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Magnetotactic Bacteria and their Application in Medicine

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Abstract

It is a known fact how the magnetic field of the Earth is very important for life. Relation between living systems and the earth magnetic field has been investigated for many years. Birds and their migration routes are the first one of the things that comes to mind when we state living things. The Earth's magnetic field is still accepted to be the main factor for birds and other flying living beings to complete their travels correctly. The changes in migration routes, which are observed from time to time, are sometimes said to be due to the changes in the magnetic field. However, no light has been shed to this matter yet.

The Earth's magnetic field has not been sufficiently studied, and its role on small living models such as bacteria has not been adequately discussed. One of the best examples in this field is relation between the Earth's magnetic field and "magnetotactic bacteria (MTB)", which were discovered by Salvatore Bellini in 1963. Currently, it is claimed that magnetotactic bacteria have a widespread use in microbiology, mineralogy, limnology, physics, biophysics, chemistry, biochemistry, geology, crystallography, and astrobiology. Although the importance of this issue magnetic bacteria is still limitedly used in medical area. Therefore more performance is necessary to explore world of this bacteria that is candidate for new therapy strategies in the medical field. Because of the reason stated here and to attract the attention of scientists to this topic this short review has been prepared.

Keywords: The Earth's magnetic field; Bacteria; Magnetotactic bacteria; Medicine

Introduction

Magnetic bacteria, which is known as magnetotactic bacteria (MTB) can produce nanosized and membrane-bound magnetite particles with regular morphology and size within the single-domain region under mild conditions at normal temperature and pressure. In other words, magnetic bacteria synthesize nano bacterial magnetic particles (BMPs), which offer high technological potential as they can be conveniently manipulated with magnetic force. BMPs are ultrafine magnetite crystals (50-100 nm diameters) of regular morphology produced by *Magnetospirillum magneticum AMB-1*. Magnetic structures aligned in chains are thought to function as biological compass needles allowing the bacterium to migrate along redox gradients through the Earth's geomagnetic field lines. Despite the discovery of this unique group of microorganisms several decades ago, the mechanisms of magnetic crystal bio mineralization have not been fully elucidated yet [1].

Magnetotactic bacteria (MTB) are a morphologically, metabolically, and phylogenetically diverse group of prokaryotes. Magnetotactic bacteria (MTB) are a gram negative prokaryote bacteria group that moves according to the Earth's magnetic field lines and can orient, migrate along geomagnetic field lines because of their intracellular single-domain magnetic nano-crystal particles with bio membrane bounded, which are referred as magnetosomes [2-4].

Biologically, MTB are prokaryotic microorganisms living in water. They move by the propulsion of their helix-shaped flagella. Another microbiological fact that makes them interesting is, although they are bigger and have less protein in their flagella, they can move about twice as fast as *Escherichia coli* [5]. MTB's different strains show different morphological properties and directions, possibly due to magnetosome order [6].

Various morphologies of MTB, such as rods, vibrio, spiral, cocco bacteria and multicellular bacteria were found in freshwater residues [7,8]. It is known that MTB produce two minerals such as iron oxide and iron sulfide [9]. Some produce Fe_3O_4 (magnetite) others produce Fe_3S_4 (greigite) [10,11], some produce both [12]. MTBs in freshwaters produce only Fe_3O_4. MTBs in lake and saltwater ecosystems produce both magnetite and greigite [13].

Development of new and fast techniques in biology and electron microscopy have revealed the presence of various microorganisms capable of producing inorganic fine crystals in the intracellular depositions or as the extracellular frames. Calcium carbonate and calcium phosphate elemental selenium, iron oxides (magnetite) and so on can be synthesized through biological processes. Some of these crystals are formed with regular morphologies at specific locations in a highly controlled manner. As it is stated above, one of these crystals is magnetite and it is one of the fine particles produced by biological processes. However, it has been using in practical applications such as a magnetic carrier in bioassay, magnetic controlling device in precision machine and memory or marker device in various electronic appliances.

Presence of magnetic bacteria is pioneer to bring new manufacturing method for production of fine-grade magnetite and new application areas including medicine. For instance, the successful isolation, cultivation and colony formation of magnetic bacteria, *Magnetospiriilum sp. AMB-1* and *MGT-1*, which are tolerant to oxygen, have brought to develop the conjugative gene transfer and transposon mutagenesis systems and to promote their metabolic and phylogenetic investigations. Although pure culture of MTB had been hardly achieved little was known about how magnetic bacteria synthesize bacterial magnetic particles (BMPs) at molecular level [14].

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The Earth's Magnetic Field

The Earth can be considered as a large magnet surrounded by a magnetic field. It is thought that the Earth's magnetic field is caused by convection flows (vertical movement caused by the temperature differences in the inner and outer layer) in the liquid crust. The outer core is the area between the inner core and the mantle, which is 1260 km thick and is made of iron and nickel. While the temperature in the outer parts of the outer mantle is 4400ºC, the temperature goes up to 6100ºC in the parts close to the inner core. The regular displacement of the atoms in a sufficient power causes the forming of magnetic fields, magnetism in other words, and this causes a permanent magnetism around the Earth. It is also thought that the eddy flows in liquid iron and nickel affects the Earth's magnetic field. The Earth's magnetic field is like a dipole magnet that has north and south poles. However, there is an 11º difference between the Earth's rotation axis and the dipole magnet's rotation. This means that the geographical and magnetic poles are different. It is reported that the Earth's magnetic field changes between 15-microtesla and 65 μT (microtesla) [15].

History and Properties of Magnetotactic Bacteria

These remarkable bacteria were first discovered by Salvatore Bellini in 1962 [16]. Bellini noticed that a group of bacteria swam towards the direction of the North Pole and dubbed this bacteria group "magneto sensitive bacteria". In 1975, Blakemore named these bacteria "magnetotactic bacteria" by deriving from the biological term "magneto taxis" [17]. The discovery of MTB caused a significant impact on research fields such as microbiology, physics, biophysics, chemistry, biochemistry, geology, limnology, crystallography, and even in astrobiology [18]. Although they can be found in any environment, especially in lake and seawater habitats, due to their unique life, it is not easy to isolate and cultivate MTB [19]. However, the fast advances in biotechnology, magneto technology and in other fields have eased the cultivation of MTB in laboratories, and sped up the researches [17].

Magnetotactic bacteria, described by Richard Blakemore, have long intrigued researchers because they synthesize intracellular nano-scale (40-100 nm) magnetic particles composed of Fe_3O_4, termed magnetosomes. It is reported that the extensively studied strains of magnetotactic bacteria include *Magnetospirillum gryphiswaldense MSR-1*, *M. magnetotacticum MS-1*, *M. Magneticum AMB*-1, *Magnetococcus sp. MC-1*, and *magneto-ovoid strain MO-1*. Interestingly, a variety of higher organisms, including bees, algae, pigeons, eels, and humans, are also capable of synthesizing intracellular magnetite. The formation and physiological function of magnetic crystals in these organisms are still not known sufficiently. However, thorough understanding of bacterial magnetosome formation will serve as a model to uncover the mechanism of magnetosome formation and function in other species. It is stated that current research focuses on the molecular mechanisms of bacterial magnetosome formation and its practical applications in biotechnology and medicine. It is also noted that functional analysis of several genes involved in magnetosome formation, e.g., mamJ, mamK, magA have revealed the roles of membrane associated proteins in transport and bio mineralization processes required for the installation of magnetosomes [20].

Magnetosome and Magnetite

Magnetosomes are a type of vesicles that is found in the cell. This structure which is surrounded by a membrane and consists of inorganic crystals containing magnetic iron is vital for MTB [4]. Magnetosomes are vesicles that are in a membrane within the cytoplasm that is full of magnetite and greigite, which help the bacteria align themselves according to the Earth's magnetic field. *Magnetospirillum magneticum* and *Magnetospirillum gryphiswaldense*, which have approximately 15-20 magnetosomes along the cell's center line aligned horizontally are magnetotactic organisms that have been researched the most. This line-up along the central line creates a structure similar to a compass needle that has the magnetic moment that is equal to the sum of each magnetosome's magnetic moment. Electron chromatographic analysis shows that there is a filament that accompanies the magnetosome chains, and that the magnetosomes are connected to this filament by acidic MamJ proteins [21] (Figure 1). In various electron microscopes, magnetosome crystals of various shapes were observed in MTB, such as cubic octagonal, bullet-shaped, prismatic and rectangular [4].

Each magnetosome consists of a magnetite (Fe_3O_4) or greigite (Fe_3S_4) crystal enveloped by a lipid bilayer membrane derived from the cytoplasmic membrane [9]. Magnetosomes are generally organized in linear chains and orient the cell body along geomagnetic field lines while flagella actively propel the cells, resulting in so-called magnetotaxis [9,22].

Magnetite (Fe_3O_4) is the main chemical component of magnetosomes characterized by the high chemical purity, fine grain size uniformity, and good biocompatibility, which can be used as a new kind of nano-magnetic materials applied in many fields of biochemistry, magnetic materials, clinical medicine and wastewater treatment etc. Magnetosome formation is the mineralization process under strict biochemical mechanisms control, including four steps: iron accumulation, membrane formation, transportation and controlled bio mineralization of Fe_3O_4 [2-4]. Magnetosomes are generally organized in linear chains and orient the cell body along geomagnetic field lines while flagella actively propel the cells, resulting in so-called magnetotaxis [9,22].

Behavior of Magnetosomes in East and North Hemisphere of Earth

Magnetotactic bacteria, which are affected by the Earth's magnetic field face north in the northern hemisphere, and face south in the southern hemisphere [3] (Figure 2).

MTB from the Southern Hemisphere swim antiparallel to the vertical component of the geomagnetic field toward the South and are termed South-seeking MTB (SS-MTB). In contrast, MTB from the Northern Hemisphere swim parallel to the vertical component of the geomagnetic field lines and are pre- dominantly North-seeking (NS-MTB) [23]. The inclination of the geomagnetic field lines is believed to direct cells downwards away from toxic concentrations of oxygen in

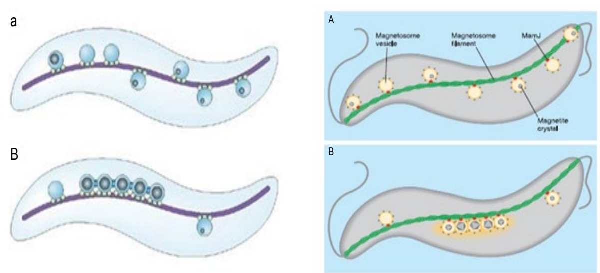

Figure 1: Magnetosomes and their alignment on the filament (Source: Nature Reviews/Microbiology and American Society for Microbiology, http://forms.asm.org/micr obe/index.asp?bid=63469)

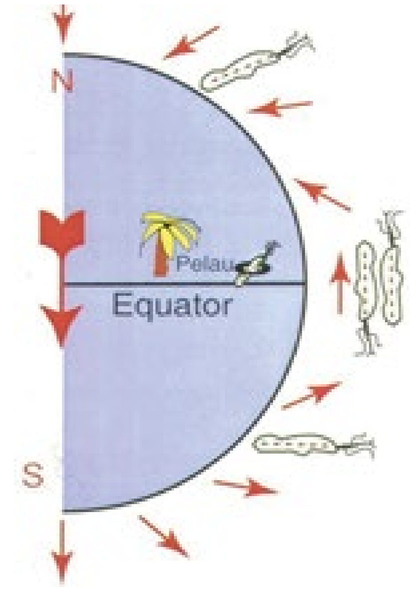

Figure 2: Inclination of the magnetotactic bacteria that move in the direction of the Earth's magnetic field.

While oxygen is vital to humans, it is abrasive and harmful to many bacteria. For this reason, magnetotactic bacteria have developed a surprising method in which the use the Earth's magnetic field like a compass to go as deep into the ground as possible. Almost all of them are anaerobic bacteria and live in conditions where the oxygen level is very low. For this reason, they prefer oxic-anoxic interfacial or anoxic areas. This is the most important characteristic of MTB (3).

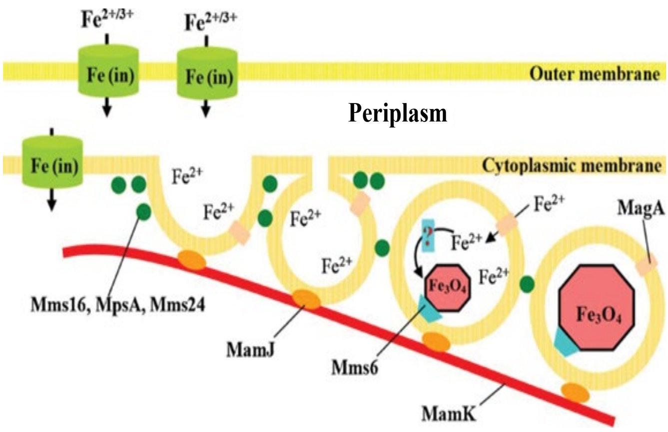

Figure 3: Formation of magnetosomes (17).

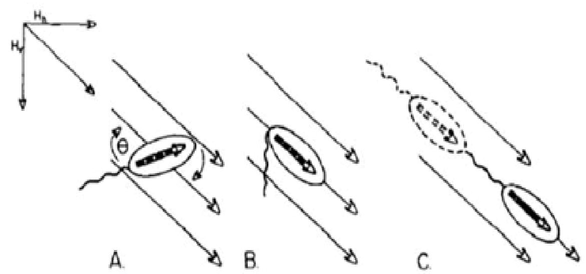

Figure 4: The alignment of magnetotactic bacteria in the direction of the outer magnetic field (H: outer magnetic field) (43).

surface waters, thereby helping them locate and maintain an optimal position in vertical gradients which is usually at or near the oxic-anoxic interface. However, there are reports of SS-MTB and NS-MTB in both hemispheres [24].

Applications of Magnetosome

Nano-technology has been identified as an area which will bring about new evolutions in material, devices and processes. New challenges of nano-biotechnology entail manufacturing more sophisticated and highly efficient biosensors and biomaterials at the nano-scale level for use in interdisciplinary fields. Matsunaga and Okamura stated that magnetic bacteria synthesize intracellular magnets which are encapsulated by lipid bilayer membranes. Sizes of bacterial magnetic particles (BMPs) vary from 50 - 100 nm in diameter, and number over 10 per cell. BMPs are composed of magnetite (Fe_3O_4) with a single magnetic domain. Easy aqueous dispersion of BMPs enables development of highly sensitive chemiluminescence enzyme immunoassays by the chemical coupling of antibodies on BMP surfaces. BMPs can likewise be used as drug delivery systems employing magnetoliposomes with high capture volumes [25].

After the discovery of MTB, it was noticed that they possess unique magnetic, physical and optical characteristics. Due to these characteristics, their use in various scientific and technological applications has been a current issue. It was claimed that by means of magnetic separation, the MTB could separate heavy metals and radionuclides [26,27]. As MTB can be retained with a magnetic recorder, the samples in water can be easily removed and the magnetically labeled cells and nanoparticles can be retained [28]. Another application field of live MTB is nanorobotics. A routing magnetic field is used to orient MTB and a torque is applied to the magnetosome chain in bacteria to propel them at a predetermined location [29].

The magnetite magnetosomes in bacteria are used to block relatively large bioactive substances. These substances can later be controlled by a magnetic field. Magnetite particles of MTB are used as carrier genes in determining nucleic acids [30-33]. With the help of the organized magnetosomes, nucleic acids can be isolated. In addition, it was found that magnetosomes are helpful in determining biomolecular interactions in medicine and diagnosis [34]. In recent times, magnetosomes gained importance as a potential drug-carrier for tumor treatment and as contrast material for MRI [35,36]. The fact that MTB can swim by following the magnetic field direction and their efficient activity provides a possibility to deliver drugs to tumors. Development of deformed capillaries, heterogeneous blood flow, high pressure between cells and other micro-environment parameters of tumors affect drug transfer to the tumors. Microorganisms who act as micro carriers help distribute drugs evenly while ailing the aforementioned conditions [37].

Again, MTB are used in magnetic hyperthermia, which is used in tumor therapy. Magnetic hyperthermia is a method in which magnetic nanoparticles are sent to the tumor and are heated by applying alternate magnetic fields. The heat generated by the nanoparticles has an anti-tumor effect. This technique can be used in the treatment of some tumors such as in lung cancer. Up to now, chemically synthesized iron oxide nanoparticles display super paramagnetic or ferromagnetic characteristics in normal human body temperature. In a magnetic field less than the threshold value, the chemically synthesized nanoparticles' specific absorption ratio (SAR) is much less than that of magnetosome nanoparticles. For this reason, it is claimed that it would be more advantageous to use magnetosomes. Hence, the interest in the "magnetic hyperthermia" by using magnetosomes for cancer treatment has increased [38].

Practical applications of magnetosomes are based on their ferrimagnetism, nanoscale size, narrow size distribution, dispersal ability, and membrane-bound structure. Bacterial magnetosomes have been used experimentally as carriers of enzymes, antibodies for highly sensitive immunoassay, and as efficient sorbents for isolation and purification of DNA or RNA. Artificial magnetic nanoparticles have been used as carriers for cancer diagnosis and targeted therapy in experimental animals. Similarly, magnetic nanoparticles enclosed in biological membranes can be linked to genes or drug molecules and thus could be used as carriers of drugs for targeted therapy of tumors. Several recent reports indicate that purified, sterilized magnetosomes from M. gryphiswaldense MSR-1 are non-toxic for mouse fibroblasts in vitro, and may be useful as carriers of genes, or drugs for cancer therapy or other diseases. However, the applications of magnetosomes have not yet been developed commercially, mainly because magnetotactic bacteria are difficult to cultivate and consistent, high yields of magnetosomes have not yet been achieved [39].

The Formation of Magnetosomes

The formation mechanism of magnetosomes is a complex process that includes the formation of magnetosome vesicles, taking the iron mineral into the cell, carrying the iron in the magnetosome vesicles and the control of magnetite or greigite biomineralisation within the vesicle [18]. While the formation of magnetosomes is not entirely understood, there are views that explain the process. In the most current abstract presented by Arakaki *et al.* [40], it was proposed that three major steps were present in magnetosome biomineralisation [17] (Figure 3).

The first step involves the cytoplasm folding inwards, creating a pocket to form the magnetosome membrane by GTPase, and the organization of these vesicles along a line to form the magnetosomes' carrier fiber. The second step is involving the carrying of iron into the cell by carrier proteins and siderophores and collecting them in the vesicles by transmembrane iron carriers. The iron within the cell is strictly controlled by oxidation and reduction mechanisms, as too much iron is highly dangerous for the cell [10, 41]. The last step is concerning the formation of magnetite in the magnetosome and the organization of the morphology. It is thought that various proteins of the magnetosome are related to collecting oversaturated iron, the core formation of minerals and the continuation of the reducing conditions [17].

In MTB, magnetite formation takes place in microaerobic and anaerobic conditions, as high oxygen content has an inhibiting effect. However, the relation between magnetite formation and low oxygen levels are unclear [17]. While the relation between magnetosome formation and sources of iron is unclear, it is known that iron is the basic element for magnetosome production. MTB require more iron compared to other bacteria for the biomineralisation of the magnetite crystal. This amount can be as high as 4% of the MTB's dry cellular weight. The maximum level for growth and magnetite formation is 100 μM, which emerges in the extra-cellular iron concentration. However, the saturation for both conditions have been observed at iron concentrations of 15-20 μM. Iron concentrations above 20 μM slightly increase the cellular yield and magnetosome content. On the other hand, iron concentrations above 200 μM show an inhibiting effect on growth [42].

Biophysics of Magnetotactic Bacteria

Each MTB has iron particles that can be seen with an electron microscope. Before knowing the chemical condition of the iron within, it was understood that every cell has a constant magnetic dipole moment. When the bacteria that were obtained from an environment rich with mud were subjected to a magnetic pulse of a few hundred Gaus for 1 μs anti-parallel to the swimming direction, it was seen that the cells were momentarily magnetized in magnetic pulses of 200-800 Gaus pulse magnitudes. It was observed that the magnetized cells made a U-turn and began to swim in the opposite direction. This result shows that each bacterium has a specific magnetic moment [43]. In order to understand how the interaction between MTB and the Earth's magnetic field takes place, the cell's magnetic moment should be taken as basis. The main factor that orientates the bacterium in the direction of the magnetic field is the interaction energy between the bacterium's magnetic moment and the outer magnetic field. The bacterium's thermal energy is the factor that makes the bacterium swim randomly, and it is directly proportional of the environment's temperature (thermal energy=kT: k-Boltzmann constant, T-Temperature). In order for the MTB to orient themselve in the direction of the magnetic field, the interaction energy and the outer magnetic field must be greater than the thermal energy. The Langevin function can be used to compare these two energies.

In the classical paramagnetic theory, the Langevin function defines the average array of the magnetic dipole groups that do not interact with each other within a magnetic field. The Langavin function is defined with the following equation. $\cos\theta = L\ (MH/kT)$

In the equation above, θ is the angle between the (H) direction of the environment's magnetic field and the bacteria's magnetic moment direction (M). The average array in an outer magnetic field is determined by the ratio of the applied magnetic field and the bacterium's magnetic field (MH) to the thermal energy of the bacterium (kT). When the MH/kT ratio exceeds 10, the magnetic moments of the particles in the bacterium become aligned with the magnetic field [43]. Therefore, the MTB become aligned with the magnetic field direction (Figure 4).

Different approaches have been used to determine the dipole moment magnitude of MTB. The studies showed that some magnetosomes contain magnetic iron oxide, also known as lodestone. In another study, the shape, size and average number of magnetosomes in a cell were studied. It was determined that each cell contains approximately 20 magnetosomes with an aligned edge length of 420 A° [23]. However, it is not completely clear how the MTB limit the magnetosome dimensions to a single active domain, and why they are in a chain. In addition, it is thought that this limitation is caused by the interaction between the membrane that encompasses the magnetic particle and the cell. The magnetic domains that are in a single chain are aligned such that the magnetic axes are parallel to each other. Therefore, the total magnetic energy of the magnetosome is the sum of all the magnetic moments of each particle. The magnetic moment of MTB is responsible of aligning according to the Earth's magnetic field. These bacteria behave as ferromagnetic biomagnets [43].

Conclusion

In the treatment of many diseases, especially cancer, it is very important that the drugs only reach the tumoral cells (as a target cell), and that a point-shot is directed at these ailing cells. In order to avoid surgery and side effects of drugs, a target subject among current researchers in having bacteria and small scale robots cooperate. The idea that the drug to be carried to problematic cells by bacteria, in other words, using bacteria as little robots have been met with enthusiasm in the scientific community. Especially since they can swim in blood and have magnets within them, the use of magnetotactic bacteria by directing them to target cells with an applied magnetic field has been raised as a topic. As a result, the aim of this paper is to attract attention to these microorganisms that have become the spotlight in research and their medical applications. The increase number of studies and supports in this research field may open up new horizons.

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