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Preliminary Report on the Isolation of Magnetotactic Bacteria from River Tamiraparani in South India

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ABSTRACT

Magnetotactic bacteria are ubiquitous in aquatic environments and cosmopolitan in distribution. These microorganisms shows characteristic behaviour: they orient and swim in the direction of magnetic field. This peculiar behaviour is due to the formation of an intracellular enveloped magnetic grains called magnetosomes. In this present study, a preliminary examination was made to find out the presence of magnetotactic bacteria in River Tamiraparani, a perennial river in Tirunelveli District of Tamilnadu State, India. Collection and isolation of magnetotactic bacteria was done by modified race track method of purification. Then the magnetotactic bacteria were subjected to streak plate method and light microscopic observation. From this study the magnetically collected bacterial cells from river Tamiraparani are rod and oval in shape, gram negative and are geomagnetically sensitive.

1. Introduction

Animals use magnetic field in various ways of their life especially for locomotion and also in homing behaviour. Bacteria, the very minute creature are also geomagnetically sensitive [1] and bacteria that involved in such activities are called magnetotactic bacteria. Magnetotactic bacteria were first identified by Blakemore (1975) [2] and are a heterogeneous group of prokaryotes which are ubiquitous in aquatic environments and cosmopolitan in distribution [3]. These microorganisms show a peculiar behaviour: they orient and swim in the direction of magnetic field. This orientation is caused by the interaction of the microorganism's magnetic moment in a magnetic field [3]. Magnetotactic bacteria are distributed worldwide and most of them are found at, or just below, the oxic anoxic transition zone (OATZ) or redoxocline in aquatic habitats [4].

Magnetotactic bacteria synthesize intracellular enveloped magnetic grains termed as magnetosomes. Magnetosomes are specialized organelles synthesized by magnetotactic bacteria (MTB) for geomagnetic navigation in their aquatic habitats. Each species of magnetotactic bacteria has a different shape of magnetosomes [5]. The magnetosomes are arranged in intracellular chains that enable the cell to align and swim along external magnetic fields, a behaviour known as “magneto taxis” [6].

Magnetotactic bacteria play an important ecological role in many types of aquatic sediment, as for instance in biogeochemical cycling of iron and other elements. However, their role remains to be fully evaluated. Furthermore, magnetosome particles remain preserved after the bacterial cells die and deposited as magnetofossils, which significantly contribute to the magnetization of sediments [7-8]. The application of magnetotactic bacteria and magnetosomes also could widely be applied in many fields such as: material science, biology, pharmacy, electronics, optics, magnetism, and electrochemistry [4]. Magnetosomes are also used in for various bio-applications, such as magnetic drug targeting, magnetic resonance imaging (MRI), magnetic fluid hyperthermia. In addition a number of magnetosome-based immunoassays were developed to detect antigens, environmental pollutants, hormones and toxic substances [9]. In this present study, a preliminary examination was made to find out the presence of magnetotactic bacteria in River Tamiraparani, a perennial river in Tirunelveli District of Tamilnadu State, India.

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2. Experimental Methods

2.1 Sample Collection

The samples were taken from the river Tamiraparani near Agastiyar falls (Latitude 8°42'15.16" N Longitude 77°21'49.23" E). At sampling site bottom water and the top 3-4 cm sediments were collected. The bottles of 1 litre capacity were used to collect samples. Bottles were filled with 30% of sediment and remaining 70% with water that overlays the sediment. The samples were transported to the laboratory and kept in a sterile environment under ambient temperature for further studies.

2.2 Collection of Bacteria

Collection of magnetotactic bacteria was done by magnetic collection method [10]. This technique is based on the cell swimming response to a magnetic field. A magnet was attached outside the jar containing water samples and kept undisturbed for four hours. After four hours, water sample near the magnet was collected with pipette and transferred to sterile tubes and analysed (Fig. 1).

Fig. 1 Magnetic collection of magnetotactic bacteria

2.3 Race Track Purification of the MTB

Purification of magnetotactic bacteria was done by the modified capillary “race track” method [11-12, 3]. A glass tube (length 9 cm) was sealed at one end and filled with the distilled water. A medical absorbent cotton plug was made and placed in the open end of the pipette. A syringe with hypodermic needle was placed on the pipette so as the tip of the

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needle penetrating cotton plug and immersed into distilled water. The set-up was autoclaved. After sterilization the hypodermic syringe filled with magnetic collected bacterial cells. The set – up was exposed to a magnetic field produced along it with a permanent magnet at the sealed end of the pipette for 3 hours. The magnetically collected bacterial cells migrated through the cotton plug towards the closed end of the glass tube. The tip containing the accumulated magnetotactic bacteria was then broken off and the organisms transferred to the sterile eppendorf tubes. This method was repeated two more times to purify the magnetotactic bacteria (Fig. 2).

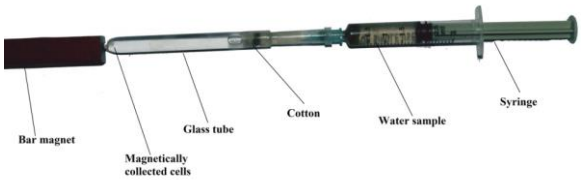

Fig. 2 Modified "race track" method for purification of magnetotactic bacteria

2.4 Media Preparation

After magnetic collection and purification methods, various media were used for the cultivation experiments. A variety of magnetotactic bacteria have been found in natural environment but their maintenance in artificial laboratory conditions is quite difficult. A modified culture medium was prepared [13-16]. The medium contains 10 mL of Wolfe's mineral solution, 10 mL of Wolfe's vitamin solution, 2 mL of ferric quinate solution 0.10 g NH_4Cl, 0.20 g $MgSO_4.7H_2O$, 0.10 g $CaCl_2.2H_2O$, 0.05 g K_2HPO_4, 20.0 mM $NaHCO_3$, 10.0 mM thiosulfate and Agar 15.00 g and the pH were adjusted to 6.5 to 6.8 to a volume of 1 litre in a sterile environment.

2.5 Assessment of Culture Magnetism

The isolates were tested for their magnetotactic response by spreading of their growth on the surface of a semisolid medium towards magnetic field [17]. The isolates were inoculated in a straight line at the centre of the medium in petriplates. The plates were incubated in a magnetic field created by placing the opposite poles of two different bar magnets on either side perpendicular to the inoculums. The growth pattern after incubation was observed for any spreading towards the magnet pole (Fig. 5).

2.6 Light Microscopy Studies

The swimming behaviour of magnetotactic bacteria were investigated by the "hanging drop" method using a light microscope (Motic BA 210) [18]. A drop of magnetically collected bacterial cells was placed on a cavity slide and the south pole of a bar magnet was placed near the drop. Magnetically collected cells were accumulated at the edge of the drop closed to the bar magnet (Fig. 3). The morphological analysis of magnetically collected cells was examined by gram staining.

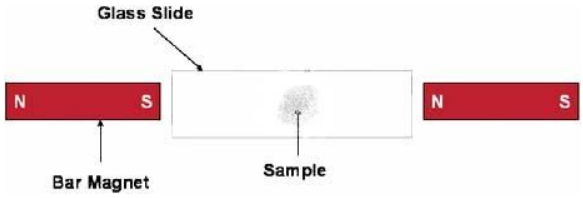

Fig. 3 Schematic diagram of Light microscopy studies

3. Results and Discussion

Magnetically collected bacterial cells were isolated from the sediment samples of river Tamiraparani, Thirunelveli District, Tamilnadu State, India (Latitude 8°42'15.16" N Longitude 77°21'49.23" E) by magnetic collection and modified race track method for purification. Then the magnetically collected bacterial cell was subjected to streak plate method and observed under light microscope.

In the light microscopic observation, the magnetically collected bacterial cells aligned and parallel to each other along the magnetic field lines and showed a migration towards the south pole of the magnet wherever it was placed (Fig. 4a and b). Gram staining results indicate that the isolated magnetotactic bacteria are gram negative and rod or oval in shape (Fig. 5).

On the streak plate method of observation, the magnetically collected cells when placed in a magnetic field showed migration towards the south and north poles. In the absence of magnetic field they did not show any migration (Fig. 6a and b).

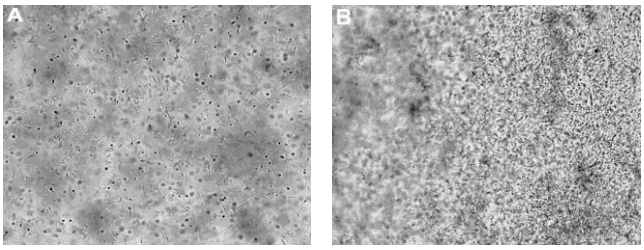

Fig. 4 Light microscopic observation of the magnetically collected bacterial cells: a) without magnetic field and b) with magnetic field.

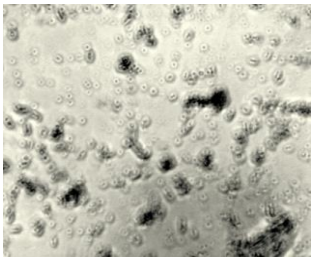

Fig. 5 Light microscopic observation of magnetically collected bacterial cells after gram staining.

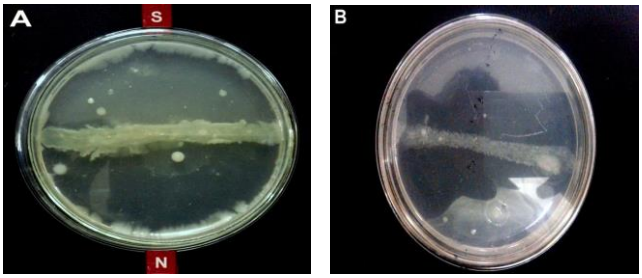

Fig. 6 Streak plate method of observation: a) presence of magnetic field and b) absence of magnetic field.

Magnetotactic bacteria are able to distinguish between south and north poles of magnet and migrate along the geomagnetic field; this behaviour is known as magnetotaxis. Magnetotactic bacteria was collected and isolated from river Tamiraparani by using race track purification method. Difficulties in isolating and cultivating of magnetotactic bacteria arise from their lifestyle, which is adapted to sediments and chemically stratified aquatic habitats. Light microscopy and streak plate method of observation is the most used technique to detect magnetotactic bacteria in environmental samples. The discovery of magnetotactic bacteria by Blakemore [2] was based on the observation of the motility of bacteria in the local magnetic field. Streak plate method of observation revealed that the cells are attracted to both poles of magnet and light microscopic observation revealed the movement of magnetically collected bacterial cells towards both poles.

In our observation magnetotactic bacteria moved towards both the north and south pole in response to magnetic field. Normally, the polarity of magnetotactic bacteria living in the northern hemisphere is north-seeking and in the southern hemisphere is south-seeking [19], magnetotactic bacteria near the geomagnetic equator are both south-seeking and north-seeking [20]. As the study area is nearer to the equator and in sub-tropics, the magnetically collected bacterial cells could be south seeking as well as north seeking as reported in the studies of Frankel et al., (1981). Magnetotactic bacteria are gram negative and cell structure include rods, vibrios, spirilla, cocci, and ovoid bacteria as well as giant and multicellular [10].

From this investigation magnetically isolated bacterial cells (MTB) from river Tamiraparani are rod and oval in shape, which are coccus and bacillus and are gram negative.

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4. Conclusion

From our study it is concluded that the magnetotactic bacteria are present in River Tamiraparani is confirmed and a thorough studies using scanning electron microscope, transmission electron microscope for the structural analysis and formation of magnetosomes and gene sequencing for the conformation and identification of the species is necessary, which will be made in our future studies.

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