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Microaerophilic Magnetotactic Bacteria from Lonar Lake, India

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Magnetotactic bacteria (MTB), the major constituents of natural microbial communities in sediments and chemically stratified water columns. The hallmark feature of MTB is the presence of unique, nanometer sized, intracellular inclusions the 'magnetosomes' that confers 'magnetotaxis 'to these cells. The 'magnetosomes', the 'magnetotaxis' and the possible applications of these bacteria grown curiosity about the MTB. Presently they received the celebrity status, as MTB might be tool for searching extraterrestrial life. Lonar Lake is the only meteorite impact crater in the world formed basaltic rock. The closed basin lake has a unique environment characterized by high alkalinity and salinity. Evidences of magnetic activity in the surrounding rocks and soil are also found, and reported. The role of MTB in biomineralization encouraged us to find such bacteria in this unique environment. The sediments samples collected from the lake were used to isolate the microaerophilic magnetotactic bacteria (mMTB). The mMTB were enriched by 'magnetic collection' method, isolated by the 'capillary racetrack' (CRT) method and cultivated under microaerophilic conditions using a chemically defined medium. The three isolated mMTB showed were tested for response to the magnetic field using magnetic field in a 'hanging drop' under a microscope and also the on semisolid agar medium. They showed migration towards the South Pole of the magnet and precise alignment at the edge of the hanging drop. On semisolid agar also they showed migration toward magnetic South Pole. The intracellular iron content of the isolated mMTB cultures was measured using atomic absorption spectroscopy (AAS), which indirectly confirms the presence of magnetosomes in these the isolated MTB; similar result was obtained by R.P. Blakemore during discovery of MTB.

Key words: Magnetosome, Magnetotaxis, Magnetic collection, Capillary race track method.

Magnetotactic bacteria (MTB), discovered by R.P. Blakemore[1] in 1975 are a general designation to a group of microorganisms which swim along geomagnetic field lines. These are are motile, aquatic prokaryotes with myriad of morphologies including coccoid to ovoid cells, rods, vibriods, spirilla and even multicellular forms[2,3,4]. The habitat of these bacteria is fresh water bodies as well as marine; where they are predominant in the sediments in the permanently stratified water columns from different water bodies (freshwater, seawater), sediment[5].

All magnetotactic bacteria contain nanometer sized intracellular inclusions; the 'Magnetosomes'[6], which are nanometer sized, enveloped, membrane bound, single magnetic domain crystal particles, composed of the iron mineral magnetite (Fe_3O_4) and/or greigite (Fe_3S_4)[1,7].Particle size of magnetosomes ranges

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between 35-120 nm; although larger, 200 nm crystals are found in some bateria[8]. The magnetosomes are the signature structures of MTB as they are uniquely present in this group of bacteria that offers them 'Magnetotaxis', a behavior in response of magnetic field by these organisms[1].

The MTB in northern hemisphere sites swim preferentially parallel to the magnetic field, which correspond to a northward migration in the geomagnetic field and known to be north-seeking (NS)[1], while in southern hemisphere they are recognized as south-seeking (SS)[9].

MTB as well as the isolated Magnetosomes have tremendous applications in biotechnology and nanotechnology. In Nanotechnology where nano-particles are synthesized for different purposes, magnetotactic bacteria are of use as they synthesize the nanometer sized particles; the magnetosomes. Small magnetic particles can be prepared synthetically but the particles formed by these methods are non-uniform, non-homogenous and also not fully crystalline which imposes problems in their application. Also the processes require the drastic regimes of temperature pH etc.[10]. The living MTB are used in non-destructive domain analysis of soft magnetic materials[11], The use of MTB can be extended to waste treatments as they are useful in removal of heavy metals and radionuclides from waste water well as removal of heavy metals and radionuclides from waste materials, the main advantage being the magnetic collection of MTB loaded with metals and radionuclides[12].

The isolated magnetosomes from this group of bacteria proved to be a good tool in biotechnology, with their various applications in this field. In medical sciences these particles are of potential use as a contrast agent in magnetite resonance imaging as well as tumor specific drug carriers based on intra-tumoral enrichment[13]. Industrial applications of magnetosomes are fascinated with small size of isolated magnetosomes particles that provide them a large surface to volume ratio which is useful for immobilization of bioactive substances such as enzymes[14], antibodies[15] etc. The magnetosomes also can be used as carriers for the introduction of DNA into cells[16].

The Lonar Lake, the only hypervelocity meteorite impact crater formed in basaltic rock, formed some 52 thousand years ago is situated in the Buldhana district of Maharashtra (India) (Lat. $19^0$58' North, Long. $76^0$34' East)[17]. The lake water is saline, alkaline (pH 9.5 – 10.0, CaCO3 alkalinity – 3.6 g/L, NaCl (as Chloride) - 3.0 g/L)[18]. As the formation of magnetosome in MTB is carried by process of biomineralization[19], these microorganisms have great importance in geobiochemical cycles[1]. Evidences of magnetic impulses in rock samples surrounding the lake (noted by us), encouraged to look for magnetotactic bacteria in the Lake environment; where they are still ignored.

MATERIAL AND METHODS

Sample collection

The littoral zone surrounding the Lonar Lake was sampled at eight different sites for the upper sediment layer and surface water. The samples were collected directly into previously disinfected (with isopropyl alcohol) plastic bottles of 1.0 to 1.5litre capacity and closed tightly with the lids. All samples were transported to the laboratory under ambient temperature conditions where they were stored under ambient conditions till use. During transportation and storage the lids of the water bottles were kept slightly open to allow gases formed under anaerobic conditions to escape.

Enrichment of magnetotactic bacteria

Enrichment of MTB was done using Magnetic collection method[20] which is based on the cell's swimming response to a magnetic field. The south pole of a permanent magnet was attached outside a jar containing the water and sediment samples, 1 cm above the sediment surface. After one to two hours; 1 - 2 ml of the water in the bottle near the wall adjoining the magnet was collected with a sterile pipette and transferred to sterile tubes to be used for further studies.

Isolation and cultivation of magnetotactic bacteria

In case of MTB, the *isolation* and cultivation techniques are separate procedures. The MTB collected by magnetic collection were purified using modified 'Capillary Race Track'

isolation of mMTB isolates from Lonar Lake sediment samples. However under the microscope it was observed that the sample obtained by these methods includes more than one morphological type. That clearly indicated presence of more than one mMTB isolates. To isolate these different mMTB the samples was subjected to the streak plate method of isolation that yielded three mMTB isolates in pure cultures. They included two Gram negative rod shaped bacteria and one gram negative slender rod. The isolates were coded as mMTB1, mMTB2, mMTB3 [mMTB-Microaerophilic Magnetotactic Bacteria]

The mixture before isolation as well as the individual isolated cultures were tested for their magnetic properties by the hanging drop technique. In the hanging drop observation, the cultures aligned parallel to each other along the magnetic field lines and showed a migration towards the magnetic south pole.

The further confirmation of the MTB with respect to their intracellular iron content which showed that magnetic bacteria accumulated eight to nine folds more iron within their cells (Fig. 1) as compared to the non-magnetic bacterial cells [*E. Coli*], similar to the observation; first reported by Blakemore *et al*. in 1979[22].

CONCLUSION

Biotechnological applications of MTB as well as isolated magnetosomes were realized soon after their discovery. Presently these organisms are receiving celebrity status due to their possible correlation to the extraterrestrial life. Recent attributes of the magnetite and iron-sulfides in the Martian meteorite ALH84001 to production of biological processes as part of the evidence for ancient life on Mars[23, 24].

That they are still short due to problems of isolation and cultivation of the fastidious life style of these organisms. Till date only few pure cultures of these bacteria are isolated in pure cultures; hence it is necessary to search new environments having these microorganisms. The results presented here clearly showed that Lonar Lake harbors MTB, which also could be exploited in Nano-Biotechnology after further studies.

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