Exploring the evolutionary perspective of magnetosome biology in magnetotactic bacteria as a potential biotechnological tool

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Abstract
This report investigates Biologically Controlled Mineralization of ferromagnetic particles within magnetotactic bacteria (MTB) and their potential role in recycling of natural resources. Iron-based nanoparticles accumulated inside MTBs are surrounded by lipid bilayer to form an organelle structure called magnetosomes which have proved to be of high evolutionary significance to the host bacteria.
In this article we review the significant attribution of scientific community towards understanding and exploring the biology of magnetotactic bacteria with respect to efficient employment of bacterial magnetic particles (BMPs) in bionanotechnology. In addition to that, this article gives an overview of the current understanding of the ecology, physiology, phylogeny and molecular biology of MTB.

IndexTerms - Magnetotactic Bacteria; MTB; Magnetosome; Magnetic Bacteria; Classification of Magnetotactic Bacteria; Evolution of MTB; Review article on MTB; Cultivation of MTB; Application of MTB; MTBs

Introduction

Increasing anoxic conditions during early Proterozoic period, paralleled with depleting inorganic elements like iron are believed to cause selective pressure on biotic community [2, 3]. Biologically induced or controlled mineralization (BIM / BCM) are such evolutionary selective criteria which are studied through metal accumulating organisms including iron storing microbes [1, 4].
Besides iron, copper, silver, gold, platinum, other similar metal compounds have been reported to be accumulated through intracellular biological processes [5, 6]. However, due to magnetic properties of iron accumulating microbes, magnetotactic bacteria have attracted high level of attention in the field of biotechnology research as well as in applied biology. Formation of minerals occurs inside the membranous structure called magnetosomes, with highly controlled processes termed as biologically induced or controlled mineralization (BIM / BCM) system, which has applications in biotechnology field in form of nanomagnets used in therapeutic techniques [7]. Magnetic nanoparticles have been synthesised by BIM process in vitro with certain advantages over chemical methods.
Magnetospirillum gryphiswaldense MSR-1, *Magnetospirillium magnetotacticum* MS-1, *Magnetospirillium magneticum* AMB-1, *Magnetococcus sp*. MC-1 and *Magneto-ovoid strain* MO-1 are some highly studied organisms containing either magnetite (Fe_3O_4), greigite (Fe_3S_4) or both as a form of iron nanoparticles complex [8]. Further studies indicate these iron complexes to be surrounded by lipid bilayer, which is connected to a filament accommodating adjacent magnetosomes by Mam J proteins. Magnetosomes crystals exhibit various morphologies like cubic octagonal, bullet shaped, prismatic and rectangular [9]. Because of this firm arrangement of magnetosomes, they behave as nanomagnets and get aligned to the earth's magnetic axis oriented from North to South Pole. MTBs are found at interface level of oxic-anoxic environment of water bodies and can move vertically with the use of flagellar locomotion. These bacteria indicate an involuntary movement due to the effect of magnetic field on their magnetosome nanomagnets. However, they also exhibit a voluntary locomotion to reach the anoxic environment in the water body, in order to find optimal conditions required to complete their life cycle. Higher level of oxygen is harmful for anaerobes and hence MTBs prefer optimum oxygen levels. MTB follow an energy conserving and magnetic field regulated path to

travel in search of optimal oxygen levels, as opposed to other bacteria that lack such a guided locomotion ability [16].

Study of molecular Structure of Magnetosome

Magnetosomes contain either magnetite, greigite or both in mineral form surrounded by lipid bilayer. Thermal energy produced by water current can directly influence magnetotaxis. Presence of one or two magnetosomes cannot overcome such heat induction and hence higher number of magnetosomes are required on filament contained by Mam J proteins to overcome the heat effect. The magnetosome filament string works as dipole on their body axis that is parallel to the earth's magnetic field. Bipolarity of magnetosomes is not constant over given period of time. This structure is comparable to compass needle that aids in navigation [15]. The MTB genome has been cloned in a fosmid library using genomic DNA from MTB (*Candidatus Magnetoglobulus bavaricum*). A physical map of MTB genome was derived using consensus region from two of the DNA sets with overlapping segments. They were found to display partial similarity to known magnetosome genes MamE and MamP. Using primers targeting these regions, about 10,000 clones from metagenomic fosmid libraries were screened with Polymerase Chain Reaction (PCR) method [18, 21]. Five clones were identified by PCR screening by amplifying either identical sequences of *mamE, mamP* or both gene regions [17].

The membrane that encloses the crystals is responsible of controlling of size and shape of the magnetosome crystals. Several studies on *Magnetospirillum* demonstrate that magnetosome formation is a complex process that involves many discrete steps, including magnetosome vesicle formation, extracellular iron uptake by the cell, iron transport into the magnetosome vesicle and biologically controlled mineralization of magnetite or greigite within magnetosome vesicle [10]. Electron microscopic studies indicate empty vesicles or partially formed magnetosemes in iron starved cells of *M. magnetotacticum* and *M. gryphiswaldense*, thereby emphasizing vesicle formation as a prior step to that of mineralisation [10]. Although formation of magnetosome is not completely understood, various hypotheses have been proposed. An early model states that magnetosome formation comprises of three major stages. First step involves uptake of ferromagnetic ions forms from outside of the cell via reductive step, followed by iron oxidation step of ferrihydrite formation and the final step of biomineralisation to form magnetite.

Detection and analysis of MTB

Magnetotactic Bacteria have oxygen dependent metabolism but their oxygen requirement range is very narrow [7]. They need oxygen concentration around 1-3% hence it becomes difficult to culture even in laboratory and especially in industries. They lack the oxygen protective enzyme, catalase which has crucial role in aerobes. *M. magnetotacticum* was the first species to get isolated as pure culture due to advancements in biotechnological instrumentation. Following this, *M. gryphiswaldense and M. AMB-1* were successfully cultured on a large scale [7].

Large volume culture of above mentioned MTB strains indicate resistance to the atmospheric air.

MTB can be separated from contaminations based on their activity towards magnetic field. They are placed inside magnetic field generated by bipolar magnet which induces movement in MTB thus they get accumulated to the specific region towards pole of magnet with concentration, after which they can be isolated. Purification is performed, however, with limited efficiency since purifying agents affect their optimum growth conditions [12].

A capillary racetrack method using a capillary tube, is another way to isolate magnetotactic bacteria and separate from other bacterial contaminants. A sterile saline is placed inside sample holder or on wide side of tube while magnetic field is applied from the closed site of the capillary tube. Other contaminants such as chemotactic bacteria and protozoa are delayed in passage through cotton while MTB move very quickly toward magnetic poles under the influence of magnetic field promoting their separation [18].

Magnetosomes can be isolated by magnetic separation, sucrose gradient or combination of both. These crystals are attracted toward magnetic forces after the dissolution of cellular membrane [11]. WGA-QDs (ZnS core/shell SIGMA-ALDRICH-748056) and SiMAG-PEI (Chemicell-1204-11204-5) are quantum dots which strongly interact with MTB. Intensity of fluorescence increases with concentration of MTB ranges from 10^2 to 10^7 cfu/ml.

Biological Perspective

The optimum conditions for *M. gryphiswaldense* MSR-1 to perform biomineralization require a pH value of 7.0 and temperature of 28 ̊C, along with Iron uptake rate at Vmax of 0.8 nmol min^{-1} (mg dry weight) [20]

Several studies have indicated influence of parameters in form of temperature shift between 4 ̊C to 35 ̊C and pH between 5.0 to 9.0 can results into the alteration of biomineralization rate. Although some MTB are found to be extremophiles the studies were restricted only to MTB strains of *M. gryphiswaldense* MSR-1 and *M. gryphiswaldense* (7).

Cultivation of MTB is difficult because of their stringent requirement of habitat and nutrition. They fail to form colonies upon extended incubation with specialized media and simulation of natural environment [19]. Pure form of MTB are very difficult to isolate from mixed cultures as some magnetic property possessing agents cause contamination making them difficult for removal.

MTB have been used for labelling and retaining heavy metals and nanoparticles. They have also been employed in the field of nanorobotics, thereby broadening their path. MTBs are placed inside magnetic field and torque is applied to the magnatosome chain in bacteria to propel them towards desired location. MTB can be used to transfer genes as carrier biogenic robot [14].

Comparison of MTBs with eukaryotic magnetosomes:

Many eukaryotes also possess magnetosomes but the purpose is not yet clearly defined as they lack two dimensional motility [1].

Magnetotaxis activity is demonstrated by human nerve tissues as clusters of cells containing magnetosomes inside the cytosol (11). The number of magnetesomes in each of cells range between "1000 to 10,000" as compared to MTBs with magnetosomes ranging from "10 to 30" per cell. Using an ultrasensitive superconducting magnetometer in a clean-lab environment, presence of ferromagnetic material has been detected in a variety of tissues from the human brain [11]. Magnetic particle extracts from solubilised brain tissues have been examined with high-resolution transmission electron microscopy, electron diffraction and elemental analyses identify minerals in the magnetite-maghemite family. The results have depicted various crystal morphologies and structures resembling ferromagnetic crystals biosynthesized by magnetotactic bacteria [13].

Distribution of MTBs amongst Bacterial Phyla:

The discovery of MTBs and their phylogenetic distribution amongst the various bacterial phyla makes them evolutionary significant in understanding the occurrence of magnetosomes in the bacterial strains.

Phylogenetic analysis done with 16sRNA sequences from isolated MTB strains indicate random distribution of the MTBs across the bacterial taxonomy [21]. The domain of *Archaea* is surprisingly found to lack any MTB strains so far. However the domain of *Bacteria* contain most of the MTB strains (fig.2), spread in the *Alpha-*, *Gamma-*, and *Deltaproteobacteria* classes of the *Proteobacteria* phylum. The *Nitrospirae* phylum and *Planctomycetes- Verrucomicrobia-Chlamydiae* (PVC) bacterial superphylum have been reported to contain some of the uncultured strains of MTBs [22]. Amongst the *Alphaproteobacteria* class, the MTB strains discovered and studied are *Rhodospirillales* (*Magnetospirillum*, *Magnetovibrio*, and *Magnetospira*) (23, 24, 25) and *Magnetococcales* (*Magnetococcus*) [26]. The *Magnetospirillum* genus forms cuboctahedral and elongated prismatic magnetite crystals [27 28].

The MTB strains belonging to *Deltaproteobacteria* class are found in two orders, the *Desulfovibrionales* (*Desulfovibrio* and *Desulfonatronum*) [29] and the *Desulfobacterales* (*Candidatus* Magnetoglobus and *Ca*ndidatus Desulfamplus) [30, 31]. The *Deltaproteobacteria* class contains MTB strains that produce magnetite or greigite magnetosomes. The sulfate reducing *Desulfovibrio magneticus* MTB strains RS-1 [32, 33] and strain FH-1 [34] also belong to this class.

The *Gammaproteobacteria* class has been reported to contain two MTB strains termed BW-2 and SS-5 [35, 36]. The MTB strain BW-2 belongs to the *Thiotrichales* order, and the MTB strain SS-5 belongs to the *Chromatiales* [37].

In the *Nitrospirae* phylum, four MTB strains are reported, namely, *Candidatus* Magnetobacterium bavaricum, a designated strain MHB-1, *Candidatus* Thermomagnetovibrio paiutensis strain HSMV-1, *Candidatus* Magnetoovum mohavensis strain LO-1 and *Candidatus* Magnetoovum mohavensis. These MTB strains are found to biomineralize bullet-shaped crystals of magnetite.

Another novel MTB strain termed SKK-01 was discovered and reported to belong to candidate OP3 division of bacteria based on 16S and 23S rRNA sequencing [38, 39].
The distribution of MTB strains amongst bacterial domains and continued discovery of novel strains indicate vast diversity and evolution of magnetotaxis and which may be greatly underestimated in the field of bacteriology.

Conclusion

Biologically Controlled Mineralization is an evolutionary conserved ability found in microorganisms to conserve minerals, including metal ions. In magnetotactic bacteria, the process is facilitated by specialized organelles called magnetosomes, which continue to get discovered in many novel bacterial strains that remain unculturable. Archaeabacteria have somehow resisted the formation of magnetosome biology within the domain, indicating the need for investigation to reason its significance.
We have reviewed the importance of magnetotaxis and magnetotactic bacteria in maintaining the natural resources as well as their employment in field of biotechnology and medical science. (fig.1). The biology of magnetosomes and their occurrence amongst the existing and newly discovered MTB strains is also reviewed with respect to their evolutionary significance.
The potential application of MTBs against oncogenesis by magnetic field generated hyperthermia to kill malignant cells gives them a gene therapeutic advantage
With the current evidence of magnetotactic bacteria with respect to their ecology, physiology, phylogeny and molecular biology, they form an intriguing scientific curiosity to understand the magnetosome biology and exploring its evolutionary path throughout prokaryotic and eukaryotic organisms.

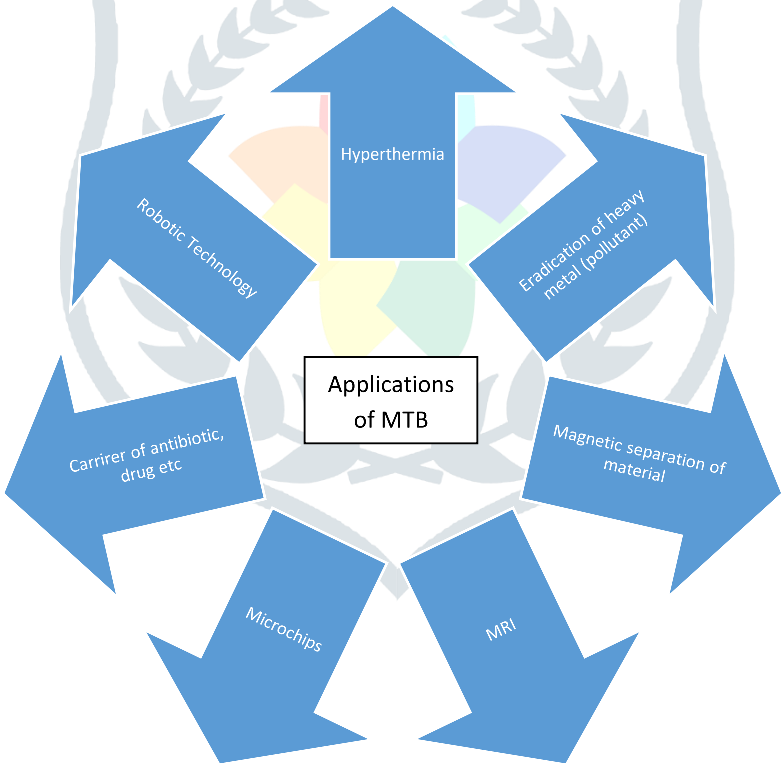

Figure 1. Role of Magnetotactic Bacteria as potential Biotechnological Tool

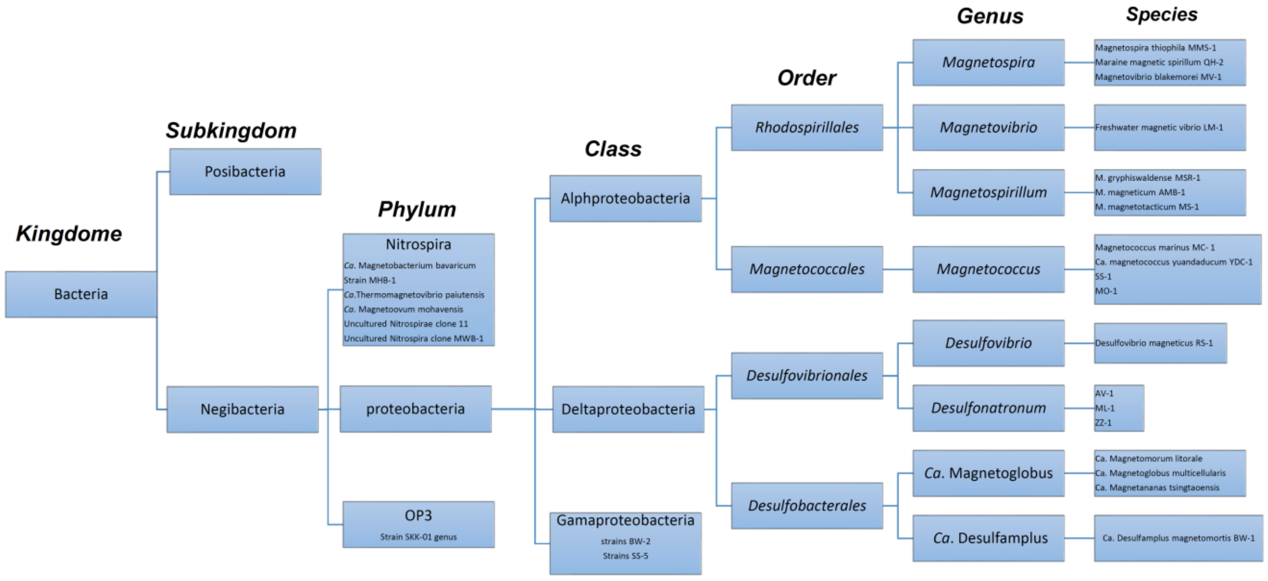

Figure 2. Taxonomic distribution of Magnetotactic Bacteria

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