



Preliminary isolation report of aerobic magnetotactic bacteria in a modified nutrient medium

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Abstract

A modified nutrient medium was developed for isolation of magnetotactic bacteria. An aerobic strain of Magnetotactic bacteria (MTB) was successfully isolated in modified nutrient medium. Ferric quinate was used as the main iron source. Macroscopic and microscopic studies were performed to study the magnetotactic response of the isolated strain. Motility towards the externally applied magnetic field direction confirmed the isolated strain to be magnetotactic in nature. Scanning electron microscopic analysis was used to study the morphology, which shows rod like shape of isolated strain. Electron dispersive X ray diffraction analysis confirmed the presence of elemental carbon as a major constituent. Moreover, elemental iron and oxygen indicated the formation of magnetic material inside the bacterial cells. Vibrating Sample Magnetometer analysis is performed to reveal the magnetic characteristics of the isolated bacterial strain.

Keywords: Culture medium, Isolation, Magnetotactic bacteria, Magnetotaxis, Motility

INTRODUCTION

Magnetotactic bacteria (MTB) can be defined as a wide range of microorganisms which have the ability to orient and migrate themselves along the geomagnetic field lines. The MTB prokaryotes show diversity for their morphological and physiological features. Different morphologies of such microorganisms include cocci, rod, vibrio, ovoid, spirilla and multicellular aggregates [1], have been found in fresh water environments (e.g. pond, lake, river etc) and marine environment (e.g. coastal environment and marine sediment surface). These microorganisms form intracellular membrane bound magnetic crystals of order of billionth fraction of a meter, known as magnetosome. The main constituent of magnetosome is magnetite (Fe_3O_4) or greigite (Fe_3S_4) [2,3]. Moreover, intracellular formation of iron pyrite (FeS_2) and weak magnetic crystals pyrrhotite (Fe_7S_8) were also reported in literature [4, 5]. Magnetic crystals formation within the cell provides a permanent magnetic dipole moment to the cell. The consequence of interaction of these intracellular nanomagnetic dipoles with geomagnetic field is alignment and movement of the cells along geomagnetic field lines. This phenomenon is known as magnetotaxis.

A variety of MTB have been found in natural environment but their maintenance in artificial laboratory conditions is quite difficult. A few strains of MTB such as *M. magnetotacticum*, *M. gryphiswaldense*, AMB-1 and MGT-1, MV-1, MV-2 and MV-4, MC-1 and RS-1 have been isolated till date [6]. Most of the MTB

isolation methods are based on their magnetotaxis [7, 8, 9, 10]. However, isolation of weakly magnetotactic bacteria RS-1 without using magnetotaxis has also been reported [11].

The isolated strains of MTB are very sophisticated in terms of their culture medium requirements. They require organic acids as a carbon source and nitrate as nitrogen source with trace of vitamins and minerals to make chemically defined medium slightly complex [8]. Beside it, culture media modification also enhances the yield of magnetotactic bacterial culture. Approximately five times yield enhancement of the isolated microaerophilic strain MS-1 with culture media modification was achieved as reported in the literature [12]. Contemporary researches are more devoted towards isolation of aerobic MTB. Some aerobic strains MG-1, MG-2 and MG-3 have been successfully isolated in such media with some modification [13]. These studies signify the possibility to isolate and grow MTB in nutrient medium with or without some modification.

The present investigation is intended to isolate aerobic MTB in modified nutrient medium using their magnetotaxis property.

MATERIAL AND METHOD

Samples were collected from pond (five different locations) located near iron scrap industries, Uttarakhand, India. As iron is the one of the key component for magnetic property of various magnetotactic bacteria, presence of iron in nearby region of the pond indicates that the collected samples may contain the concerned microorganism. While collecting the samples, permanent bar magnets were placed just above the surface of water for one hour, facing the south pole of magnets towards the surface of water. The movement characteristic of MTB was influenced by the externally applied magnetic field and resulted to the densification of the naturally occurring MTB near the south magnetic pole. To maintain the oxygen availability, the samples were half filled in clean plastic bottles. Moreover, the caps were partially closed to create temperature equilibrium and air exchange with the surroundings. The

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bottles containing microorganisms were transported to the lab and stored with the loose caps at ambient room temperature until use[14].

To prepare ferric quinate solution, 0.27 g of ferric chloride (FeCl_3) and 0.19 g of quinic acid were dissolved in 100 ml of distilled water. Nutrient broth was made by mixing 5 g of Sodium chloride, 3 g of Beef extract and 5 g of Peptone in 1.0 liter of distilled water and then modified with 2 ml of ferric quinate solution. The pH of the medium was adjusted to 6.7 using 0.1 N NaOH. For the preparation of solid medium, 15 g/l agar was added to the above prepared culture medium. Medium was sterilized by autoclaving.

Before inoculating in the modified nutrient broth (growth media), densification of MTB in the sample was further carried out by using bar magnet. One day exposure of magnetic field was carried out onto the surface of collected sample. 0.1 ml of sample was pipette out for inoculation into the freshly prepared petri dish and vigorously spreaded all over the media surface with the help of sterile T-shaped glass spreader. The incubation temperature was maintained same as the temperature of sample collection arena. After 24 hours of incubation at 25°C, various colonies were observed in the petri dish. Each colony was isolated by an inoculation loop and further cultivated in a 250 ml flask filled with modified nutrient broth medium. From this investigation three different types of strains (Strain-A, B and C) were isolated. They were further studied for their various properties.

The isolated strains were inspected for their motility under the effect of the externally applied magnetic field. The motility test was performed with 0.2 % agar in the culture medium. In a laminar chamber, all the three isolated strains were transferred to the center of separate petri dishes by using inoculating needles. An additional set of three petri dishes, containing individual strains, was prepared. First set of petri dishes was incubated under an externally applied magnetic field whereas the second set was incubated in the absence of magnetic field. Preliminary observations showed that only one strain (strain-A) responded for the externally applied magnetic field. Other strains (strain-B and strain-C) did not respond for magnetic field. Strain-A was further tested for its magnetic motility.

For microscopic observation of the motility of strain-A, another motility test was performed in hemocytometer. A small aliquot

of the cell suspension was mixed with equal volume of 0.4% trypan blue dye. One drop of mixed suspension was poured into the grids of pre-cleaned hemocytometer. Then the grids were overlaid with a cover slip and external magnetic field was applied around the hemocytometer using permanent bar magnet [15].

Scanning Electron Microscope and Electron Dispersive X-ray Diffraction analysis were done for morphological and elemental analysis, broth of strain-A was centrifuged at 10000g in REMI research centrifuge for 10 minutes and suspended in 4% glutaraldehyde solution for around 12 hours at 5°C for the cell fixation. Five different gradients (30%, 50%, 70%, 90% and 100%) of ethyl alcohol were prepared and then the cells were incubated one by one in each gradient for 20 minutes at room temperature. It completely replaces the water of cells with ethyl alcohol [12]. The cells were finally centrifuged and separated cell pellet was examined under SEM (QUANTA 200 F, FEI, Netherland).

For magnetic property detection in isolated strain concentrated suspension of centrifuged bacterial cells is washed many times in distilled water to remove any trace of growth media. Cell suspension was again centrifuged at 10000g for 10 min to collect the cells for vibrating sample magnetometer test. The bacterial sample was filled in sample holder for analysis at the room temperature.

RESULT AND DISCUSSION

Growth characteristics of the various isolated strains were measured under UV spectrophotometer (UV210 A, Shimadzu, Shanghai) at 610 nm. Growth curves were plotted against incubation time. Experimental values were mean of five replicates and data of each set were in close proximity. Result revealed that the optical density, which is consequently the concentration, of strain-A in culture medium was comparatively poor. The log phase remained for 8 to 20 hour and growth ceased after 24 hours, after which, death phase dominated. Strain-B came in stationary phase in 24 hours. The optical density of strain-C was found to be highest among all the three strains, showed its concentration was highest among all the three strains. The poor biomass formation of strain-A may be due to absence of some unknown required vitamins and minerals in growth media.

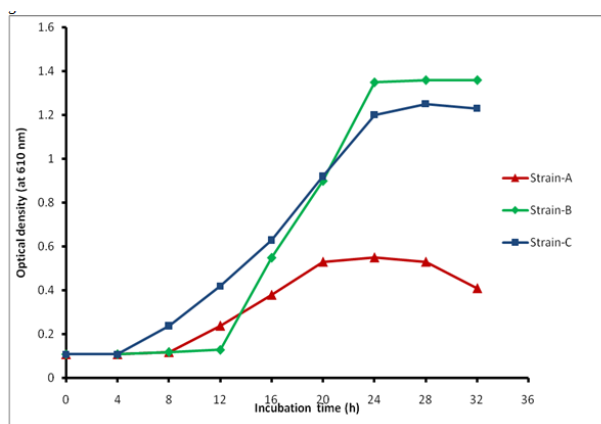


Fig. 1. Optical density of various isolates

In the macroscopic observation of motility test, the growth of all the strains was visualized. In the second set, each strain was found to spread in all the directions to make a circular halo. While in the first set, colony of strain-A was found to be elongated in the direction

of externally applied magnetic field. However, strain-B and C did not respond to the magnetic field and growth was dispersed in all the directions. This experiment confirmed that strain-A is a south seeking bacteria.

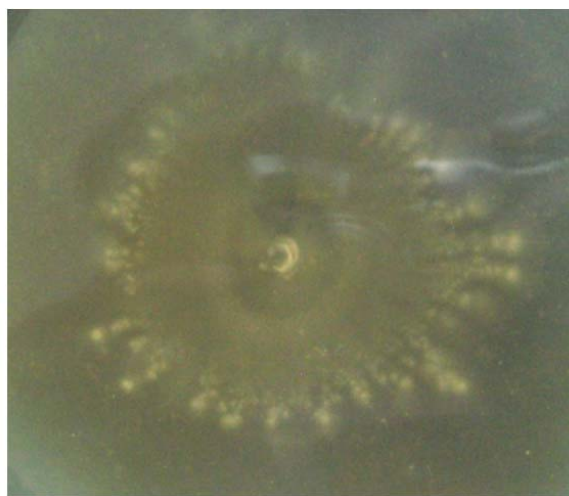


Fig. 2. (a) Cells motility in all directions in the absence of applied magnetic field.



Fig. 2. (b) Migration of cells towards the South Pole in applied magnetic field.

Under microscopic observation, all the dead cells were stained with trypan dye and appeared as blue colour. Live cells remained unstained and appeared as blackish cloudy. The photographs of the cells were taken at the interval of 5 second. Grids of the

hemocytometer were used to find out the relative change in position of the motile cells. Movement of the cells was clearly observed towards the south pole of the magnet (Fig. 3 b). This test confirms the magnetotactic nature of the isolated strain-A.

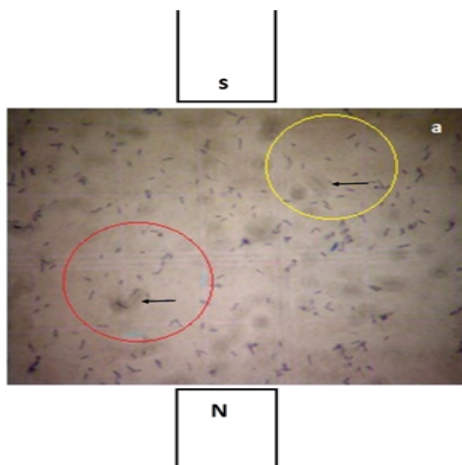


Fig. 3. (a) Initial positions of the cells.

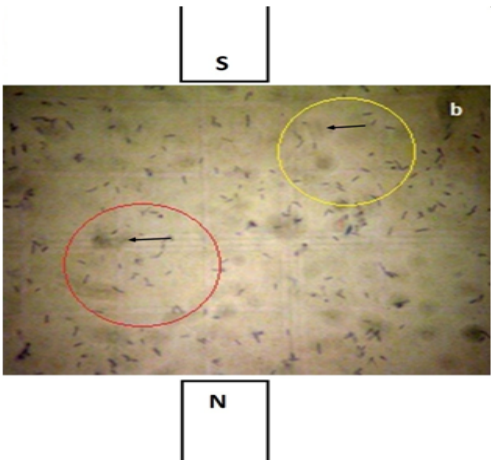


Fig. 3. (b) Positions of the cells after 5 seconds.

Under the SEM inspection, all the cells were found to be cylindrical in shape with a diameter of 0.3-0.5 μm and length of 0.9-1.6 μm (Fig. 4). An energy dispersive X-ray spectrum shows the elemental composition of the pallet (Fig. 5). Carbon content of the

pellet was found to be 70.47%, confirming it to be the main component. The other elements of the cell pellet were 8.28% iron and 10.56% oxygen. These two elements are responsible for the formation of magnetic materials within the bacterial cells.

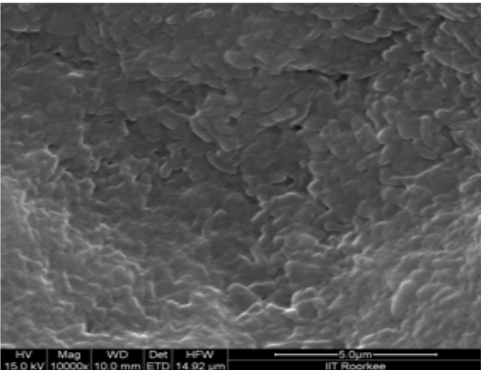


Fig. 4. SEM analysis of cell pellet.

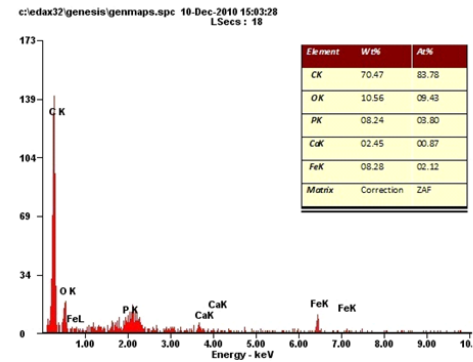


Fig. 5. EDAX analysis of cell pellet

The magnetization of the cell sample was studied as a function of external magnetic field. Fig.6 shows hysteresis loops in the measurement which attained a saturation point, indicates the presence of magnetic material in the sample. The saturation magnetization and coercivity were found to be 0.68 $\mu\text{emu/g}$ and 190 Oe respectively indicates that the magnetic material formed inside

the biomass falls under hard magnetic material. Interestingly, the obtained hysteresis curve is quite comparable with the hysteresis curve for MS-1 culture. The saturation magnetization of isolated strain was approximately half of the MS-1 culture shows the sample contains lesser magnetic material [12].

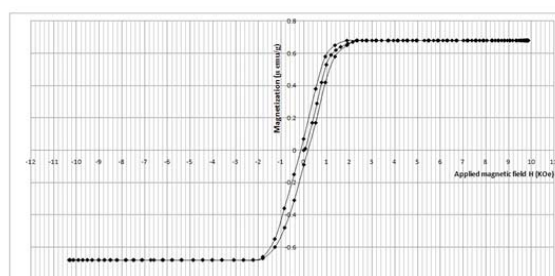


Fig. 6. M-H curve for isolated magnetotactic bacterial culture.

Table 1. Properties of various isolated strains

Isolate codes	Gram test response	Morphology	Growth period	Magnetic motility response
Strain-A	Gram negative	Rod like structure	24 h	South seeking
Strain-B	Gram negative	Rod like structure	20 h	No motility
Strain-C	Gram negative	Spherical structure	24 h	No motility

CONCLUSION

Initial investigation of all the three strains is summarized in the table 1.

The isolation of oxygen tolerable strain of MTB in modified nutrient medium was successfully achieved from this investigation. The isolated strain takes relatively lesser time (20 hours) for growth as compared to the other MTB species reported in literature [6, 13]. However, growth of the isolated magnetotactic strain was observed to be poor. This fact may be due to absence of required unknown growth components. The saturation magnetization of isolated strain is about half of MS-1 strain [12]. Present isolation method is advantageous over other methods because it requires less complex culturing condition for MTB. Further study regarding the isolated strain may be helpful for the growth component requirement and hence, biomass yield and magnetic properties enhancement.

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