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RESEARCH ARTICLE

ISOLATION, IDENTIFICATION OF MAGNETOTACTIC BACTERIA AND THEIR MAGNETOSOMES FROM LONAR LAKE

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ABSTRACT

Magnetotactic bacteria (MTB) compose a group of diverse, motile prokaryotes that have the ability to passively align themselves along earth's geomagnetic field due to the presence of intracellular organelles called magnetosomes. From literature study we came to know that Lonar lake is a rich source of diverse species of magnetotactic bacteria. So we selected this ecosystem for the isolation of magnetotactic bacteria. We collected water samples from the Lonar Lake and were used for the isolation of magnetotactic bacteria. In the present work, we isolated the magnetotactic bacteria, from lonar lake sample and confirmed it by using hanging drop technique and Agar plate method. Their response to a magnetic field was observed employing the 'hanging drop technique' under a microscope and use of a semisolid medium. The bacteria showed a typical response in the form of movement towards the respective magnetic poles and precise alignment at the edge of hanging drop. Biochemical identification of isolated magnetotactic bacteria was done by performing various tests like Gram's staining, Catalase, oxidase, nitrate reduction, sugar fermentation test, starch hydrolysis, casein hydrolysis, growth at different temperatures and pH, etc. Also, magnetosomes were isolated from these magnetotactic bacteria and which was confirmed by FTIR analysis to ensure the presence of Fe-O group.

Key words: Magnetotactic bacteria, Hanging drop technique, Agar Plate method, Biochemical Identification Magnetosome, FTIR.

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INTRODUCTION

Magnetotactic bacteria which orient and swim along a geomagnetic field have been found in fresh and marine sediments. Richard Blakemore firstly discovered the magnetotactic bacteria. (Tadashi Matsunaga[1],Kohji Hashimoto[1],Noriyuki Nakamura[1],Kayoko Nakamura[2], and Shozo Hashimoto[2]). Magnetotactic bacteria abundantly inhabit diverse environments (especially aquatic) such as lakes, rivers, ponds, estuaries, soils as well as some extreme ecosystem. (Hoda Karbary, Mohamed F.Eida). Magnetotactic bacteria (MTB) are a heterogeneous group of aquatic microorganisms that have an ability to orient and migrate along geomagnetic field lines, a behaviour referred to as "magnetotaxis". This property is based on specific intracellular structures, the magnetosomes which, in most MTB are nanometre-sized; membrane bound magnetic particles, composed of the iron mineral magnetite (FeO3) or rarely greigite (Fe3S4). These magnetosomes are organized in one or more straight chains parallel to the long axis of the cells. Such an arrangement confers a magnetic moment to the cell. (South Seeking Magnetic Bacteria from Lonar Lake: Mahesh S. Chavadar and Shyam S. Bajekal).

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MTBs have reported to have important applications in determining south magnetic poles in meteorites and rocks containing fine-grained magnetic materials. MTBs are been used in easy separation of blood cells like granulocytes and monocytes by introducing MTBs in the cells by phagocytosis. (Tadashi Matsunaga[1],Kohji Hashimoto[1], Noriyuki Nakamura[1],Kayoko Nakamura[2], and Shozo Hashimoto[2]). Magnetic particles isolated from magnetotactic bacteria have been used for enzyme immobilization (Matsunaga and Kamiya 1987). Magnetosomes attracted much attention for biotechnological applications such as those in magnetic resonance imaging (MRI), magnetic hyperthermia, and drug delivery (Edouard Alphandery). In the present study, Lonar Lake water sample was analyzed for the presence of magnetotactic bacteria and effective method was developed to isolate magnetotactic bacteria from Lonar Lake. Pure isolates obtained were characterized on the basis of morphology, motility, and various biochemical tests. Furthermore, the intracellular magnetosomes produced by these magnetotactic bacteria were extracted and was studied by the FTIR spectroscopy to characterize the functional groups of magnetosome membrane and existence of Fe-O group.

MATERIALS AND METHODS

Collection: Water sample was collected from Lonar Lake, Buldhana dist., Maharashtra, India, in a presterilized plastic

bottle. The pH of the water was checked with the help of pH strips. Beaker containing Lonar Lake water was placed in between South and North poles of bar magnets for 48hrs at room temperature. The water settled near the two respective magnetic poles was collected in two separate test-tubes. The test-tubes were named as N and S according to the water sample collected from the North and South poles of magnet respectively.

Enrichment of bacterial samples

Four 250ml flasks each containing 50ml Alkaline peptone broth (pH=9.5 to 10) were taken. 1ml of different test water samples (collected from north side, south side, north and south side and only broth as control) were inoculated in different flask. These 4 flasks were kept for incubation at 37^0C for 48 hrs.

Isolation of magnetotactic bacteria

Each bacterial sample was streaked horizontally in the centre on separate agar plates containing alkaline peptone agar (pH 9.5 to 10). These plates were incubated at 37^0C for 48hrs in the presence of respective magnetic poles. After incubation different isolated colonies of bacteria which were dragged towards the magnetic poles were streaked on separate Alkaline peptone agar (pH=9.5 to 10) plates. These plates were kept for incubation at 37^0C for 24hrs.

Identification of MTB by using :-

- **Hanging drop technique**
- **Agar Plate Method**

Motility test by hanging drop technique

Hanging drop method was done to determine the swimming manner of the magnetotactic bacteria. Magnetotaxis of the microorganisms was observed by placing the bar magnet on the stage of the microscope (Richard Blakemore).

Motility test on agar plate

A loopful of bacterial culture from each ependorff was streaked horizontally in the centre of separate Petri plates containing alkaline peptone agar (pH=9.5to10). The plates were incubated for 48hrs at 37^0C in the presence of magnetic field. (Mahesh S. Chavadar and Shyam S. Bajekal).

Biochemical identification of isolated MTB

Various biochemical tests like Gram's stain, IMViC, catalase, oxidase, nitrate reduction, sugar fermentation test, growth at different pH and temperature, reaction with HCl and H_2SO_4 etc. were carried out for isolated MTB. The results of each biochemical test for each sample were noted down.

Extraction of iron (Fe)

Each isolated magnetotactic bacterial culture was analyzed for iron particle extraction. In this, media that contained iron bounded in the form of ferric chloride (pH=9) was used. This broth was distributed in different test tubes. Each tube contained 10ml of the broth. A loopful of each isolated magnetotactic bacterial cultures were taken and inoculated in separate test tube. A non- inoculated tube was kept as control. These tubes were incubated at 37^0C for 72hrs. The extracted free iron (Fe) was confirmed by two chemistry based methods

Reaction with H_2SO_4 : Free iron vigorously reacts with H_2SO_4 to produce iron sulphates. It is an exothermic reaction. A green-violet color is obtained.

$$Fe_{(s)} + H_2SO_{4\,(aq.)} \longrightarrow FeSO_{4\,(aq.)} + H_{2\,(g)}$$

In this test, 1ml of test sample from each tube is taken in separate ependorff tubes. 2-3 drops of H_2SO_4 was added in each ependorff. The ependorff tubes were observed for colour change.

Reaction with HCl: Free iron reacts with HCl to produce pale green iron chloride. Exposure to air oxidizes iron (II) to $FeCl_3$. $FeCl_3$ is a yellow-orange color.

$$Fe_{(s)} + 2HCl_{\,(aq.)} \longrightarrow FeCl_{2\,(aq.)} + H_{2\,(g)}$$

In this test, 1ml of test sample from each tube is taken in separate ependorff tubes. 2-3 drops of HCl was added in each ependorff. The ependorff tubes were observed for colour change.

Isolation of Magnetosomes from magnetotactic bacteria

Two types of isolates (magnetotactic bacteria) were obtained. These magnetotactic bacteria were grown for 48hrs. After this the culture was centrifuged at 4◦C for 8000g, and washed with distilled water. The cells were then digested with 5M NaOH for 12hrs. And washed with distilled water until the supernatant fluid becomes clear. Magnetic particles were collected by centrifugation. (Tadashi Matsunaga and Shinji Kamiya). Isolated pellet as well as supernatant of each isolate (Yellow & white) was then further utilized for analysis of Fe-O bonding and presence of magnetosomes.

Confirmation of isolated magnetosomes by FTIR

The surface nature of extracted magnetosomes was studied by the FTIR spectroscopy to characterize the functional groups of magnetosomes membrane.

RESULTS

Four flasks each containing 50ml of alkaline peptone broth (pH=9) and respective water samples were kept for incubation and were observed for growth after 48hrs. The growth was determined by looking for the turbidity in the flasks. Growth was observed in the three flasks which were inoculated with respective water sample. Growth was not observed in the control flask which was uninoculated. 0.1ml of culture from flask was spread over the alkaline peptone agar plates & incubate at 37^0C for 24 hrs and colonies were selected from this plate. Two types of isolates (white & yellow colour colony) were obtained (Fig 2) & Colony characteristics were noted down of isolated colonies from each plate. (Table1).
Motility of the two isolated bacteria was checked by using hanging drop technique (Fig. 3& 4) and motility on agar plate by streaking the colony at the middle of plate. Isolated bacteria had shown precise alignment at the edge of the hanging drop. It was also observed that some colonies on the plates kept in the magnetic field were dragged towards the respective magnetic poles. (Fig.5& 6).

- **Results of reaction with $H_2SO_{4\ (aq.)}$** : The formation of green or greenish- yellow color gave the confirmation of presence of free iron (Fe). Thus it was concluded that iron was extracted in the samples that showed the color change. (Fig.7).
- **Results of reaction with HCl:** - Free iron reacts with HCl to produce pale green iron chloride. Exposure to air oxidizes iron (II) to $FeCl_3$. $FeCl_3$ is a yellow-orange color. Thus it was concluded that iron was extracted in the samples that showed the color change. (Fig.8)

Fig 1. Isolation of MTB

Fig2. Two different isolates (isolate 1& isolate2)

Hanging drop technique

Fig 3. Before placing magnet

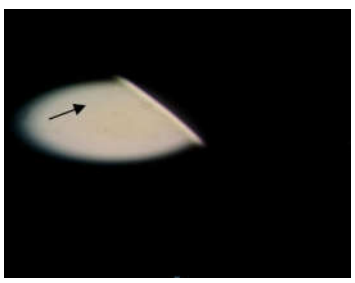

Fig 4. After placing magnet

Plates kept in the magnetic field

Fig.5. Plate kept in presence of both magnetic poles

Fig.6. Plate kept in presence of north pole

Fig.7. Reaction with H_2So_4

Fig.8. Reaction with HCl

Colony characteristics

Two different bacterial colonies were selected from different plates which were observed to be dragged towards the magnetic poles and the results were as follows: - (Table1)

Table 1. Colony Characteristics of both the isolates

Sr.No.	Character	Isolate 1	Isolate 2
1.	Shape	Circular	Circular
2.	Margin	Smooth	Smooth
3.	Elevation	Concave	Concave
4.	Opacity	Opaque	Transparent
5.	Colony color	White	Yellow
6.	Motility	Motile	Motile
7.	Arrangement	Pairs	Chains

Various biochemical tests, such as Indole test, methyl red test, citrates utilization test, motility test, Gram's staining, catalase, oxidase test etc., were performed over the pure isolates of magnetotactic bacterial cultures. The results of the various biochemical tests were as follows: - (Table 2).

Table 2. Results of Biochemical tests

Sr. No.	Name of the Test	Isolate – 1	Isolate-2
1.	Gram's staining	Gram positive rods	Gram positive rods
2.	Confirmation of free iron		
	a)Reaction with H_2So_4	Positive	Positive
	b)Reaction with HCl	Positive	Positive
3.	Catalase test	Positive	Negative
4.	Oxidase test	Positive	Negative
5.	Nitrate reduction test	Negative	Negative
6.	Starch hydrolysis	Positive	Positive
7.	Casein Decomposition	Positive	Positive
8.	Sugar fermentation test		
	a) Lactose	Negative	Negative
	b)Sucrose	Negative	Negative
	c) Fructose	Negative	Negative
	d) Glucose	Negative	Negative
9.	Growth at different pH		
	a)At pH 3	Negative	Negative
	b) At pH 5	Positive	Negative
	c) At pH 7	Positive	Positive
	d) At pH 9	Positive	Positive
10.	Growth at different temperature		
	a)At 35 ◦ C	Positive	Positive
	b) At 37◦ C	Positive	Positive
	c) At 50◦ C	Negative	Negative
11.	Indole production	Positive	Positive
12.	Methyl red	Negative	Negative
13.	Voges-Proskauer	Negative	Negative
14.	Citrate	Positive	Positive

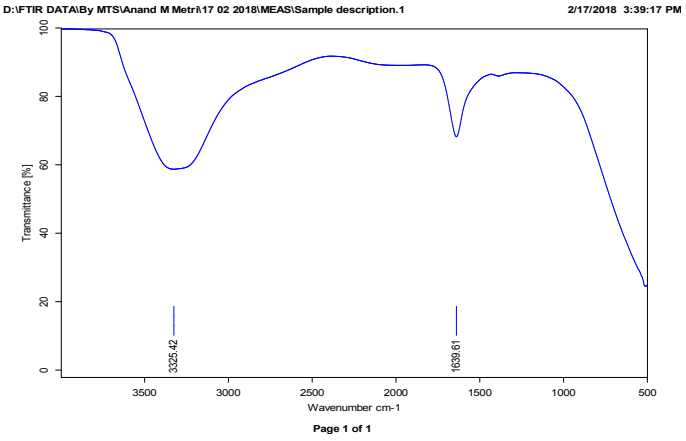

Fig. 9. FTIR spectra of extracted magnetosomes

Magnetosomes were isolated from obtained isolates of magnetotactic bacteria. The FTIR analysis was carried out to find out the Fe-O bonding in the magnetosomes. The obtained FTIR spectrum revealed the existence of H-O and N-H group at 3850 and 3325.17 cm^{-1} respectively. The peaks at 2950 cm^{-1}, 1639.38cm^{-1}, 1190 cm^{-1} can be assigned to the presence of C-H, C=C and C-C group, correspondingly. Additionally the weak peak observed at 750.00 cm^{-1} was attributable to Fe-O group, while vibration band at around 610 cm^{-1} along with a small band at 547 cm^{-1m} is due to the Fe- O bending mode. In this respect, Fe-O group increases the probability of presence of magnetosomes in the isolated MTBs. While the presence of other organic groups confirm the synthesis of a protein membrane, coating the magnetosomes. (Fig.9)

DISCUSSION

Magnetotactic bacteria navigate along the magnetic field this process is known as magnetotaxis **(**Farhad Farza). On the semisolid medium too, the MTB when placed in a magnetic field showed pronounced migration towards the south pole referred as south seeking, while in the absence of magnetic field they did not show any migration towards any pole (Mahesh S. Chavadar). The isolated cultures were tested for their magnetic properties by the hanging drop technique and the semisolid medium method (Mahesh S.Chavadar). The biochemical properties and effect of various temperature and pH on growth of isolates were studied (Vidya Karande & Rupali Patil). The magnetosomes were isolated from MTB by using Centrifugation technique (Tadashi Matsunaga and Shinji Kamiya). The surface nature of extracted magnetosomes was studied by the FTIR spectroscopy characterize the functional groups of magnetosome membrane (Hoda Kabary[1] ,Mohamed F . Eida). From the present work it is concluded that MTB were successfully isolated from Lonar Lake.

Conclusion

Isolated MTB was confirmed by using Hanging drop technique and Agar plate method. On the semisolid medium too the MTB when placed in magnetic field showed pronounced migration towards both the magnetic poles. Biochemical identification of isolated MTB was performed by using different biochemical test like catalase, oxidase, sugar fermentation, IMViC, Grams nature, starch hydrolysis, nitrate reduction etc. The biochemical properties and effect of various temperature and pH on growth of both the isolates were studied systematically. It is shown that the change in temperature and pH influences the growth as well as magnetic response of cultures. The growth of MTB was not seen at higher temperature. Magnetosomes particles were successfully isolated from MTB and confirmed by using FTIR analysis as it shows presence of Fe-O bonding at wavelength 750.00 cm^{-1}. The result gives the direction for future work, that isolated magnetosomes can be used further for enzyme immobilization. (Tadashi Matsunaga and Shinji Kamiya).

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