Analysis of Magnetotactic Behavior by Swimming Assay

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Prokaryotic organelles called magnetosomes allow magnetotactic bacteria to navigate along geomagnetic field lines. In this study, we modified a swimming assay commonly used to assess bacterial motility to develop a new method of assessing magnetotactic motility. By this method, the swimming assay was performed in an artificial magnetic field. Magnetotactic bacteria formed a wedge-shaped swimming halo that elongated parallel to the magnetic field. Magnetotactic motility was qualitatively assessed by comparing halo shapes. We termed this method the magnetic swimming assay. On the magnetic swimming assay, the mamK deletion strain formed a shorter halo than the wild type, indicating that the assay sensitively detects differences in magnetotactic motility. Moreover, we isolated two spontaneous magnetotactic motility mutants using magnetic swimming plates. Our findings indicate that the magnetic swimming assay is a useful method for the sensitive analysis of magnetotaxis phenotypes and mutant screening.

Key words: magnetotactic bacteria; motility; swimming; cytoskeleton; magnetosome

Magnetotactic bacteria synthesize unique prokaryotic organelles called magnetosomes, which function as cellular compasses for navigation along Earth’s magnetic field.1–5) Magnetosomes have a well-ordered chain-like structure, comprising membrane-enveloped nano-sized magnetic crystals and various specifically associated proteins. Magnetotactic bacteria are generally found at the oxic-anoxic transition zone in aquatic habitats, and magnetotaxis is thought to guide cells downward to less oxygenated regions in aquatic habitats.6,7)

Several methods of measuring cellular magnetism in analyzing the molecular mechanisms underlying the magnetotaxis of magnetotactic bacteria have been reported. The simple methods have been developed using microscopic observation and the image analysis techniques to assay swimming behavior and the magnetic responses of individual living cells.8–10) Although these methods are easy to apply, they cannot be used to measure cell magnetism quantitatively. Magnetism moments have also been determined in cells by light-scattering and birefringence techniques, as reported by Rosenblatt et al.11,12) Schuler et al. developed a light-scattering method to measure magnetism quantitatively, and this is now generally accepted as the standard method of testing the magnetism of magnetotactic bacteria.13) They defined $C_{mag}$ as the ratio of the value of light-scattering in the direction of the magnetic field parallel to the light path to that perpendicular to the light path. This is a fast and sensitive method of monitoring magnetite formation in growing cell cultures. Based on the same principle, Zhao et al. developed a simple measuring apparatus to be used with a spectrophotometer14) (Supplemental Fig. S1; see Biosci. Biotechnol. Biochem. Web site).

Recent progress in molecular biological studies of magnetotactic bacteria have revealed that several tens of magnetosome-associated proteins are encoded in a large genetic island, referred to as the magnetosome island, that is essential for the synthesis of magnetosomes.15–17) These proteins are thought to function in magnetite biomineralization, magnetic sensing, the formation of magnetosome vesicle, and the construction of magnetosomal structures. Although the details of the functions of individual proteins are unclear, comprehensive analysis of the genes encoded in the magnetosome island have provided clues to the mechanisms of magnetosome biogenesis, maintenance, and magnetoreception.18–20) Characterizations of deletion mutants of the individual genes in the magnetosome island have elucidated individual magnetosome-associated protein functions. For example, the MamJ protein is implicated in the control of magnetosome chain assembly. The mamJ deletion mutant shows no change in the synthesis of magnetite crystals, but does not produce a straight magnetosome chain.21,22) Four small proteins, MamG, MamF, MamD, and MamC, control the grain size of magnetite crystals. Transmission electron microscopy (TEM) of mamGFDC mutant cells revealed the presence of small magnetosome crystals of a cuboidal shape that were aligned in irregular, widely spaced chains.23) Thus, the functions of some magnetosome-associated proteins have been clarified.

The mamAB operon, which encodes 18 genes, is highly conserved in several magnetotactic bacteria species, and is essential for magnetosome formation.15,17) Murat et al. generated individual single-gene deletions of the mamAB operon in Magnetospirillum...
They measured the $C_{\text{mag}}$ values of these mutants. Although the $C_{\text{mag}}$ values were diminished compared with the wild type in most of the mutants, the $C_{\text{mag}}$ values did not show clear differences between individual mutants. Thus the light-scattering method is powerful tool to assess cellular magnetism, but it is not enough to analyze or compare the phenotypes of magnetosome associated gene mutants.

The term "magnetotaxis" is used to describe the orientation and migration of bacteria along geomagnetic field lines. Magnetotaxis is thought to separate two phenotypes of magnetosome associated gene mutants. But it is not enough to analyze or compare the method is powerful tool to assess cellular magnetism, between individual mutants. Thus the light-scattering medium,27) under an O$_2$ field lines.25) Magnetotaxis is thought to separate two phenotypes of magnetosome associated gene mutants.

To establish a new magnetotaxis assay, we improved a bacterial swimming assay that assesses bacterial motility. Indeed, the method is needed to assessing both magnetism and motility to investigate magnetotactic behavior.

Bacterial strains and culture conditions. The strains and plasmids used in this study are listed in Table 1. The *M. magneticum* AMB-1 (ATCC 700264) wild type (WT) and the mamK deletion strain (ΔmamK strain) were cultivated in a chemically defined liquid medium, MS-1 medium,27) under an O$_2$ atmosphere at 28 °C in the dark.

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Table 1. Bacterial Strains and Plasmids Used in This Study

<table>
<thead>
<tr>
<th>Strains and plasmids</th>
<th>Genotype and makers</th>
<th>Source or reference</th>
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<tr>
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<td>mamK deletion derivate of WT</td>
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<td>Insertion of control vector (pBBR-tac) in wild type, Gm'</td>
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<tr>
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<td>pBBR-tac</td>
<td>With tac promoter inserting into BAD promoter</td>
<td>This study</td>
</tr>
<tr>
<td>pBBR-tac-mamK</td>
<td>pBBR-tac with mamK fragment inserting into MCS</td>
<td>This study</td>
</tr>
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</table>
fluorescence data were collected using a luminescent image analyzer LAS 3000 (Fujifilm, Tokyo, Japan). The band intensities were quantified using Multi Gauge version 2.2 software (Fujifilm).

For immunolocalization of MamK filaments, cells were grown for 48 h to the early stationary phase, fixed in 2% formaldehyde in phosphate buffered saline (PBS; 140 mM NaCl, 3 mM KCl, 8 mM Na2HPO4, and 1.5 mM KH2PO4) for 10 min at 25 °C. The cells were washed 2 times with PBS and suspended in sterile distilled water. They were treated with varying amounts of lysozyme (5-50 mg/mL) and adhered to the glass slides and dried completely. The slides were immersed in PBS, 0.5% bovine serum albumin for 10 min and then incubated with primary anti-MamK antibody at a dilution of 1:100 in the same buffer for 30 min. The slides were washed twice in PBS and incubated with secondary fluorescein isothiocyanate-conjugated anti-rabbit immunoglobulin G (GEY Laboratories, California, USA) at a dilution of 1:1500.

Fluorescence microscopy images were observed with Eclipse E600 (Nikon, Tokyo, Japan). Images were acquired with a DS-Qi1MC monochrome quantitative digital camera (Nikon) and processed with NIS-Elements software (Nikon) and/or Adobe Photoshop CS2 (Adobe, San Jose, California).

Magnetic swimming assays. Magnetic swimming assays were done by inoculating cells into a semi-solid 0.28% (weight/volume) enriched mineral solution (containing 0.1 g of FeSO4·7H2O in 1,000 mL water, at an iron concentration 0.36 mM), 2 mL of ferric quinate solution and 2 mL of modified ferric quinate solution at final iron concentrations between the inoculated cell site and the magnet. The samples were removed, and fixed in 2% formaldehyde in PBS for 10 min at 25 °C. The slides were washed twice with PBS and suspended in sterile distilled water. The samples were observed by TEM, and the number of magnetite crystals per cell was counted for at least 50 cells.

Spontaneous magnetotaxis mutants were identified by screening using the swim plate. WT cells were inoculated onto the plate and incubated under microaerobic conditions at 28 °C for 7 d. The area between point 1 and point 2 of the swimming halo was transferred to liquid MS-1 medium and incubated under microaerobic conditions at 28 °C in the dark. The culture was inoculated onto a new swim plate and incubated under microaerobic conditions at 28 °C for 7 d. These screening manipulations were repeated until a different swimming halo was observed as compared to the WT. The cell samples were observed by TEM.

To determine the number of magnetosomes per cell in the magnetic swimming assay, the swim plates were divided at 4 points with a 2 cm distance of the inoculated cell site from the magnet. The samples were removed at each point on the cultures with a Pasteur pipette and fixed in 2% formaldehyde in PBS for 10 min at 25 °C. The cells were washed 2 times with PBS and suspended in sterile distilled water. The samples were observed by TEM, and the mean number of magnetite crystals per cell was counted for at least 50 cells.

Magnetic swimming assays were done by inoculating cells into a semi-solid 0.28% (weight/volume) enriched mineral solution (EMSVM) agar plate. To demonstrate magnetotaxis, a neodymium magnet (15 x 15 x 1.5 mm, magnetic flux density = 180 mT) was attached to the edge opposite the inoculation site on the swim plate. The swim plate was put in a 15 °C demonstration magnetotaxis, a neodymium magnet (Shimazu Rika, Tokyo, Japan).

Microscopic observation of swimming speeds in liquid cultures. To assay swimming speed, cultures of the WT, the ΔmamK, and the mamK complementation strain were grown on MS-1 medium under an O2 (1%)–N2 (99%) atmosphere at 28 °C in the dark for 7 d. Then 4 mL of each culture was dropped onto a slide glass and sealed with a cover glass. The swimming of each strain was observed immediately by dark-field microscopy, and the swimming speeds were calculated as the length of the swimming trajectory of individual cells during a 1 s exposure time. All results shown are mean swimming speeds of 50 cells.

Electron microscopic observation of the number of magnetite crystals per cell in liquid cultures. To assay swimming speed, cultures of the WT, ΔmamK, and mamK complementation strains were inoculated in liquid EMSGM at various concentrations of iron (35 μM, 18 μM, and 9 μM) under an O2 (1%)–N2 (99%) atmosphere at 25 °C in the dark. These cultures were grown for 24 h to mid-log phase or 48 h to early stationary phase. The cell cultures were observed by TEM, and the number of magnetite crystals per cell was counted for at least 50 cells.

Electron microscopy. Cells were adsorbed on carbon-coated copper grids. The samples were viewed and recorded with a JEOL JEM 2000EX transmission electron microscope (JEOL, Tokyo, Japan) at an accelerating voltage of 120 kV in bright-field mode. Some of the grids were negatively stained with 2% (weight/volume) uranyl acetate for several seconds.

Results and Discussion

Development of the magnetic swimming assay

The bacterial swimming assay is a simple method of assessing bacterial chemotaxis and motility. In this assay, bacterial cells were inoculated onto a semi-solid medium with 0.2–0.5% (weight/volume) agar. After incubation for several days, the bacteria formed a circular swimming halo. The swimming halo was extended by bacterial flagellar motility. The swimming assay allows for visualization of bacterial motility based on the diameter of the swimming halo. This assay is a commonly used method of assessing the bacterial motility of several species, including E. coli, Bacillus subtilis, Vibrio parahaemolyticus, and Pseudomonas aeruginosa.

In the present study, we modified the swimming assay to assess magnetotaxis qualitatively. Figure 1a shows the swim plate after 7 d of incubation. In the magnetized plates, a wedge-shaped halo formed from the inoculation site to the south pole of neodymium magnet (Fig. 1a). In the nonmagnetized plates, however, the cells formed a circular swimming halo (Fig. 1b). When the nonmagnetic M. magneticum AMB-1 cells were inoculated onto the swim plate, a circular halo formed in the magnetic field (Fig. 1c). Hence we concluded that the wedge-shaped halo demonstrated the magnetic motile property of the magnetotactic bacteria. We labeled this method the magnetic swimming assay.

Why did the magnetotactic bacteria form a wedge-shaped halo? The magnetic field lines are concentrated into a pole of the neodymium magnet. Magnetotactic bacteria migrated in agar medium along the magnetic field to the magnetic pole. Thus the magnetotactic bacteria formed a wedge-shaped halo in the swim plate.

We examined to determine whether the magnetosomes of the cells differed depending on the position of the swimming halo. Figure 1e shows the sampling points where we harvested the cells. The distance between sampling points was 2 cm. The cells were removed, and fixed in 2% formaldehyde in PBS for
The numbers of magnetite crystals at several points in the cells were counted by TEM. As shown in Fig. 1d, the cells collected from point 1 contained only a small number of magnetite crystals (1.0 ± 0.3 crystals/cell, n = 50). This result indicates that the cells at point 1 were nonmagnetic. It has been reported that the culture of *Magneto spirillum* contains small amounts of spontaneous nonmagnetic cells. Such cells are probably attributable to the genetic instability of the magnetosome island. The spontaneous nonmagnetic cells in the culture could not migrate to the magnet, so they formed a circular halo at the inoculation site (Fig. 1e, arrow). The numbers of magnetite crystals per cell in the bacteria at points 2, 3, and 4 were 11 ± 3/60, 15 ± 6/15 crystals/cell (n = 50), and 16 ± 2/16 crystals/cell (n = 50) respectively (Fig. 1d).

These results indicate that the length of the swimming halo depended on the number of magnetite crystals per cell. Cells containing a sufficient number of magnetite crystals migrated from the inoculation site to the magnet along magnetic field lines, and then formed swimming halos at points 2, 3, and 4.

**Isolation of magnetotactic motility mutants using the magnetic swimming assay**

Swimming assay is used to screen for motile and chemotactic mutants. We used the magnetic swimming assay as a screening method to isolate magnetotactic motility mutants. We isolated two spontaneous mutants, mutant 1 and mutant 2 (Fig. 2).

The swimming halo of mutant 1 showed a circular shape (Fig. 2b), while mutant 1 had no magnetite crystals according to TEM observation (Fig. 2e). This result suggests that the circular halo of mutant 1 resulted from a defect in magnetite synthesis. The shape of the swimming halo of mutant 2 was different from that of WT (Fig. 2a, c). The swimming halo near the magnet followed a magnetic field line, while the swimming halo remote from the magnet was not oriented on a magnetic field line (Fig. 2c). According to TEM observation, the magnetite crystal size of mutant 2 was 23.7 ± 1.5 nm (n = 60) (Fig. 2f, h) and that of WT was 47.5 ± 1.3 nm (n = 73) (Fig. 2d, g). *M. magneticum* AMB-1 WT synthesizes single domain paramagnetic magnetite crystals that range from 30 to 50 nm. On the other hand, mutant 2 synthesized the magnetite crystal of less than 30 nm. That magnetite crystals was superparamagnetic. It is likely that due to the superparamagnetic particle in the magnetosomes, mutant 2 showed magnetotaxis only near the magnet in the swimming plate, and they did not orient to a magnetic field in remote areas from the magnet (Fig. 2c). The phenotypes of the two spontaneous mutants in the swimming plate were involved in magnetosome formation. This finding suggests that the magnetic swimming assay is applicable in screening mutants involving magnetotaxis.

**Functional analysis of the MamK cytoskeleton by a magnetic swimming assay**

If the magnetic swimming assay can be used to analyze the phenotypes of mutants of magnetosome-associated proteins, it should prove useful to investigate the functions of magnetosome-associated proteins. We analyzed the phenotype of the *mamK* deletion strain by the swimming assay. MamK is a new member of the...
bacterial actin homologs and is conserved specifically among magnetotactic bacteria. MamK polymerized in filamentous structure in vivo and in vitro. Komeili et al. found MamK filaments running parallel along magnetosome chains in a M. magneticum AMB-1 cell by electron cryotomography. Moreover, they reported that in ΔmamK cell, small groups of a few neighboring magnetosomes separated by large gaps appeared dispersed throughout the cell. They suggested that MamK is important in maintaining the magnetosome chain after the production of magnetosomes, but the details of the function of the MamK filaments remain unclear. To determine the function of MamK, we analyzed the phenotype of the ΔmamK deletion strain by magnetic swimming assay.

Figure 3 shows the results of magnetic swimming assay of the ΔmamK strain. The strain formed a wedge-like shaped halo similar to that of WT. The length of the swimming halo of the ΔmamK strain was shorter than that of WT (Fig. 3a, b), in that the length of the swimming halo of ΔmamK was approximately 70% that of the WT (Fig. 4a). The expression and localization of MamK in the mamK complementary strain were confirmed by immunoblotting (Supplemental Fig. S2a) and immunofluorescence (Supplemental Fig. S2b). The length of the swimming halo of the complementary strain was restored to that of WT (Fig. 3c). These results indicate that the magnetic swimming assay showed differences between ΔmamK and WT, even though there were no significant differences in the Cmag values (Table 2) or the TEM observations (Fig. 3d, e) between ΔmamK or WT. In addition, these strains had similar swimming speeds in liquid medium and had similar sizes of magnetite crystals (Table 2). These results clearly indicate that the magnetic swimming assay is useful in investigating the function of magnetosome-associated proteins in magnetotaxis.

Effects of iron concentration on the swimming halos of the ΔmamK strain
There were no differences in magnetism (Cmag), swimming speeds, or sizes of magnetite crystals as
between WT and ΔmamK strain (Table 2). However, the length of the swimming halo was significantly different as between WT and ΔmamK strain. To determine the reason for this difference, we conducted the magnetic swimming assay at different iron concentrations. The numbers of magnetite crystals decreased in the magnetotactic bacteria with decreasing iron concentrations in the medium.3) We examined to determine the reason for this difference, we conducted the magnetic swimming assay at different iron concentrations. The numbers of magnetite crystals decreased in the WT, ΔmamK, and complementary strains were cultured at 28 °C for 24 h (mid-log phase) and 48 h (early stationary phase) in EMSSGM at different iron concentrations (Table 3). The numbers of magnetite crystals in WT cells were not affected by the iron concentration in the media in the mid-log (24 h) or the early stationary (48 h) phase (Table 3). The WT cells contained 12–14 magnetite crystals at the mid-log phase, while the WT cells contained 16–18 magnetite crystals at the early stationary phase. The numbers of magnetite crystals in the cells were constant at each iron concentration of the media. In the mid-log phase (24 h) and the early stationary phase (48 h), the numbers of magnetite crystals in the ΔmamK cells were almost constant (7–10 particles for 24 h, 15–17 particles for 48 h) at each iron concentration. The numbers of magnetite crystals of the ΔmamK cells at the mid-log phase (24 h) were significantly lower than that of the WT cells, while the numbers of magnetite crystals in the complementary strain were similar to that in WT at both the mid-log phase (24 h) and the early stationary phase (48 h).

We checked the distribution of the numbers of magnetite crystals in these strains by TEM observation (Fig. 5). Although the distribution of the numbers of magnetite crystals in the ΔmamK cells was similar to that of the WT cells at the early stationary phase (48 h), that of the ΔmamK cells at the mid-log phase (24 h)
Table 3. Mean Numbers of Magnetite Crystals per Cells at Each Iron Concentration at Various Growth Phases

<table>
<thead>
<tr>
<th>Strains</th>
<th>The mid-log phase (24h)</th>
<th>The early stationary phase (48h)</th>
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<tr>
<td></td>
<td>Fe 35 µM</td>
<td>Fe 18 µM</td>
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<tr>
<td>WT</td>
<td>13.8 ± 0.7</td>
<td>13.6 ± 0.6</td>
</tr>
<tr>
<td>ΔmamK</td>
<td>9.5 ± 0.9</td>
<td>8.9 ± 0.8</td>
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<tr>
<td>ΔmamK pBBR-tac-mamK</td>
<td>13.9 ± 0.8</td>
<td>13.8 ± 0.7</td>
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</tbody>
</table>

*The mean numbers of magnetite crystals in cells, for which at least 50 cells was measured.

*K Growth phase of each strain of cells.

*Iron concentration of each culture.

differed from that of the WT. Notably, 13–17% of the ΔmamK cells had no magnetite crystals at the mid-log phase (24 h) at each iron concentrations (Fig. 5a, b, c). The distribution of the numbers of magnetite crystals in the complementary strain cells was same as that in WT (Fig. 5).

These results indicate that the numbers of magnetite crystals in the ΔmamK cells decreased at the mid-log phase (Fig. 5, Table 3). The length of the swimming halo of the ΔmamK strain was shorter than that of WT (Figs. 3 and 4). The length of the swimming halo was dependent on magnetite crystals in the cells (Fig. 1d). Hence, the shorter swimming halo of ΔmamK strain was due to the lower numbers of magnetite crystals per cell at the mid-log phase. During magnetic swimming assay, magnetotactic bacteria grew continuously in the semi-solid medium plate and formed a swimming halo, suggesting that the cells grew logistically during this assay.

A previous study of the ΔmamK strain indicated that MamK is dynamically involved in the positioning and assembly of the magnetosome chain. Katzmann et al. suggested that the MamK filament is required for proper magnetosome chain positioning and segregation in Magnetospirillum gryphiswaldense MSR-1. Our results indicate that some ΔmamK cells had no magnetite crystals at the mid-log phase (Fig. 5). The lack of magnetite crystals in the ΔmamK cells was due to the absence of the MamK filament. Thus, MamK filaments might be required to segregate magnetosomes to daughter cells. Because the ΔmamK cells did not segregate the magnetosomes to the daughter cells, some of daughter cells did not orient magnetic field line. Hence the length of the swimming halo of the ΔmamK strains was shorter than that of the WT.

In the magnetic swimming assay, the length of the swimming halo indicated the numbers of magnetite crystals in a cell. In this study, we found that this method can assess the magnetism in magnetotaxis. On the other hand, the swimming assay can assess the bacterial motility based on the elongation of the swimming halo. Accordingly, in the magnetic swimming assay, the wedge-shaped halo can be formed by magnetism and by motility. Therefore, the magnetic swimming assay can...
assess magnetotaxis. By the magnetic swimming assay, it might be possible to isolate magnetotactic motility mutants. Hence the assay is a useful tool to ascertain magnetotaxis.

Conclusions
In the present study, we established a qualitative method of assessing the magnetotactic motility of magnetotactic bacteria. This method is based on a bacterial swimming assay with a magnet. In this method, magnetotactic motility is represented as the length of the swimming halo. We named the method the magnetic swimming assay with a magnet. In this method, magnetotactic bacteria. This method is based on a method of assessing magnetotaxis. It might be possible to isolate magnetotaxis mutants and utilize magnetosomes. It is applicable to the screening of magnetotaxis mutants involved in the magnetosome island. In addition, this method should be useful for assessing magnetotaxis by comparing the swimming halos of the deletion mutants of individual genes involved in the magnetosome island. In addition, this method is suitable for the screening of magnetotaxis mutants and can thus be used to study bacterial magnetotaxis for potential utilization of magnetosomes.

Acknowledgments
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References