Magneto bacteria from estuarine, mangrove and coral reef environs in Gulf of Mannar

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Totally 37 strains from the three biota were isolated with predominance of Bacillus spp. followed by Pseudomonas spp., Spirillum spp. and Vibrio spp. Based on the fatty acid profile, a few of the strains were identified as Pseudomonas mendocina, Pseudomonas carophylli and Bacillus cereus. Of the three biota studied, the coral reef harbours higher percentage of magnetotactic bacteria followed by the mangroves and estuaries. The strains were further studied for growth, salinity optimum, magnetotaxis and survival. The bacterial growth was observed as a ring formation between 7 and 14 days of incubation. The distance to the ring from the top surface of culture medium ranged from 0.5 to 4.6 cm. The ring was prominent in a salinity of 20 ppt. Regarding magnetotaxis, a 3 h exposure to the external magnetic field was required for 100% aggregation of bacterial cells. The magnetobacteria isolated from coral reef were able to survive for 5 to 6 months whereas those from mangroves and estuaries survived for 4 and 2 months respectively.

Magnetic bacteria were discovered by Blakemore during 1970's. These bacteria are motile and passively align with north - south poles of magnetic fields. This magnetotactic behaviour is due to the presence of unique iron-rich intracellular crystals called "magnetites" present in cytoplasm of the bacterial cells. Though the magnetite is not necessary for growth or survival of the magnetic bacteria under defined laboratory conditions, the role of the particles in the phenomenon of magnetotaxis is becoming clear. The magnetotactic response may allow the magnetobacteria to locate anaerobic or microaerophilic environments by following the vertical components of earth's geomagnetic field down into the sediments of aquatic environments which are more favourable for their growth. Their magnetotaxis would also cause cells to localize in regions of high magnetic flux surrounding the materials of high magnetic susceptibility particularly iron in their environment. The magnetotactic behaviour is exhibited by spirillum, vibrio, cocci, etc. The recent discovery of a diverse group of such bacteria are collectively referred to as the magnetotactic bacteria. Studies on the magnetobacteria in aquatic environments have been carried out by many workers. There is no such report on magnetobacteria in Indian waters and hence is this work.

The present study was carried out in three different marine environs i.e. station 1—Vellar estuary (lat. 11°29'N and long. 79°46'E); station 2—Pichavaram mangroves (lat. 11°27'N and long. 79°48'E) and station 3—coral reef of the Gulf of Mannar (lat. from 78°08' to 79°35'N and long. 9°25' to 8°35'N). From these stations, sediment samples were collected and dried at 45°C for 48 hours and pulverized. The magnetobacteria were isolated using agar mud medium. The occurrence of magnetobacterium was confirmed by the appearance of white line in the test tubes after a minimum incubation period of 10 days in darkness. The line was like a floating or suspended white ring. The ring was located at a minimum of 1 cm to 4 cm below the top surface of the culture medium. The time taken for the ring formation and the distance from the top surface of the agar mud medium was recorded. A loopful of culture from the ring was taken and streaked on to nutrient agar plate. After 24 h, the isolated individual colonies were then transferred to fresh nutrient agar slants for identification.

The isolates were identified up to the generic level and a few strains were identified up to species level based on the fatty acid profiles of the microbes, using the Microbial Identification System (Hewlett Packards 5890 U.S.A.).
The motility or non motility of the isolated magnetotactic strains was ascertained by microscopic examination of the culture using the hanging drop or wet mount method. 18-24 h pure culture from nutrient broth was transferred to a cover slip, fixed to a cavity slide and observed under a binocular microscope (×1000). A bar magnet was placed on the stage of the microscope in such a way that the south pole of the magnet was in the vicinity of the hanging drop or edge of the mount. Observations were made for every 30 minutes.

To study the effect of salinity on the growth of magnetobacteria in the agar-mud medium, culture media were prepared in three different salinities (5, 20 and 35 ppt.). Each tube was inoculated with known magnetobacterial isolates and incubated at room temperature in darkness. The influence of salinity on the magnetobacteria was studied by observing the ring formation and their intensity.

The test tube cultures containing the magnetotactic population as a white line were selected and incubated at room temperature for a period of 6 months in darkness as such without any protective measures. At the end of the incubation period, the viability of magento-bacteria was tested by streaking a loopful of culture taken out from the ring on to nutrient agar plate and the growth was noted.

A total of 37 strains were isolated from all the three study stations and identified. *Bacillus* spp. (16) were predominant followed by *Pseudomonas* (9), *Spirillium* (7) and *Vibrio* (5). Predominant strains identified were *Bacillus cereus, Pseudomonas mesophilica* and *P. caryophylli*, based on their fatty acid profile. The ring formation was prominent by the magnetotactic bacteria collected at the south pole of the magnetic field than that at the north pole. The magnetobacterial isolates from three different marine biota are shown in Table 1.

At station 1, the time required for the formation of the ring was between 7 and 14 days. The distance to the ring from the top surface of the agar mud medium ranged from 0.5 cm to 1.0 cm. The ring produced by the bacteria of the south pole origin was prominent and clear but not in the north pole (Table 1). At station 2, the period required for the formation of the ring was between 13 and 18 days. The distance between the ring and the top surface of culture medium ranged from 1.3 cm to 1.5 cm. The south pole origin inoculum showed a prominent ring formation but not the north pole inoculum. At station 3, the time required for the ring formation ranged from 8 to 12 days. The distance between the ring and the top surface of culture medium was 1.0 cm and 14.6 cm. Both the inocula of south pole and north pole origins showed prominent ring formation.

The aggregation of the bacterial cells towards the magnetic pole was gradual and increased with time. In general, a minimum period of 3 h exposure to the external magnetic field was required for 100% aggregation of cells. After complete aggregation (within 3 to 4 hours), almost all the bacterial cells lost their motility and became static. Non magnetic cells did not show any preferential alignment. Majority of bacteria were long or short rods, spiral and very few of them were coccoid.

After 10 days of incubation, growth of magnetobacteria (white ring formation) in the media of different salinities was observed. Prominent ring formation was noticed in 20 ppt salinity. Faint ring formations were found in 30 ppt salinity. Very faint and or no ring formation was seen in 5 ppt salinity. All the isolates inoculated were of south pole inoculum.

The magnetobacteria isolated from coral reefs of the Gulf of Mannar were able to survive in the agar mud media for 5 to 6 months whereas the magnetobacteria isolated from estuarine and mangrove habitats showed survival for 2 to 4 months. The estuarine magnetobacteria showed diffused orange zone in the place of white ring in the test tubes after a month of incubation and lost survival after two months.

A white suspended ring was formed much below the top surface of the agar mud medium by the magnetobacteria. The distance between the ring formation and the top surface of the medium varied

<table>
<thead>
<tr>
<th>Stations</th>
<th>No. of isolates</th>
<th>No. of strains forming ring</th>
<th>Mean distance between ring and surface of the medium</th>
<th>Incubation period</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estuary</td>
<td>36</td>
<td>17</td>
<td>0.96 ± 0.34</td>
<td>10.40 ± 1.77</td>
</tr>
<tr>
<td>Mangrove</td>
<td>8</td>
<td>5</td>
<td>1.38 ± 0.07</td>
<td>15.26 ± 1.71</td>
</tr>
<tr>
<td>Coral reef</td>
<td>20</td>
<td>15</td>
<td>1.90 ± 1.98</td>
<td>10.31 ± 1.04</td>
</tr>
</tbody>
</table>

Table 1—Magnetobacterial isolates from estuarine, mangroves and coral reef environs
with different strains of magnetotactic bacteria. The possible reason could be the oxygen tension. Due to low oxygen tension the bacteria formed a ring closer to the surface of the medium (between 0.5 to 1.5 cm). This phenomenon was well exhibited by the magnetotaxic bacteria collected from estuarine and mangrove environs. If the oxygen tension was high, the magnetotaxic bacteria formed the white ring much lower from the surface of the medium (with a range of 2 to 4.5 cm). This was well exhibited by the coral reef magnetobacteria.

The salinity plays vital role in the growth of magnetotaxic bacteria. In media containing 20 ppt salinity, there was a prominent ring formation after 10 days of incubation. In media containing 30 ppt salinity, there was only faint ring formation. This indistinctive ring formation indicates the partial growth of magnetobacteria i.e. partial tolerance to salinity. In media containing 5 ppt salinity, there was no prominent ring formation which indicates the absence of growth and their intolerance to low salinity.

The magnetotaxis of the bacterial strains was confirmed by imposing an external magnetic field on the cavity slide containing the bacterial cultures. The aggregation of the bacterial cells was in the north and south axis of the magnetic field. Similar observation was made by Blackmore. The reason for magnetotaxis was due to the presence of ferromagnetic particles inside the bacterial cells. Although these particles are not essential for growth and survival of the magnetotaxic bacteria under laboratory conditions, their adaptive significance has been suggested. Magnetotactic response might help the bacteria to locate anaerobic or microaerophilic environment which is more favourable for their growth. Of the various strains observed, long rods and spiral forms showed higher magnetotactic activity as was evident by their complete aggregation towards the magnetic pole within a short time. The velocity of magnetotactic bacteria was reported earlier. Cell velocities and motility patterns were determined using videotape technique. The velocity of magnetococci to travel a distance of 400 μm was measured, the average transit time was 5.8 ± 1.1 sec. The Spirillum species exhibited a velocity of 44 ± 8 μm/sec. From these reports and also from the present study, it is concluded that the magnetotactic bacteria are motile forms, showing high motility in the presence of magnetic field.

The survival of the magnetobacteria in the culture media varied. Jar culture with natural waters showed growth and survival for 1 to 2 months. In the test tube culture containing standard agar mud medium, after the appearance of magnetotaxic bacteria populations as a white line in 10 days of incubation, the white line began to fade slightly after 1 month of culture and a more diffuse orange zone appeared just above it. The magnetobacteria survived and persisted in the test tubes atleast for 3 months. In the present study, the magnetobacteria of coral reef habitat survived and persisted in the test tubes containing the agar mud medium up to 6 months. The estuarine and mangrove magnetotaxic populations showed survival for 1 to 4 months. The estuarine and mangrove magnetobacteria showed diffused orange zone in the test tube after a month of incubation. The reason could be the water quality and suspended organic matter. Hence in the test tube, the biodegradation and formation of opaque surface zone would have led complete depletion of oxygen thereby the cells would have lost their viability.

The present study has been limited with the isolation, confirmation and identification of magnetobacteria. Further studies on physiology, ecology and genetics of the magnetobacteria are warranted to reveal their possible role in iron biogeochemistry and in contributing to the palaeomagnetic record of the earth.

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References