RESEARCH ARTICLE

INTERNATIONAL MICROBIOLOGY (2006) 9:267-272
www.im.microbios.org

INTERNATIONAL MICROBIOLOGY

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Cell viability in magnetotactic multicellular prokaryotes

Received 20 July 2006
Accepted 15 November 2006

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Summary. A magnetotactic multicellular prokaryote (MMP) is an assembly of bacterial cells organized side by side in a hollow sphere in which each cell faces both the external environment and an internal acellular compartment in the center of the multicellular organism. MMPs swim as a unit propelled by the coordinated beating of the many flagella on the external surface of each cell. At every stage of its life cycle, MMPs are multicellular. Initially, a spherical MMP grows by enlarging the size of each of its cells, which then divide. Later, the cells separate into two identical spheres. Swimming individual cells of MMPs have never been observed. Here we have used fluorescent dyes and electron microscopy to study the viability of individual MMP cells. When separated from the MMP, the cells cease to move and they no longer respond to magnetic fields. Viability tests indicated that, although several cells could separate from a MMP before completely losing their motility and viability, all of the separated cells were dead. Our data show that the high level of cellular organization in MMPs is essential for their motility, magnetotactic behavior, and viability. [**Int Microbiol** 2006; 9(4):267-272]

Key words: magnetotactic multicellular prokaryotes (MMP) · multicellularity · cell viability · magnetotaxis

Introduction

Magnetotactic bacteria are characterized by the presence of magnetosomes, which are membrane-bound, nano-sized organelles containing magnetic crystals that most likely help the bacteria to navigate along geomagnetic field lines and to find preferred habitats [1]. The magnetosome is a highly regulated biomineral structure composed of either magnetite (Fe_3O_4) or greigite (Fe_3S_4) arranged in one or more linear chains in the cell. A diversity of morphologies, such as cuboctahedral, pseudo-hexahedral, pleiomorphic, parallelepiped, and bullet-shaped, has been described for magnetosomes [1,6].

Magnetotactic bacteria are unicellular, except for the spherical multicellular magnetotactic multicellular prokaryotes (MMPs), which have been described in several locations around the globe, including Araruama lagoon, a hypersaline lagoon near Rio de Janeiro, Brazil [3,4,11]. MMPs are gram-negative and highly motile, as evidenced by their complex and coordinated swimming behavior [4,11]. In addition, they contain electron-dense particles that have been identified as iron-sulfide magnetosomes [1]. In a preliminary phylogenetic analysis of the 16S rDNA sequence of MMPs from Araruama lagoon, the bacteria were found to belong to the class of δ-Proteobacteria [5]. Ultrastructural analysis showed that MMP cells are arranged side by side around an internal, acellular compartment, such that all cells maintain contact with both the external environment and the internal compartment [4]. The cells are tightly bound to each other and their pyramidal shape allows them to fit together to form a spherical organism.

MMPs have an unusual life cycle: each multicellular organism grows by enlarging the volume of its cells; these

cells then divide synchronously so that their number in the MMP doubles. In the next step, the multicellular organism elongates, becomes figure-eight-shaped, and finally splits into two equal spherical multicellular organisms [5]. No individual swimming cell of a MMP has been observed, which indicates that they are multicellular in all stages of their life cycle [5]. The present study shows that this multicellular organization is crucial for the survival of MMPs.

Materials and methods

Sampling and magnetic concentration. Samples consisting of water and sediment (1:2) were collected from Araruama lagoon, the largest hypersaline coastal lagoon in Rio de Janeiro State, Brazil (22° 50′ 21″ S, 42° 13′ 44″ W), and stored in a 10-liter microcosm under dim light and at room temperature. The concentration of magnetic bacteria was determined as previously described [7]. Briefly, samples were put in a specially designed chamber and exposed to a properly aligned magnetic field that oriented magnetotactic bacteria towards the end of a capillary tube, from which the MMPs were collected. The MMPs were imaged by bright field or differential interference contrast microscopy as described below.

Viability assessment. Magnetically isolated MMPs from freshly collected samples were stained using the LIVE/DEAD *Bac*light Bacterial Viability Kit (Molecular Probes Inc., USA) or double-stained with propidium iodide (10 mg/ml) and rhodamine 123 (5 mg/ml) for determination of cell viability. Trypan blue (0.2%) was also used for this purpose. Freshly collected MMPs were treated with azide (0.1%), EGTA, and EDTA (12.5 mM) in sodium cacodylate buffer (0.1 M) and stained with the same dyes.

Microscopy. To visualize the cell profiles, MMPs were stained with 5 μg FM 1-43 (Molecular Probes Inc., USA)/ml. Stained MMPs were placed on

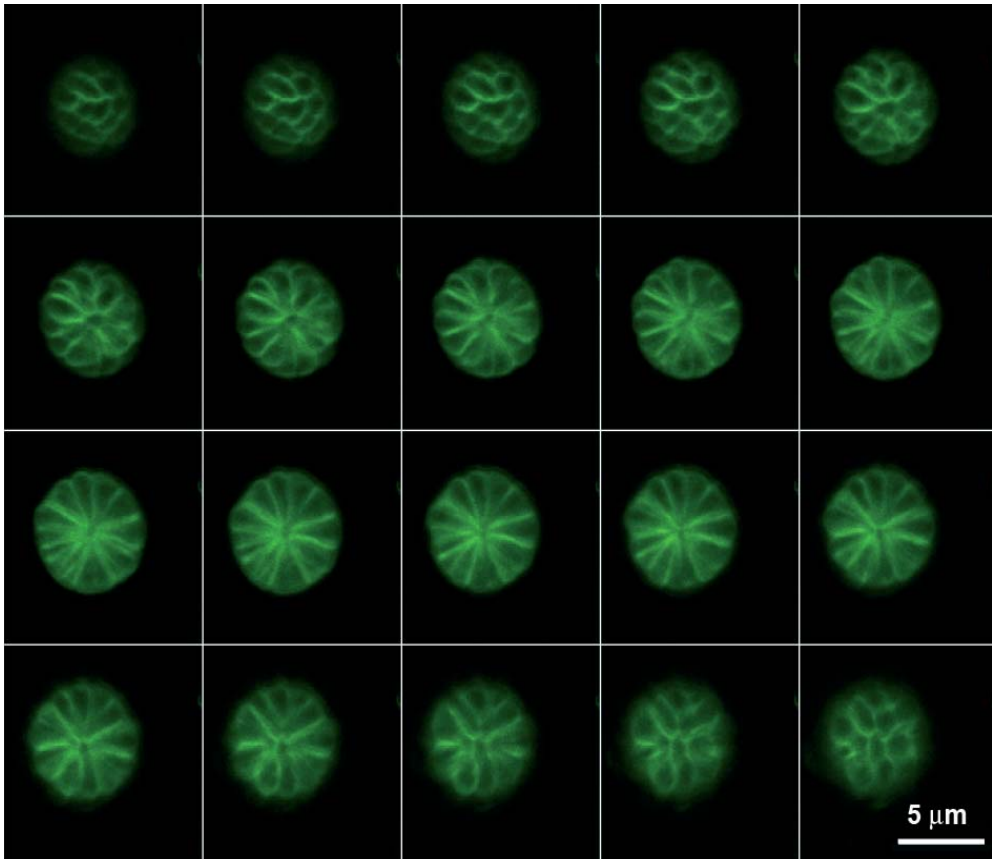

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Fig. 1. Confocal laser scanning microscopy focus series of a MMP stained with the lipophilic dye FM 1-43, which outlines the cell profiles. Note the highly organized disposition of the pyramidal cells within the MMP.

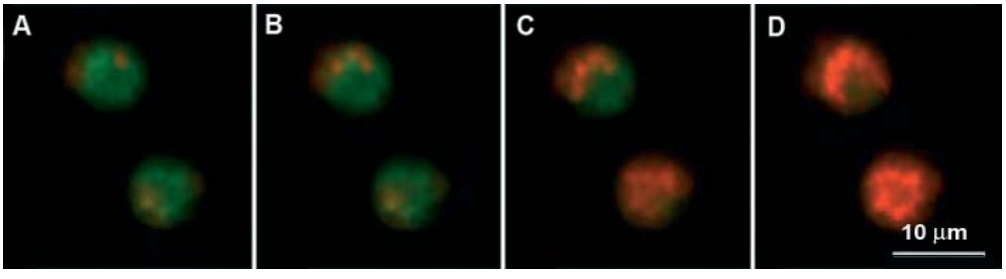

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Fig. 2. Two MMPs stained using the *Bac*Light kit. Note the sequential loss of cell viability from live (green) to dead (orange).

cover slips over excavated slides and observed with a Zeiss Axioplan 2 microscope equipped for fluorescence microscopy. Confocal laser scanning microscopy was done with a Zeiss 510 Meta LSM microscope equipped with a 488-nm argon laser and a 100 × 1.45 Planapo objective lens. For scanning electron microscopy, MMPs were fixed for 1 h in 2.5% glutaraldehyde in 0.1 M cacodylate buffer prepared with lagoon water. After washing in buffer, the samples were post-fixed in 1% OsO_4 for 1 h and washed again. They were then dehydrated in a graded ethanol series, critical-point-dried with CO_2, and gold-sputtered prior to observation in a JEOL JSM5310 scanning electron microscope operating at 15 kV.

Results and Discussion

Live individuals of MMPs stained with the lipophilic dye FM 1-43 and observed by confocal microscopy showed a high level of cellular organization (Fig. 1). After magnetic isolation, live MMPs were spherical and moving, with active movement that lasted for up to 1 h. Afterwards, MMPs slowed down and gradually lost their motility and viability (Fig. 2). While under observation, many of the MMPs became disorganized, and some cells separated from the spherical microorganism. The separated cells lost their membrane integrity, motility, and capacity to align along a magnetic field, although they still contained magnetosomes. Individual cells did not respond to the applied magnetic field because, under these conditions, the magnetic moment of the magnetosome was probably damaged by the collapse of the chain.

When exposed to different disruptive treatments, MMP cells (Fig. 3A) lost their organized arrangement faster than non-treated MMPs (Figs. 3B-D). Freshly collected MMPs

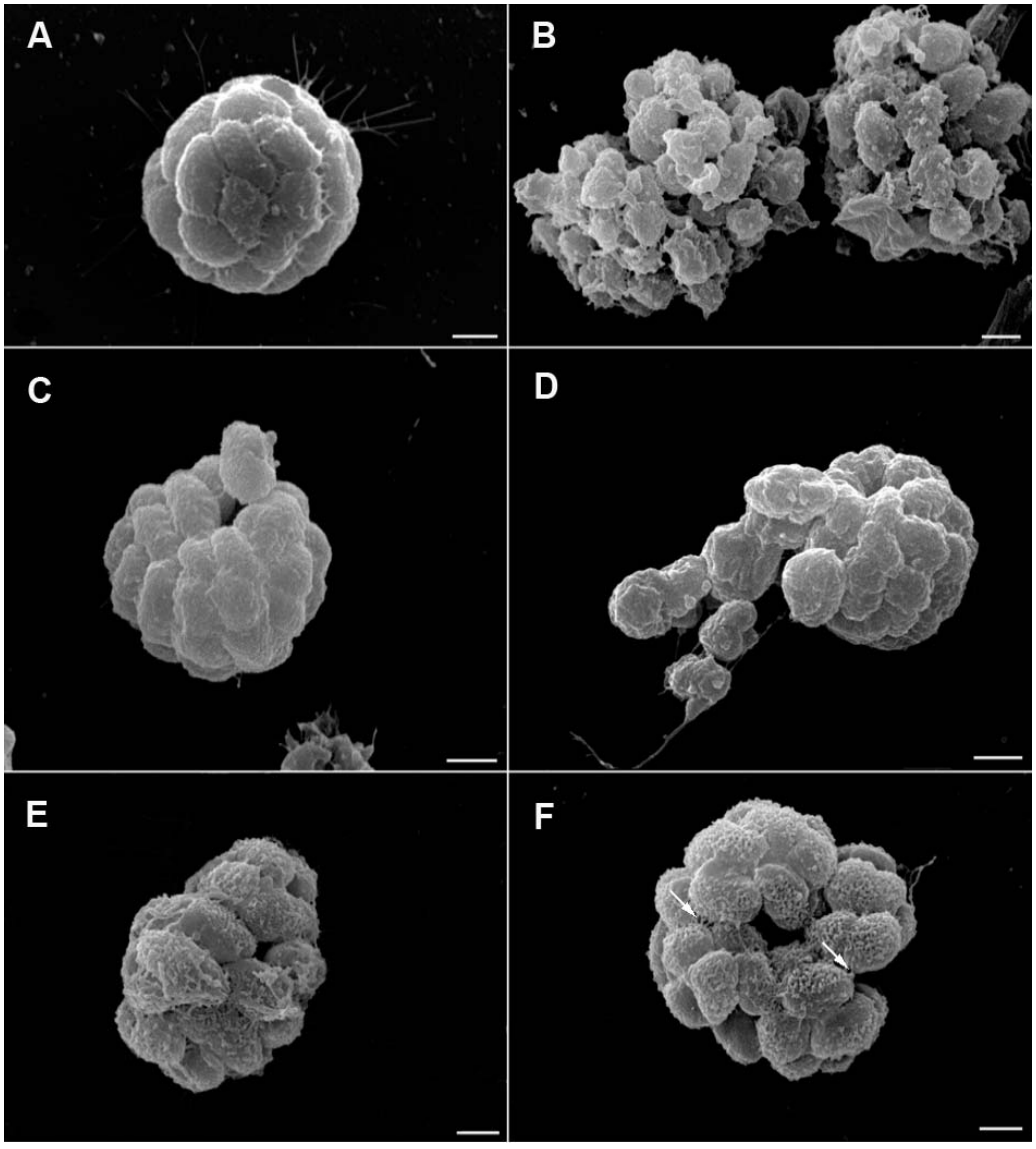

Fig. 3. Scanning electron microscopy of a MMP. (**A**) Untreated MMP with itsspherical morphology and tightly bound cells. (**B**) After treatment with distilled water, the cells are totally degraded and the spherical morphology is severely altered. (**C**) An untreated MMP fixed after natural disaggregation reveals a cell detached from the MMP without complete loss of the spherical morphology. (**D**) In this untreated MMP, fixed after natural disaggregation, a chain of cells has detached. **(E**) EDTA treatment of MMP causes cells to loosen such that the organism is no longer spherical. (**F**) In this EGTA-treated MMP, the cells are still loosely attached to each other by focal contacts (arrows). Scale bars indicate 1 μm.

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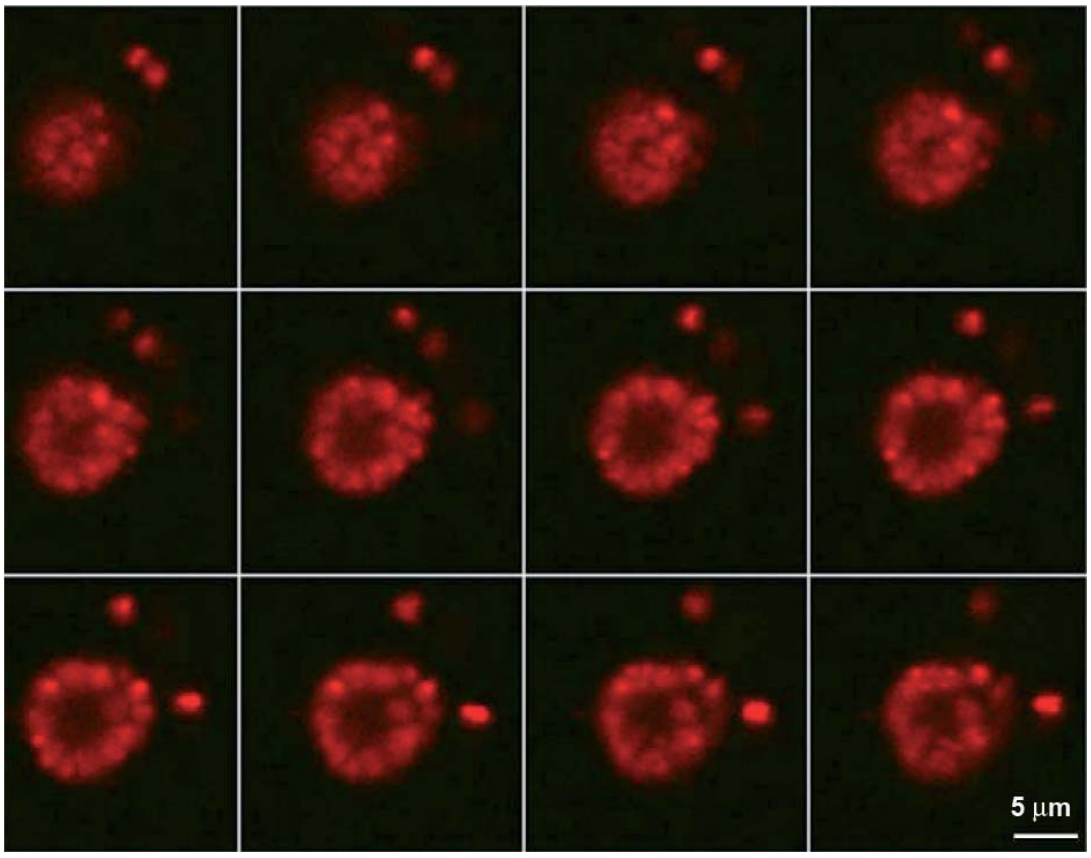

Fig. 4. Confocal laser scanning light microscopy focus series of a MMP stained according to the LIVE/DEAD *Bac*-Light kit. Note the cells stained red (dead) and that there is an increase in the internal compartment of the MMP.

treated with 0.1% azide became disorganized and the cells gradually died. EDTA and EGTA damaged the cell membrane in all cells at the same time, which altered the general morphology of the MMP (Figs. 3E,F), although focal contacts between cells persisted (Fig. 3F, arrows). Occasionally, EGTA/EDTA treatment caused complete disaggregation of the MMPs into separated dead cells, but most of the time the cells were still loosely connected. After such treatments, the cells might have been held together by the thick capsule surrounding the MMP and/or by filaments such as those already observed between cells [4]. Treatment with cacodylate buffer caused simultaneous loss of the membrane integrity of all cells, and sometimes disaggregation. MMPs that had lost a few cells or in which only a few cells had damaged membranes were still motile and able to respond to changes in the surrounding magnetic field. However, when the spherical morphology of MMPs was altered and the structures contained swollen cells, motility was completely lost as was the capacity to align along a magnetic field.

Confocal laser scanning microscopy showed that the cells of non-motile MMPs were not as well-organized spatially as those of intact motile MMPs (Fig. 4). The loss of viability was associated with an increase in the size of the internal compartment of the organism, which loosened the cell contacts. Occasionally, small motile MMPs with more rounded large cells were observed (Fig. 5). However, these MMPs

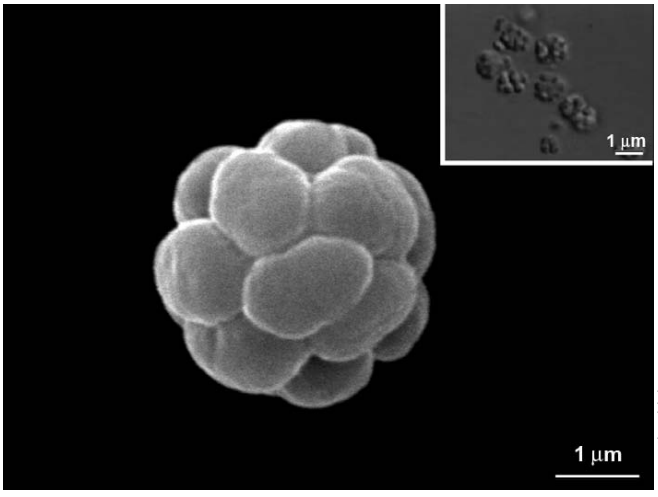

Fig. 5. Scanning electron microscopy image of a small MMP with rounded large cells. The inset shows a light microscopy image of small MMPs that are less motile than the usually observed MMPs.

were less motile than those of the usual size and they eventually lost their viability.

Swimming is one of the most easily recognizable behaviors of living prokaryotes [8]. However, a lack of motility is not definitive in determining whether a prokaryotic cell is dead or alive. The present study shows, for the first time, that the spatial organization of the cells is essential to the movement and viability of MMPs, since cell loss eventually induced the death of the whole microorganism. A previous report [3] showed that, after disaggregation, MMPs lose their motility and capacity to align in an applied magnetic field, suggesting that the multicellular microorganisms die under these conditions. The present results also indicated that individual cells are not viable, which emphasizes the completely multicellular nature of MMPs. This is the first direct evidence that the separation of cells from the whole MMP induces cell death. The fluorescence probes could distinguish not only between living and dead but also between vigorous and frail microorganisms.

Transmission electron microscopy of ultra-thin sections and freeze-fracture replicas showed that the MMPs cells are tightly bound to each other and their pyramidal shape allows them to fit together to form a spherically shaped organism. In fact, MMP cells are polarized, as evidenced by the presence of flagella on the part of the cell in contact with the outer environment and the capsule [5]. This part also has a higher concentration of intramembrane particles than the part in contact with another cell [5], further reinforcing the idea of membrane specialization. Therefore, loss of the specific spatial arrangement of the cells within a MMP leads to a loss of cell-to-cell specialization and coordination, eventually resulting in the death of the whole multicellular organism. The presence of focal contacts between cells of partially disrupted MMPs, as observed by scanning electron microscopy (Fig. 3C), supports this hypothesis.

Calcium seems to be involved in maintaining the multicellular structure of MMPs. The structural modifications induced by EDTA or EGTA treatment indicated that the intercellular junctions described in MMPs [4,11] are calcium stabilized, which again points to the organized and coordinated aspects of MMP individuals. Calcium is involved in many processes in prokaryotes, including cell cycle and division, and its absence induces morphological changes in *Escherichia coli* L-forms, which subsequently become spherical [10].

The unique multicellular nature of MMPs may be related to their survival in the environment. As a multicellular unit, a MMP can efficiently swim and navigate along geomagnetic field lines to lower levels in the sediment, where oxygen levels are zero (data not shown). This may allow it to avoid predation from flagellates or ciliates present in the upper layers of the sediment. The multicellular nature of MMPs might provide additional protection from predation that could not be accomplished by unicellular non-motile individuals [2,9].

In summary, the highly unusual prokaryotic multicellular nature of MMPs is reflected by: (i) their high level of structural organization; (ii) the interdependence of their cells, as suggested by the loss of viability when MMPs disaggregate; and (iii) the cell coordination required for both their complex swimming behavior and the orientation of their magnetic crystals to provide a net magnetic moment.

Acknowledgements. Financial support was granted from CNPq, FAPERJ (Pronex) and CAPES Brazilian programs.

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Viabilidad celular en procariotas multicelulares magnetotácticos

Resumen. Un procariota multicelular magnetotáctico (MMP en inglés) es un conjunto de células bacterianas dispuestas una al lado de otra en una esfera hueca en la que cada célula se enfrenta tanto hacia el ambiente externo como hacia un compartimento acelular interno en el centro del organismo multicelular. Los MMPs pueden nadar como una unidad propulsadas por el batir coordinado de los numerosos flagelos de la superficie exterior de cada célula. Todas las fases del ciclo vital de los MMPs son multicelulares. Inicialmente, un MMP esférico crece debido al aumento del tamaño de cada célula, y entonces las células se dividen. Después, las células se separan, formando dos esferas idénticas. Nunca se han observado células individuales de MMPs nadando. Describimos el estudio de la viabilidad de las células individuales de MMP realizado con tinciones fluorescentes y el microscopio electrónico. Cuando se separan del MMP, las células dejan de moverse y ya no responden a campos magnéticos. Las pruebas de viabilidad indican que todas las células separadas están muertas. Varias células se pueden separar de un MMP antes de que ésta pierda completamente su movilidad y viabilidad. Nuestros datos indican que el alto nivel de organización celular de los MMPs es esencial para su movilidad, comportamiento magnetotáctico y viabilidad. [**Int Microbiol** 2006; 9(4):267-272]

Palabras clave: procariotas multicelulares magnetotácticos (MMP) · multicelularidad · viabilidad celular · magnetotaxis

Viabilidade celular em procariontes multicelulares magnetotáticos

Resumo. Um procarionte multicelular magnetotático (MMP en inglês) é um conjunto de células bacterianas organizadas lado a lado em uma esfera onde cada célula é voltada para o ambiente externo e um compartimento interno acelular no centro do microrganismos. Os MMPs nadam como uma unidade propelida pelo batimento coordenado dos numerosos flagelos na superfície externa de cada célula. O ciclo de vida dos MMPs é multicelular. Inicialmente, um MMP esférica cresce através do aumento do tamanho de cada célula, e em seguida as células se dividem. Depois, as células se separam em dois esferas idênticas. Células individuais dos MMPs que nadam nunca foram observadas. Aquí describimos a viabilidade celular das células MMP individuales com corantes fluorescentes e microscopia eletrônica. Quando separada do MMP, as células páram de se mover e responder a campos magnéticos. Testes de viabilidade indicam que todas as células separadas estão mortas. Algumas células podem ser perdidas por um MMP antes que ele perca completamente sua motilidade e viabilidade. Nossos dados indicam que o alto nível de organização celular nos MMPs é essencial para sua motilidade, comportamento magnetotático e viabilidade. [**Int Microbiol** 2006; 9(4):267-272]

Palavras chave: procariontes multicelulares magnetotáticos (MMP) · multicelularidade · viabilidade celular · magnetotaxia