrochemistry (Bazylinski, 2004). Therefore, to establish an effective method to isolate magnetotactic bacteria is the crucial precondition for research on and application of them. Since the discovery of magnetotactic bacteria in 1975, magnetotactic bacteria differing in morphology have been isolated by the adscititious magnetic field based on their magnetotaxis from natural environment or

enriched samples. The methods of isolating magnetotactic bacteria could be roughly divided into two groups: (1) to isolate magnetotactic bacteria with motility and intracellular magnetosomes by the adscititious magnetic field, for example: magnetic collection and "race track" purification of magnetotactic bacteria (Moench and Konetzka, 1978; Wolfe et al., 1987), or to design an apparatus to harvest magnetotactic bacteria (Ulysses et al., 2003; Wei Yangbao et al., 1994); and (2) to isolate non-motile and non- or weekly-magnetotactic bacteria as a result of environmental factors from enriched samples by colony formation, and optimization of conditions for growth and synthesis of magnetosomes (Sakaguchi et al., 1996). A sulphate-reducing magnetic anaerobe, RS-1, which is weakly magnetotactic, was isolated by this method.

The present paper reports an apparatus for harvesting magnetotactic bacteria and the method of isolating them, which have apparent advantages such as: being easy to perform, inexpensive, and effective to isolate a large number of novel magnetotactic bacteria. Several novel magnetotactic bacteria have been successfully isolated and purified by this apparatus and method.

2 Isolation, identification and phylogenetic analysis of magnetotactic bacteria

2.1 Design of the home-made apparatus for harvesting magnetotactic bacteria

This apparatus is used to enrich and isolate motile-magnetotactic bacteria. It is made up of two parts (Fig. 1), one part is the separating chamber, which is a flask used for enriching sample before the harvest procedure. The other is a sampling chamber which is refitted from tundish. At the top of the sampling chamber is a sampling hole, and there is a control piston at the joint of the two parts. The principle of the apparatus is: the arrangement of single-magnetic-domain magnetosomes in chains maximizes the dipole moment of the cell, when exposed to the magnetic field (approximately 4 Gauss) which is produced by placing the south pole of a circular magnet near the sampling hole, the motile-magnetotactic bacteria with intracellular magnetosomes would swim to the sampling chamber and could be isolated after a few hours.

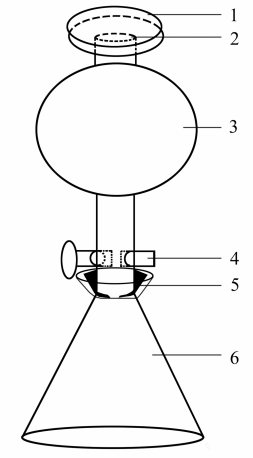

Fig. 1. Home-made apparatus for harvesting magnetotactic bacteria. 1. Magnet; 2. sampling hole; 3. sampling chamber; 4. piston; 5. rubber plug; 6. isolating chamber.

2.2 Isolation of magnetotactic bacteria

2.2.1 Sampling and enrichment

Laterite or the soil with a relatively dark color is usually selected as terrene samples. 20 g of soil were measured and put into a 300-mL flask filled with 200 mL of sterilized liquid-enriched medium (Blakemore et al., 1979), and the flask was covered by pledget and left undisturbed in dim light at room temperature (22–28℃). In this paper, we sampled a paleosol layer with high magnetic susceptibility from a Chinese loess section at Qingyang, Gansu Province. As to aquatic samples, we usually select water and sediment samples on the surface of the boundary between water and sediment at about 1-m depth. In this paper, one liter of sample was collected at 1-m depth from a freshwater pond in Nanjing. Sediment and water in a proportion of approximately 1:2 were stored in loosely capped bottles, and left to be undisturbed for 1 month under dim light at room temperature (22–28℃).

2.2.2 Magnetic harvesting of magnetotactic bacteria

About 80 mL of sterilized enriched medium were taken to sterilize the sampling chamber of the home-made apparatus (Fig. 1), a sterilized filter paper was put at the joint of the sampling chamber and the separating chamber, the two chambers were joined by a rubber plug, and the piston was opened up to connect the water column in the two chambers of the apparatus. A magnet was put at the top of the apparatus with its south pole pointing downwards. Meanwhile, two control experiments were made with the north pole pointing downwards with no magnet. Under the effect of the magnetic field, magnetotactic bacteria swam to the south pole of the magnet and penetrated the filter paper to enter the sampling chamber. The piston was closed after 3 hours, and then the harvested sample containing magnetotactic bacteria was extracted by means of a sterilized injector from the sampling hole. The harvested sample was transferred to a 150-mL sterilized flask and

cultured at 100 r/min at 30℃ to enrich magnetotactic bacteria secondarily.

2.2.3 Isolation of magnetotactic bacteria

This paper aimed at isolating oxygen-tolerant magnetotactic bacteria. The enriched solid medium was used and the magnetotactic bacteria were isolated and purified in Petri dishes by the method of agar plate colony isolation from the collected liquor at 28℃ (Blakemore et al., 1979).

2.2.4 Observation of magnetotactic bacteria under electron microscope and by energy-dispersive X-ray analysis (EDXA)

2.2.4.1 Observation of the harvested sample One drop of the harvested sample was deposited on a grid. The drop was placed partially covering the grid surface. A magnet was positioned on the opposite side of the drop with an alignment of the magnetic field in such a way that the bacteria would swim to the side close to the middle of the grid (Ulysses et al., 2003). In about 6 minutes, the bacteria were accumulated on the drop edge. One drop of distilled water was deposited by the side of the drop that did not cover the grid, and then the grid was air-dried. Usually through these procedures, the bacteria could spread evenly in some grid fields. The specimens were viewed under a HITACHI H600A-2 transmission electron microscope (TEM) operating at voltages of 75 kV.

2.2.4.2 Observation of isolated bacteria under TEM

Purified cells of the isolated magnetotactic bacteria were prepared by centrifugation their liquid culture or scraping their colony formed on agar plate, and then the pure cells were diluted by sterilized phosphate buffer solution (pH=6.7，10 mM). The specimen was dropped directly to a grid without immobility or negative stain, the grid was air-dried, and then viewed under TEM operating at voltages of 75 kV.

2.2.4.3 Observation under Scanning Electron Microscope (SEM) and Energy-Dispersive X-ray Analysis (EDXA) of isolated bacteria Samples prepared by the method described in 2.2.4.2 were placed onto the surface of double-faced tape attached to a copper stub and then the specimens were air-dried. A coating of gold was vacuum-deposited on the specimens, which were then viewed under a JSM-5610LV SEM operating at voltages of 15 kV.

Qualitative elemental composition of the magnetosome region and magnetosome-free region in isolated magnetotactic bacterial cells was determined by using VANTAGE energy-dispersive X-ray analysis system coupled to the SEM.

2.3 Phylogenetic relationships between the two new magnetotactic bacteria and other known magnetic strains

2.3.1 Cultivation of strains

The two presently isolated magnetotactic bacteria MG-1 and MG-2 were cultured in a liquid growth medium to exponential phase (Blakemore et al., 1979).

2.3.2 Amplification and sequencing of 16SrDNA

Bacterial genomic DNA was extracted and purified from each strain by Genomic DNA isolation Kit K713 (Shenergy Biocolor). 16SrDNA of MG-1 and MG-2 were amplified in a reaction containing 1.5 U of *Thermus aquaticum* DNA polymerase (Promega, Madison, Wis.), 10 nmol of each deoxynucleoside triphosphate, 5 μL of 10×PCR buffer (Promega), 75 nmol of $MgCl_2$, and 50 pmol of each universal primer (Ksiazek et al., 2003; Peiris et al., 2003). For the PCR template, 4 μL of extracted DNA was added. The reaction volume was made up to 50 μL with sterile distilled water. A thermal cycler (PTC-100) was used, with 5 min of initial denaturation at 94℃, 35 cycles of 1 min at 94℃, 1 min at 47℃, and 1.5 min at 72℃, and a 10 min extension to the last cycle at 72℃. PCR products were electrophoresed at 10 Vcm^{-1} in 1% agarose gel in Tris-borate-EDTA buffer, stained in ethidium bromide solution, and photographed under UV illumination. PCR products were sent for sequencing if they are single bands confirmed by agarose gel electrophoresis.

2.3.3 Phylogenetic analysis of 16SrDNA sequences

Six published 16SrDNA sequences of magnetotactic bacteria were selected from GenBank: *Magnetospirillum magneticum* MGT-1, *M.magneticum* AMB-1, *M.magnetotacticum* MS-1, *Desulfovibrio magneticus* RS-1, *Magnetobacterium bavaricum* and iron sulfide containing magnetotactic bacterium MMP1991. In addition, eight other bacterial 16SrDNA sequences were selected from GenBank in order to infer the phylogenetic position of the two new magnetotactic bacteria in relation to other Proteobacteria, such as: *Rhodospirillum molischianum*, *Agrobacterium tumefaciens*, *Rhodobacter sphaeroides*, *Pseudomonas testosteron*e, Uncultured *gamma proteobacterium* SP_A2，*Desulfovibrio desulfuricans*, *Leptospirillum ferrooxidans* CF12 and *Anacystis nidulans*.

The 16SrDNA sequences obtained were aligned

with other 16SrDNA sequences on the basis of secondary structure and conserved sequence. Multiple alignments of the sixteen 16SrDNA sequences were carried out on a computer using the ClustalX (1.8) algorithm and fine-tuned manually. Phylogenetic trees were constructed from evolutionary distance data by the neighbor joining method.

3 Results and discussion

3.1 Enrichment and harvesting of magnetotactic bacteria by the home-made apparatus

After one month of enrichment, brown coating and deposits appeared on the surface of sample and sediment. This is inferred to as the influence from a mass of enriched magnetotactic bacteria and dissociative magnetosomes after autolysis of cells, consistent with previous report (Wei Yangbao et al., 1994).

The bacteria in harvested samples are mostly bacillus and cocci under TEM. Most bacteria contain 3–8 of intracellular electron dense particles (Fig. 2). The arrangement of these particles is in a single chain along the long-axis of the most cells (Fig. 2a, b, c), as well as in a cluster in minor cells (Fig. 2d). The dense particles are about 80 nm in diameter when measured along their long axes, which is consistent with the earlier report (Bazylinski and Frankel, 1995).

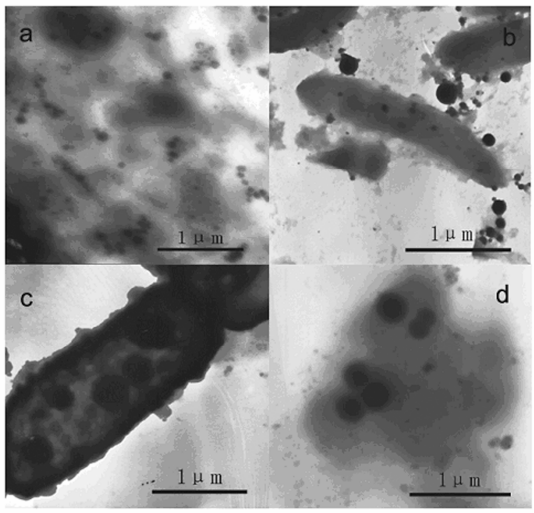

Fig. 2. TEM images of some harvested MTB. a. A group of MTB with various cell morphologies; b. a vibrio; c. a rod; d. several cocci.

In harvesting procedure, we set two control experiments: with a magnet and without a magnet with N pole downwards the apparatus. The result of control experiment showed that there are no bacteria or electron dense particles as shown above. This suggests that some bacteria with magnetotaxis swam to the S pole of the adscititious magnetic field and reached the sampling chamber.

3.2 Isolation and purification of magnetotactic bacteria

Through isolating single colony on agar plate aerobically from the harvested samples, three novel oxygen tolerant magnetotactic bacteria were purified successfully, named MG-1, MG-2, and MG-3, respectively (Fig. 3). After cultivation for 4 days, TEM results showed that all cells of the three strains contain 3–8 of electron dense particles with a section of being circular-like and a diameter of about 80 nm (Fig. 3).

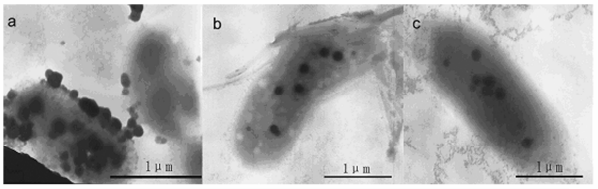

Fig. 3. TEM images of the three purified MTB. a. IMG-1; b. MG-2; c. MG-3.

3.3 Observation under SEM and EDXA of magnetotactic bacteria MG-1

Strain MG-1 was selected for further research. MG-1 is rod-shaped, with a size of (0.4–0.7) μm×(1.1–1.5) μm as observed under SEM (Fig. 4).

Figure 5 shows the elemental composition of the magnetosome region and magnetosome-free region in a cell of MG-1 by EDXA. In magnetosome region of the cell (Fig. 5a), except for C, O, P, Ca, the main metallic element is Fe, and its content accounts for 10.23% of the total elemental composition of the cell, in addition, the content of Co is 1.54% and Ni is 0.53%. However, the content of Fe is merely 0.43% while Co is 0.21% and no Ni is present in

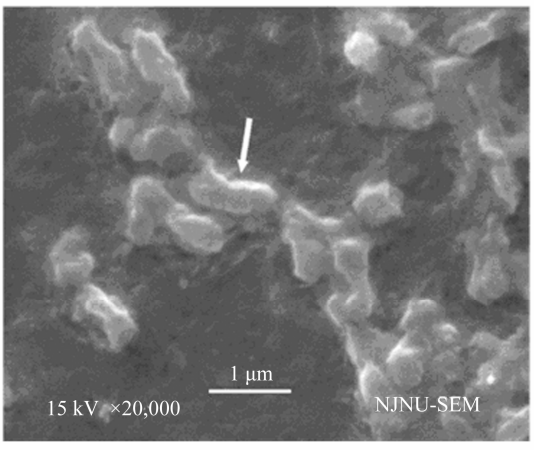

Fig. 4. SEM image of MG-1.

magnetosome-free region of the cell (Fig. 5b). This indicates that the main elemental composition of the magnetosomes of MG-1 is Fe, while a small quantity of Co and Ni exists. All these three elements are significant constituents of magnetic materials.

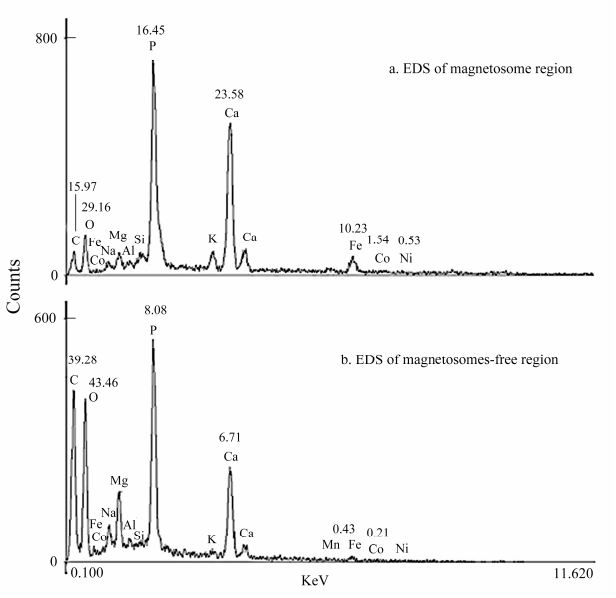

Fig.5. Energy-dispersive X-ray spectra of a cell of MG-1. a. Spectrum obtained from magnetosome region of a cell; b. spectrum obtained from magnetosome-free region of a cell.

3.4 Phylogenetic analysis of 16S rDNA sequences

Amplified and sequenced 16S rDNA of the two new strains of magnetotactic bacteria MG-1 and MG-2, as well as the 1417bp and 1414bp sequences were obtained, respectively. The two sequences were aligned with six published sequences of magnetotactic bacteria and eight other bacterial 16S rDNA sequences which represent members of each subclass of the *Proteobacteria*. Phylogenetic analysis was carried out after 16 bacterial sequences were aligned, and the phylogenetic tree is constructed by the neighbor joining method (Fig. 6).

According to literature (Spring and Scheifer, 1995), we designate magnetotactic bacteria into α-subclass, β-subclass, γ-subclass, and δ-subclass of the *Proteobacteria*, *Cyanobacteria* phylum and *Nitrospira* phylum. Since the majority of the present magnetotactic bacteria are proven to be affiliated with α-subclass of *Proteobacteria* (Bazylinski and Frankel, 2000; DeLong et al., 1993), we divide α-subclass into α-1 subgroup, α-2 subgroup and α-3 subgroup according to literature (Burgess et al., 1993), so as to further position magnetotactic bacteria within the α-subclass. As is shown in Fig. 6, among the four strains of magnetotactic bacteria with magnetosomes of Fe_3O_4, three (AMB-1, MGT-1, MS-1) are affiliated with the α-1 subgroup of the α-subclass of *Proteobacteria* while a magnetic sulfate-reducing bacterium RS-1 belonged to the δ-subclass. A magnetotactic, many-celled procaryote MMP1991 with magnetosomes of Fe_3S_4 and a magnetic sulfate-reducing bacterium RS-1 with magnetosomes of Fe_3O_4 both belong to the δ-subclass of *Proteobacteria*. A new magnetotactic bacterium MG-1 is closely related to an uncultured *gamma proteobacterium* SP_A2, and both of them are affiliated with the γ-subclass of *Proteobacteria*. The other new magnetotactic bacterium MG-2 and a morphologically distinct large magnetic rod *Magnetobacterium bavaricum* were assigned to the

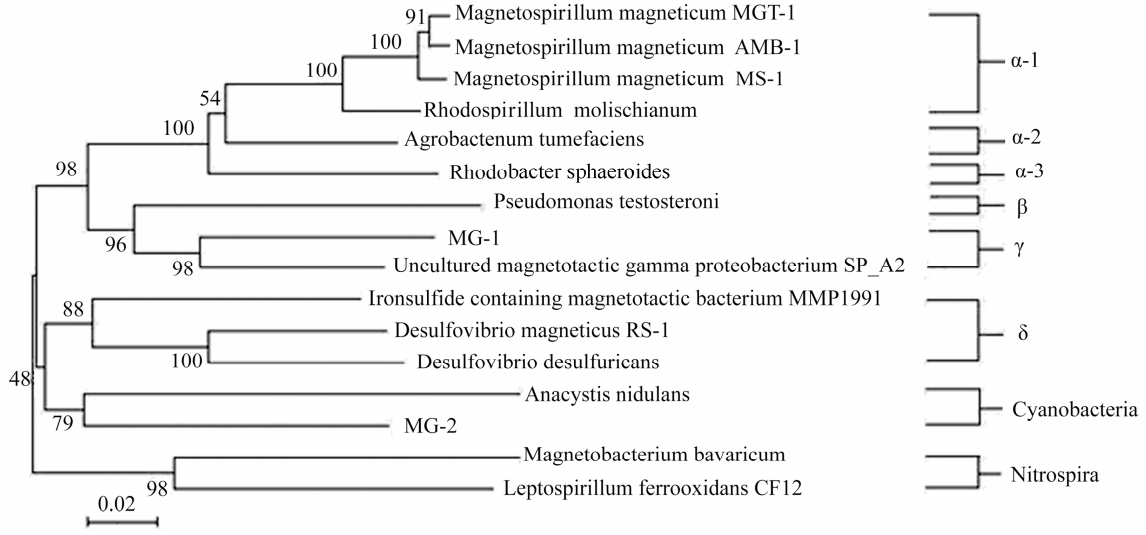

Fig. 6. Phylogenetic tree showing the positions of the new magnetic strains MG-1 and MG-2 in relation to representative Proteobacteria. *A. nidulans* and *L. ferrooxidans* on the basis of comparative sequence analysis of 16SrRNA genes; Bar = 2% estimated sequence divergence.

Cyanobacteria phylum and *Nitrospira* phylum, respectively.

Previously DeLong considered that magnetotactic bacteria with magnetosomes of Fe_3O_4 are related to the α-subclass of *Proteobacteria* (DeLong et al., 1993), while magnetotactic bacteria with magnetosomes of Fe_3S_4 are related to sulfate-reducing bacteria of the δ-subclass of *Proteobacteria*, and the ability to form magnetosomes has been evolved from separate evolutionary origins. However, from the phylogenetic tree in Fig. 6, we could see that not all the magnetotactic bacteria with magnetosomes of Fe_3O_4 belong to the α-subclass of *Proteobacteria*. For example, a magnetic sulfate-reducing bacterium RS-1 with magnetosomes of Fe_3O_4 is affiliated with the δ-subclass of *Proteobacteria*, and *Magnetobacterium bavaricum* with magnetosomes of Fe_3O_4 is assigned to the *Nitrospira* phylum. This means that the phylogenetic origin of magnetotactic bacteria is of magnetotaxis which as a characteristic feature may has been multi-evolved. There is no genus particularity of magnetotaxis to bacteria. It is proven through the novel magnetotactic bacteria with phylogenetic diversity that the home-made apparatus could harvest and isolate novel magnetotactic bacteria efficiently.

4 Conclusions

Several strains of magnetotactic bacteria were harvested successfully by the home-made apparatus. Through aerobical plate colony isolation, we obtained three strains of pure oxygen tolerant magnetotactic bacteria MG-1, MG-2, and MG-3. This apparatus has many unique advantages compared with the similar domestic and foreign apparatuses reported so far, for example: (1) low-cost, simple to assemble, and easy to implement; (2) the flask for enrichment directly links with the separating chamber of the apparatus, which significantly simplifies the working procedures; (3) the sampling chamber is filled with enrichment medium to balance the concentrations of solution in two chambers of the apparatus, so as to reduce the influence on the swimming of bacteria caused by molecule diffusion; (4) the enrichment medium in the sampling chamber could enrich a few harvested bacteria secondarily; (5) high efficiency as evidenced by the fact that we isolated three strains successfully after merely 3 hours of harvesting in this paper; (6) the isolated magnetotactic bacteria are of novelty on morphology and phylogenesis through comparing their phylogenetic relationship with other known magnetic strains. However, our apparatus has the deficiency that depends on the magnetotaxisto to retrieve a large number of magnetic bacteria. There are non-motile and non- or weekly-magnetotactic bacteria as a result of environmental factors in natural environments (Sakaguchi et al., 1996; Schuler and Frankel, 1999). So it relies on the method that does not use magnetotaxis to retrieve magnetic bacteria (Sakaguchi et al., 1996), which consists of several steps that include incubation of sediments, enrichment of bacteria in the medium and isolation by colony formation. Although our approach may not retrieve all possible forms of magnetotactic microorganisms, we believe that both methods are complementary and can be used in different situations or for different purposes, and it is proven to be easy to use and retrieve a large number of novel magnetotactic bacteria efficiently by our apparatus and method.

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References

Bazylinski D.A. (2004) Magnetosome formation in prokaryotes [J]. *Nature Reviews Microbiology*. **2**, 217–230.

Bazylinski D.A. and Frankel R.B. (1995) Controlled biomineralization of magnetite (Fe_3O_4) and greigite (Fe_3S_4) in a magnetotactic bacterium [J]. *Applied and Environmental Microbiology*. **61**, 3232–3239.

Bazylinski D.A. and Frankel R.B. (1995) Controlled biomineralization of magnetite (Fe_3O_4) and greigite (Fe_3S_4) in a magnetotactic bacterium [J]. *Applied and Environmental Microbiology*. **61**, 3232–3239.

Bazylinski D.A. and Frankel R.B. (2000) *Environmental Microbe-Mineral Interactions* [M]. pp.109–144. ASM Press, Washington DC.

Bazylinski D.A. and Garratt-Reed A.J. (1993) Copper association with iron sulfide magnetosomes in a magnetotactic bacterium [J]. *Arch. Microbiol*. **160**, 35–42.

Bazylinski D.A., Frankel R.B., Garratt-Reed A.J., and Mann S. (1988) Anaerobic Production of magnetite by a marine magnetotactic bacterium [J]. *Nature*. **334**, 518–519.

Bazylinski D.A., Garratt-Reed A., and Frankel R.B. (1994) Electron-microscopic studies of magnetosomes in magnetotactic bacteria [J]. *Microscopy Research Technology*. **27**, 389–401.

Blakemore R.P., Maratea D., and Wolfe R.S. (1979) Isolation and pure culture of a freshwater magnetic spirillum in chemically defined medium [J]. *J. Bacteriol*. **14**, 720–729.

Burgess G.J., Kawaguchi R., Sakaguchi T., Richard H., and Matsunaga T. (1993) Evolutionary relationships among magnetospirillum strains inferred from phylogenetic analysis of 16SrDNA sequences [J]. *Journal of Bacteriology*. **20**, 6689–6694.

DeLong E.F., Frankel R.B., and Bazylinski D.A. (1993) Multiple evolutionary origins of magnetotaxis in bacteria [J]. *Science*. **259**, 803–806.

Dunn J.R. (1995) Magnetic Material in the Human Hippocampus [J]. *Brain Research Bulletin*. **36**, 149–153.

Farina M., Esquivel D.M.S., and Lins de Barros H.G.P. (1990) Magnetic iron-sulphur crystals from a magnetotactic microorganism [J]. *Nature*. **343**, 256–258.

Kathie L., Thomas-Keprta, and Simon J. (2002) Magnetofossils from Ancient Mars: A Robust Biosignature in the Martian Meteorite ALH84001 [J]. *Applied and Environmental Microbiology*. **68**, 3663–3672.

Kirschvink J.L. and Chang S.R. (1984) Ultrafine-grained magnetite in deep-sea sediments: Possible bacterial magnetofossils [J]. *Geology*. **12**, 559–562.

Ksiazek T.G., Erdman D., and Goldsmith C.S. (2003) A novel Coronavirus associated with severe acute respiratory syndrome [J]. *The New England Journal of Medicine*. **348**, 53–66.

Lida A. and Akai J. (1996) TEM study on magnetotactic bacteria and contained magnetite grains as biogenic minerals, mainly from Hokuriku-Niigata region [J]. *Japan, Sci. Rep. Niigata Univ, Ser. E (Geology)*. **11**, 43–66.

Mann S., Sparks N.C.H., Frankel R.B., Bazylinski D.A., and Jannasch H.W. (1990) Biomineralization of ferrimagnetic greigite (Fe_3S_4) and iron pyrite (FeS_2) in a magnetotactic bacterium [J]. *Nature*. **343**, 258–261.

Moench T.T. and Konetzka W.A. (1978) A novel method for the isolation and study of a magnetotactic bacterium [J]. *Arch. Microbiol*. **119**, 203–212.

Peiris J.S. M., Lai S.T., and Poom L.L.M. (2003) Coronavirus as a possible cause of severe acute respiratory syndrome [J]. *The Lancet*. **361**, 1319–1325.

Peng Xianzhi, Jia Rongfen, Li Rongsen, Dai, Shunying, and Liu Tungshen (2002) Simulation experiments of MB and MSs contributions to the magnetic susceptibility of Chinese loess-paleosol sequences [J]. *Quaternary Sciences*. **22**, 188-194 (in Chinese with English abstract).

Rodgers F.G., Blakemore R.P., and Blakemore N. (1990) Intercellular connections in a many-celled magnetotactic prokaryote [J]. *Archives of Microbiology*. **154**, 18–22.

Sadaf Naseem, Shahid Naseem, Erum Bashir, Khaula Shirin, and Shamin A. Sheikh (2005) Biogeochemical evaluation of *Nannorrhops ritchiana* A Mg-flora from Khuzdar, Balochistan, Pakistan [J]. *Chinese Journal of Geochemistry*. **24**, 327–337.

Sakaguchi T., Tsujimura N., and Matsunaga T. (1996) A noval method for isolation of magnetic bacteria without magnetic collection using magnetotaxis [J]. *J. Microbiol. Meth*. **26**, 139–145.

Schuler D. and Frankel R.B. (1999) Bacterial magnetosomes: Microbiology, biomineralization and biotechnological applications [J]. *Appl. Microbiol. Biotechnol*. **52**, 464–473.

Spring S. and Scheifer K.L. (1995) Diversity of magnetotactic bacteria [J]. *System. Appl. Microbiol*. **18**, 147–153

Ulysses Lins, Flavia Freitas, and Carolina Neumann Keim (2003) Simple homemade apparatus for harvesting uncultured magnetotactic microorganisms [J]. *Brazilian Journal of Microbiology*. **34**, 111–116.

Wei Yangbao, Zhang Hongxia, Jiang Wei, and Qiu Jinfeng (1994) Morphogical characteristics of magnetotactic bacterium from the east Lake in Wuchang and isolation of its strain WD-1 [J]. *Journal of Wuhan University*. **6**, 115–120 (in Chinese with English abstract).

Wolfe R.S., Thauer R.K., and Pfennig N. (1987) A capillary racetrack method for isolation of magnetotactic bacteria [J]. *FEMS Microbiol. Ecol*. **45**, 31–36.

Yang Yuangen, Liu Congqiang, Xu Lei, Wu Pan, and Zhang Guoping (2004) Effects of heavy metal contamination on microbial biomass and community structure in soils [J]. *Chinese Journal of Geochemistry*. **23**, 319–328.