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Collection, Isolation and Characterization of Magnetotactic Bacteria From Fresh Water Lake Sediment

Science

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Vidya A Karande	Center for Interdisciplinary Research, D. Y. Patil University, Kolhapur - 416 006, [MS] India.
Rupali N Patil	Center for Interdisciplinary Research, D. Y. Patil University, Kolhapur - 416 006, [MS] India.
Saral J Ghosh	Center for Interdisciplinary Research, D. Y. Patil University, Kolhapur - 416 006, [MS] India.
Shivaji H Pawar	Center for Interdisciplinary Research, D. Y. Patil University, Kolhapur - 416 006, [MS] India.

ABSTRACT

Magnetotactic Bacteria (MTB) is important resource for extraction of nanomagnet. It is a major constituent of natural microbial community and plays a significant role in biomineralization. The chemical analysis and biodiversity of the Rankala lake environment have been studied. In this study, collection method was designed to isolate MTB from Rankala lake sediment based on their magnetotaxis. The obtained four isolates were cultivated in modified enrichment medium at 260 C with microaerophilic condition under the field of permanent magnet. The results of Atomic Absorption Spectroscopy (AAS) estimated the iron content of isolates is 10 times more than the nonmagnetic bacteria. The present study reports the significance of combined approach of simple magnetic assembly and modified enrichment medium for cultivation of magnetotactic bacteria. Isolation and mass cultivation of magnetotactic bacteria for magnetosomes have been great potential in nanotechnology.

Introduction

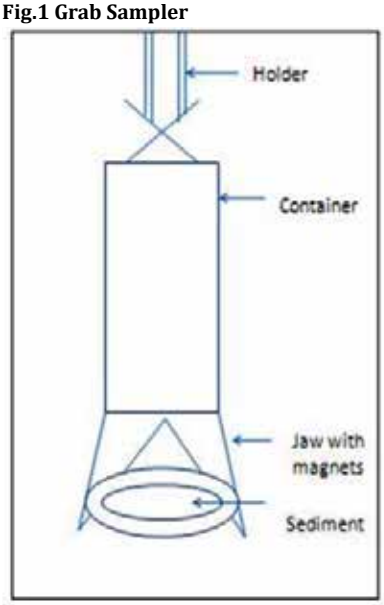
MTB is a unique prokaryote contains a special cytoplasmic inclusion as magnetosome and shows response to magnetic field lines. MTB is noted first in 1963 by Bellini and then move widely reported by Blakemore in 1975. The unique character of MTB is their magnetotaxis due to the presence of magnetosomes having highly defined morphology and narrow size distribution for each strain indicating a large degree of genetic control over the process. The MTB have been found varieties of morphologies such as cocci, rod, vibrio, and spiral shaped and in diverse bacterial families such as Nitrospira phylum-alpha, beta and gamma proteobacteria. MTB is globally distributed under the oxic-anoxic interface in aquatic environment (Wolf 1987). In present study, selected Rankala Lake has elevation of 550M above mean sea level and situated on 16° 42'N altitude and 74° 14' E longitudes.

Nanoscale magnetosome have unique characteristics and potential advantages as compare to chemically produced Magnetic nanoparticles. MTB represents a new bioconsortium which offer an ideal model for bioimonic and biomagnetism. (Staniland et al., 2009, Pawar et al, 2011). The unique and superior characteristics of MTB and magnetosome will greatly facilitate their future application in biomedicine, geobiology and Nanobiotechnology that relay on the availability of large amount of bacteria (Patil et al 2011). Thus, the study of morphology and physiology of MTB is useful to generate new insights in Nanobiotechnology. In this initial study, a designed grab sampler is used to collect MTB from Rankala Lake. The cell morphology, motility, growth features, iron content and magnetic behavior characteristics were studied systematically.

Materials and Methods

Designing of Grab Sampler to collect sediment sample for MTB The grab sampler was constructed with two spring loaded jaws which was made from of a magnet, holder and container, when it triggered then it is shut and dig into the sediment. The sediment was collected with magnetic field from upper sediment layer of Rankala Lake. The sediment and water in a proportion of approximately 1:2 were transferred in loosely capped bottles and these microcosms left undisturbed for several weeks under dim light at room temperature (Fig.1).

Fig.1 Grab Sampler



The sediment was collected with grab sampler with magnetic field from upper sediment layer of Rankala Lake. The sediment samples were gathered in plastic bottles (previsiouly disinfectant) of about 1 liter capacity with slightly loose lids. All samples were transported to the laboratory under ambient temperature conditions and stored in dark.

Enrichment of MTB cultures

During sampling, initial concentration of MTB in sediment is significantly low and therefore the modified enrichment medium was designed to survive and increase the number of Magnetotactic consortium (Kundu et al 2010).The lake water sediment was diluted in the proportion of 1:10 with water. It was then filtered through 0.45 micrometer filter membrane to obtain lake water extract. The enrichment medium consisted of 100 ml of lake water sediment extract, 0.037 gm of Tartaric acid, 0.037 gm of succinic acid, 0.012 gm of sodium nitrate, 0.005 gm of sodium acetate, 0.0035 gm of ascorbic acid, mineral solution 1ml, vitamin solution 1 ml, 0.1 gm of peptone, 0.5 ml of 0.01M

ferric quinate, 0.1gm of yeast extract, 0.05 gm of sodium chloride and 0.05gm of sodium thioglycolate (all media component purchase from Himedia, Bombay). The pH of the medium was adjusted to 7.0-7.2. The medium was autoclaved at 121˚C for 20 min.The collected sample (20 ml) was inoculated in 100 ml of enrichment medium incubated at room temperature in dark for 7 days. Initially, 2 ml enriched samples were distributed in each 20 ml of enrichment medium in capped bottles and incubated same as previously described.

Enrichment and Purification of the MTB with modified Capillary Race-Track (CRT) method This method was used to enrich and purify the MTB with Capillary Race-Track (CRT). It was constructed with a reservoir (100 ml capacity) connected to a capillary tube. The enriched sediment was filled in the reservoir and exposed with permanent magnetic field for 1 hr. A capillary tube (length 6-9 cm) sealed at one end in a glass flame was filled with the modified enrichment medium (described above) by hypodermic syringe (Schuler et al., 1999., Chavadar et al., 2008).The MTB migrated through the cotton plug to words the closed end of the capillary. The tip containing the accumulated MTB was then broken off and the organisms were transferred to the cultivation medium with the help of a sterile hypodermic needle.

Magnetic Assessment of MTB cultures

The culture was tested for their magnetotactic response by two ways. In the first way hanging drop technique under an optical microscope with the south pole of a bar magnet being placed about 10 cm distant distance from the slide. In the second way magnetic response was tested in terms spreading of their growth on the surface of a semisolid medium containing 0.5% agar and motility response. The growth pattern after incubation was observed for any spreading towards the magnetic pole. The obtained cultures were further separated by streak plate method and purified isolates was processing for the CRT and hanging drop technique to confirmation test (Fig.2).

Fig.2 Schematic experimental representation of enrichment, isolation and magnetic assessment of isolate

Optimization of Growth Parameters

The optimization of cultivation parameters was done for growth of MTB. The cell responses to pH, temperature, incubation period, $^{0}_{2}$ concentration and chemical reducing agent were determined. The isolation medium was constructed for growth of MTB. Four purified isolates of MTB were selected and named as isolates 1, 2, 3 and 4 for study of biochemical properties based on magnetic assessment. These four isolates were grown on modified cultivation medium in microaerophilic conditions and incubated at room temperature for 48 h.

The morphology and biochemical properties of these isolates were studied. The magnetic response of isolates was evaluated under different conditions (Starr et al., Joyce et al., 1986). The effect of physical parameters such as temperature, pH and incubation period on the growth and magnetism were tested. The biochemical properties of organisms involving catalase, oxidase, and nitrate or sulphate reduction ability were estimated (Bazylinski et al., 1990, 1993). Nitrate reduction in cultivation media was determined with alpha-naphthyl amine and sulphanillic acid. Oxidase test was performed by scrubbing the growth on filter paper with oxidase reagent. The Catalase activity was determined by examining the gas evolved by interaction of growing cells with 3 to10% H2O2 solutions.

Estimation of Iron content of isolates

The isolated MTB cultures and a known non magnetic bacterial culture (Escherichia coli) as control were cultivated in the ferric quinate (iron source) containing medium. The sufficient cell mass was digested by Triacid wet digestion method. For iron analysis, 1 gm of wet microbial mass added with sulphuric acid, perchloric acid and nitric acid (9:12:1).The mixture was heated until all of acid had boiled off. The resulting clear, yellow cell digest was taken up in 1 ml of 1N hydrochloric acid and diluted into 10:90 ml with deionised distilled water (DDW) before analysis. The iron content of the cell mass was determined by Atomic Absorption Spectroscopy (AAS). A reagent blank and control non MTB (Escherichia coli) was prepared in the same manner.

Results and Discussion

The biodiversity of Rankala lake environment is found to be dynamic and peculiar. The nutritive qualities of aquatic environment depend on active microbial metabolism. The different physicochemical characters of lake water were determined (APHA et al., 1981) and shown in Table no.1.

Table no.1 physicochemical parameters of lake water

No	parameter	Unit	Lake water			Upper Sediment layer
			Top	Middle	Bottom	
1	Temperature	°c	23.5	22	21	21.5
2	pH	-	8.5	8	6.2	6.9
3	Total Suspended solids	mg/lit	38.8	44.2	48.2	45.8
4	Total Dissolved solid	mg/lit	252.8	298.7	307.5	300.7
5	BOD at 27°c/3days	mg/lit	30.7	40.2	51.5	48.2
6	MPN	No./100ml	>2400			>2400
7	SPC	CFU/ml	8-9×10^{7}			11-12×10^{7}

The oligotrophic and Mesotrophic Lake is more convenient to collect the MTB than the eutrophic. It is seen that the Rankala lake is nutritionally moderate and hence it is belongs to mesotrophic nature than oligotrophic or eutrophic. The microbial community is found to vary with habitat and nature of nutritional conditions.

Isolation and Cultivation of MTB is a complex process as the sample contains mixed Magnetotactic and non Magnetotactic consortium that is difficult to separate. Thus to overcome this problem here novel collection procedure was employed. The sampling for MTB was done from oxic-anoxic zone of lake water column with the help of Grab sampler. It collects sediment only from oxic-anoxic zone while conventional technique unable to collects sediment from specified zone. The advantage with the grab sampler is easy to use and obtain relatively large volumes of desired MTB sample with avoiding the contamination of surface aquatic microflora The stratified oxic-anoxic layer of lake water collected with Grab sampler is found to rich in MTB. The count of MTB from modified enrichment medium is more as it creates selective growth conditions o for MTB and inhibits other microorganism. It acts as a highly effective tool for initial step in cultivation of MTB.

MTB being fastidious and requires natural environment for survival and growth. The similar natural environment was obtained by addition of sediment lake water extract in the medium. Magnetic field environment was designed and implemented while enrichment to facilitate the growth of MTB. The Capillary Race-Track (CRT) was used for further purification of MTB. It is found that Capillary Race-Track (CRT) is helped to separate MTB from mixed microflora.

MTB was successfully enriched from the sediment samples of the Rankala Lake using modified collection and cultivation method. The study was made to find morphological characters as gram nature, shape, size, arrangement, motility, spore formation, Magnetic assessment for four different isolates and are shown in Table no.2.

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Table no. 2 The Morphological characters of MTB Isolates.

No	Character	ISOLATE-1	ISOLATE-2	ISOLATE-3	ISOLATE-4
1	Gram nature	Gram Negative	Gram Negative	Gram Negative	Gram Negative
2	Shape	Rod	Short rod	Rod	Rod
3	size	2-3.4×0.7-0.9 µ	1.1×0.5-0.6µ	1.5×0.1-0.3 µ	1.7-2.1×0.5 µ
4	Arrangement	Pairs	Chains	Pairs	Pairs
5	Colony Color	Pink	Yellow	white	white
6	Motility	motile	motile	motile	motile
7	Spore formation	-	-	-	-
8	Magnetic Assessment	South seeking	South seeking	South seeking	South seeking

(-) Absent

It is revealed that all isolates were gram negative, motile with south seeking assessment. However, there is difference in size, arrangement and colony color of isolates. The pair arrangement of MTB is attributed to the natural tendency of MTB.

The biochemical properties and effect of various temperature and pH on growth of isolates were studied systematically and results are listed in Table no.3 with positive and negative sign that indicated growth and no growth respectively.

Table no.3 The Biochemical properties of MTB Isolates.

No	Character	ISOLATE-1	ISOLATE-2	ISOLATE-3	ISOLATE-4
1	Oxidase Production	-	-	-	-
2	Catalase Production	-	-	-	-
3	Nitrate Reduction	+	+	-	+
4	Sulphate Reduction	-	-	+	-
5	Growth at 10˚C	-	+	-	-
6	Growth at 28˚C	+	+	+	+
7	Growth at 37˚C	-	-	-	-
8	Growth at 50˚C	-	-	-	-
9	Growth at pH 3	-	-	-	-
10	Growth at pH 5	-	-	-	-
11	Growth at pH 7	+	+	+	+
12	Growth at pH 9	-	-	-	-

(+)Growth ;(-) No Growth

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 lower and higher temperature. The effect of magnetic field on the growth of cultures observed that it moved towards the south pole of magnet. All isolates were best grown at ambient temperature and at neutral pH. It was found that the cultures were unable to produce oxidase and catalase.

The effect of various time intervals on the magnetic assessment of isolates with sulphate reduction and nitrate reduction were studied and results are shown in Table no.4.

Table No.4 The effect of various time intervals on the biochemical properties of Isolates.

Organisms	Time Interval											
	24 h				48 h				72 h			
	pH	NR	SR	MA	pH	NR	SR	MA	pH	NR	SR	MA
ISOLATE-1	6.9	-	-	-	6.4	+	-	+	6.3	+	-	+
ISOLATE-2	6.9	-	-	-	6.4	+	-	+	6.3	+	-	+
ISOLATE-3	6.8	-	-	-	6.4	-	+	+	6.5	-	+	+
ISOLATE-4	6.7	-	-	-	6.5	+	-	+	6.3	+	-	+

NR: Nitrate Reduction; SR: Sulphate Reduction; MA: South Seeking Magnetic Assessment

The nitrate reduction performed by all isolates except isolate no.3 which performed sulphate reduction. Magnetotactic property gets to isolates only after 48 hours incubation period. From the Table no.4 it was analyzed that the microaerophilic conditions required for the growth of MTB and further depletion of oxygen favors the magnetosome formation. The reduction of nitrate or sulphate acts as a terminal electron acceptor and creates favorable conditions for magnetic behavior. In iron rich medium metabolic activities of MTB changes the physiological conditions such as pH, O2 level suitable for magnetosome formation.

AAS reveals that the magnetic bacteria accumulate more iron content than the non magnetic cells an observation was first reported by Blakemore et al in1979. There is definite relation between magnetic response and iron content of the cell. The iron content of isolates was determined and results are listed in Table no.5.

Table No.5 The iron content of isolates cultivated on modified medium incubated at room temperature for 48 hrs.

MTB Isolates	Iron content in ppm
Isolate 1	5.090
Isolate 2	9.270
Isolate 3	6.250
Isolate 4	3.540
Control (E.coli)	1.007

The attempts of isolation and cultivation of MTB in pure form have limitations as the sample contains mixed Magnetotactic and non magnetotactic consortium that is difficult to separate. Thus to overcome this problem here novel collection procedure was employed. The Grab sampler collects sediment sample from desired location that is from oxic-anoxic zone of lake water. Then modified media with sediment extract will greatly support the growth of MTB as well as the series of process which help to isolate MTB from natural environment.

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