A method for monitoring the chemotactic behavior of magnetotactic bacteria

Minoru Wada

MBL summer course student in 1993
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Abstract:
Magnetotactic bacteria are commonly observed in samples from freshwater and marine sediments. However, there have been very few reports of isolation of these bacteria. This is probably due to difficulty in the establishment of suitable microaerophilic conditions for bacterial growth in the laboratory and fastidious nutrient requirements. Any information of physiological and behavioral responses of the bacteria would thus be useful for development of an isolation procedure. The present study was begun in an attempt to monitor the chemotactic behavior of the magnetotactic bacteria. Using a capillary racetrack method (R.S.Wolfe et al.1987), magnetotactic bacteria were collected from a freshwater sediment sample, which contained many magnetotactic cells. The collected cells were concentrated by centrifugation and then introduced into a flat capillary sealed with vaspar and agar containing a substrate to be tested. The capillary was left on a flat surface at right angles to a magnetic field. Under this condition, most of the magnetotactic cells were actively moving for more than 20 min and easily observed under the optical microscope. In the presence of citric acid, propionic acid, succinic acid and tartaric acid (10mM each in agar), most of the magnetotactic cells were located away from the agar end in comparison to the control. Yeast extract (0.1%) did not cause chemotactic response of the cells. Although the present results do not show clear indication of chemotactic behavior, this capillary system would be useful for assessing the behavioral responses in magnetotactic bacteria.
Introduction:

Magnetotactic bacteria differ from all other bacteria in their unique ability to swim along the lines of a magnetic field. Although they are common in marine and freshwater sediments, there have been few reports of isolation of these bacteria. It is probably due to the difficulty in establishment of suitable microaerophilic conditions for their growth in the laboratory and fastidiousness of their nutrient requirements. Any information of physiological and behavioral responses of the bacteria thus would be useful for development of an isolation procedure. The objects of the present study are 1) to establish the assay system for chemotactic responses of the magnetotactic bacteria and 2) to survey the substances which cause chemotaxis on the bacteria.

Methods:

Collecting the magnetotactic cells by the 'race track' method: The freshwater sediment samples, which has been kept in MBL since 1988, were used in this study. The sediment contained many magnetotactic cells, including spirillum, cocci and small rods. A small portion of the sediment suspensions (about 1ml) were applied to the race track capillary tube (R.S.Wolfe et al.1987). The capillary itself was filled with the water from the sediment sample after filtration with 0.22 um filter.

Assay system for chemotaxis: After collecting the magnetotactic cells in the race track capillary, about 100 ul of the cell suspension, were centrifuged and a small portion of the supernatant fluid was removed in order to concentrate the cells. The concentrated cell suspension was introduced into a flat capillary, which is 10 mm long and 0.1 mm in path length. The one end was sealed with vaspar and 1.5 % agar, containing substrate to be tested, and the other end was sealed with vaspar. After putting the north or south end of stirring-bar magnet right in the middle of the capillary for 5 min, the flat capillary was left on a flat surface making a right angle with a natural geomagnetic field for 5 min, and then observed under the optical microscope with "pseudo dark field", also placing the capillary at right angles to the geomagnetic lines. The distribution patterns of the magnetotactic cells in the capillary were observed at least twice within 15 minutes. Chemotaxis for an applied substrates was monitored in a triplicate assay.

Substrates; Four organic acids, including citric acid, propionic acid, succinic acid and tartaric acid (10mM each in agar), and 0.1 % yeast extract (Difco) were used without pH adjustment.

Results and Discussions:

The magnetotactic bacteria observed in the capillary were mostly coccoid type cells. Although the magnetotactic cells seemed to lose their motility gradually during incubation, most of the cells were moving for at least 20 min, under the condition described above in Methods. The magnetotactic cells distributed with a slight peak around the middle, going down almost evenly toward the both sealed ends of the capillary. It is probably
because tube the magnet was put on the right in the middle of the capillary before observation. In the presence of the organic acids, the distribution pattern was changed. The magnetotactic cells in the region close to agar end were apparently less in number, suggesting a repellent response. Yeast extract did not work as a chemo-attractant neither.

Although the present results do not show clear indication of chemo-attractive behavior, this capillary system would be useful for assessing the behavioral responses in magnetotactic cells.

References:


Observation of the interface among Myxobacterial colonies

Minoru Wada

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Observation of the interface among Myxobacterial colonies

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Abstract:

Myxobacteria are very distinctive because of their complex developmental life cycle culminating in fruiting body formation. They glide on media and make swarms. I was thus interested in seeing what would happen if the different types of myxobacteria are put on the same place adjacently. I used 9 myxobacterial isolates, including Myxococcus xanthus, M. fulvus, M. virescens and Coralloccoccus. Most of the initial interface relations among isolates could be summarized into 3 categories: (1) No inhibition (2) Bi-lateral inhibition (3) Uni-lateral inhibition. However, the relationships were dynamic and variable during colony growth. In addition, bi- and uni-lateral inhibitions were observed even with strains of the same species, suggesting that differences in the site from which the bacteria were isolated could affect the interface phenomena. These results may imply a certain degree of territoriality for myxobacteria in nature.
Introduction:

The myxobacteria are Gram-negative, unicellular, gliding bacteria with rod-shaped vegetative cells. They have a very distinctive life cycle, culminating fruiting body formation. The bacteria are also characteristic in their spreading swarms of colonies. Although various types of myxobacteria have often been found in soils and tree barks, knowledge of interaction among myxobacteria is still restricted. The purpose of this study is to get information on the interactions among various types of myxobacteria.

Methods:

Myxobacterial isolates; Myxobacteria were isolated from soil and tree bark by using dung pellets and cycloheximide treatment (Reichenbach, H. and Dworkin, M. 1991). The fruiting body or swarming vegetative cells grown on the primary cultures were transferred to new agar plates, including CY, CTT, 0.1NA and rabbit dung (K/K) medium, at 32°C (Reichenbach, H. and Dworkin, M. 1991). After growing on the agar plates, vegetative cells were reinoculated on to the same medium and checked for purity by monitoring growth in Nutrient Broth at 37°C.

Observation of interface among myxobacterial colonies; Isolated myxobacterial vegetative cells were inoculated on to CY and CTT plates. However, as almost all the myxobacterial isolates used grew better on CY medium than on CTT, most of the observation was done on CY medium. In each combination, two isolates were put on adjacent to each other within 5 mm. During incubation at 32°C, the interface among bacterial colonies was observed periodically under a stereo microscope.

Identification of myxobacterial isolates; To identify the myxobacterial isolates, cells was transferred to K/K and TPM plates, consisted of 10mM Tris-HCl (pH 7.6), 1mM K2HPO4 and 10mM MgSO4.7H2O. The fruiting bodies developed on the media were observed and identified as myxobacterial taxa.

Results & Discussion:

The interface relations were observed in each combination of the myxobacterial cells, after 2-3 days of inoculation on to agar media. Each colony developed a thin, spreading swarms on the agar media, increasing in diameter from the inoculum site. However, the developmental processes of the swarms were different in size and shape among the combinations (Table 2).

In many cases, development of the swarms was retarded at the interface between the colonies, in comparison to that of the combination with the same isolates. Within combinations of the same isolates and also some other combinations, swarms merged each other during the colony growth. In addition, there were some combinations showing the slow development of one colony was heavily inhibited by the adjacent colony at the interface. Most of the initial interface relations among myxobacterial isolates can thus be summerized into 3 major categories; (1) No inhibition, (2) Bi-lateral inhibition, (3) Uni-lateral inhibition.
However, the interface phenomena were variable during colony growth. In some cases, the interface which was judged to be unilateral inhibition went into more like a bi-lateral inhibition, because of the development of swarm spreadings. In addition to that, the observation of either the uni or bi-lateral inhibition in combinations with the same myxobacterial species makes the interpretation of the present results difficult.

The absence of a correlation between the criteria for species of Myxococcus such as in Bergey's manual, and the present results may suggest the possibility that species, as such, in Myxococcus may not exist, but rather that myxococcus may exist in nature as separate clonal populations. Furthermore, the inhibition observed among myxobacterial colonies could be related to their territoriality in natural habitat and explained by production of anti-bacterial substances, as being suggested by D.R Smith & M. Dworkin (unpublished).

References:


Table 1. Myxobacterial isolates used in the study

<table>
<thead>
<tr>
<th>Species</th>
<th>Isolates</th>
<th>Source</th>
</tr>
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<tbody>
<tr>
<td>Myxococcus fulvus</td>
<td># 1</td>
<td>tree bark</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>soil (dung pellet)</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>soil (dung pellet)</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>soil (dung pellet)</td>
</tr>
<tr>
<td>M. xanthus</td>
<td># 1</td>
<td>soil (dung pellet)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>soil (dung pellet)</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>soil (dung pellet)</td>
</tr>
<tr>
<td>M. virescens</td>
<td></td>
<td>tree bark</td>
</tr>
<tr>
<td>Corallococcus sp.</td>
<td></td>
<td>tree bark</td>
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</table>
Table 2. The initial interface relations among myxobacterial isolates

<table>
<thead>
<tr>
<th></th>
<th>M.f.</th>
<th>M.x.</th>
<th>M.v.</th>
<th>C.</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>N</td>
<td>U</td>
<td>B</td>
<td>N</td>
</tr>
<tr>
<td>2</td>
<td>N</td>
<td>B</td>
<td>B</td>
<td>N</td>
</tr>
<tr>
<td>3</td>
<td>N</td>
<td>B</td>
<td>B</td>
<td>N</td>
</tr>
<tr>
<td>4</td>
<td>B</td>
<td>B</td>
<td>B</td>
<td>B</td>
</tr>
</tbody>
</table>

*M.f.: Myxococcus fulvus #1-4  
M.x.: M. xanthus #1-3  
M.v.: M. virescens  
C.: Coralloccocus sp.*

**N; No inhibition  
B; Bi-lateral inhibition  
U; Uni-lateral inhibition  
nd; not determined