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Salinity dependence of the distribution of multicellular magnetotactic prokaryotes in a hypersaline lagoon

Juliana L. Martins, Thaís S. Silveira, Karen T. Silva, Ulysses Lins*

Department of General Microbiology, Professor Paulo de Góes Institute of Microbiology,
Federal University of Rio de Janeiro, Rio de Janeiro, Brazil

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Summary. *Candidatus* Magnetoglobus multicellularis is an unusual magnetotactic multicellular microorganism composed of a highly organized assemblage of gram-negative bacterial cells. In this work, the salinity dependence of *Ca.* M. multicellularis and its abundance in the hypersaline Araruama Lagoon, Brazil were studied. Viability experiments showed that *Ca.* M. multicellularis died in salinities >55‰ and <40‰. Low salinities were also observed to modify the cellular assemblage. In microcosms prepared with different salinities, the microorganism grew better at intermediate salinities whereas in high or low salinities, the size of the population did not increase over time. The concentrations of *Ca.* M. multicellularis in the lagoon were related to salinity; sites with lower and higher salinities than the lagoon average contained less *Ca.* M. multicellularis. These results demonstrate the influence of salinity on the survival and distribution of *Ca.* M. multicellularis in the environment. In sediments, the abundance of *Ca.* M. multicellularis ranged from 0 to 10^3 microorganisms/ml, which represented 0.001% of the counts of total bacteria. The ability of *Ca.* M. multicellularis to accumulate iron and sulfur in high numbers of magnetosomes (up to 905 per microorganism) suggests that its impact on the sequestration of these elements (0.1% for biogenic bacterial iron) is not proportional to its abundance in the lagoon. [**Int Microbiol** 2009; 12(3):193-201]

Keywords: *Candidatus* Magnetoglobus multicellularis · multicellular magnetotactic prokaryotes · salinity dependence · iron in waters

Introduction

Magnetotactic bacteria are gram-negative microorganisms that, propelled by flagella, are capable of aligning and migrating along the lines of a magnetic field. This behavior, referred to as magnetotaxis, depends on the biomineralization of iron-rich, crystalline, membrane-bound magnetic crystals (magnetosomes) [26]. Magnetosome crystals contain either magnetite (Fe_3O_4) or greigite (Fe_3S_4), and their precursors [24]. It is thought that the magnetotactic behavior of magnetotactic bacteria serves to facilitate their positioning within the oxygen transition zone in the water column, with the geomagnetic field used for spatial orientation and navigation [11]. Magnetotactic bacteria are distributed worldwide in aquatic habitats that have a chemically stratified vertical concentration gradient [6]. Magnetite-producing magnetotactic bacteria occur in freshwater and saline environments whereas greigite-producing magnetotactic bacteria have been described only in saline or hypersaline habitats. In natural environments, uncultivated magnetotactic bacteria prefer the oxic-anoxic interface, where they are detected at relative high numbers, about 10^4 cells/ml [7]. This interface occurs in the water column or in the top few millimeters to centimeters of sediments, depending on the environment [7].

***Corresponding author:** U. Lins
Departamento de Microbiologia Geral
Instituto de Microbiologia Professor Paulo de Góes
Universidade Federal do Rio de Janeiro
21941-590 Rio de Janeiro, RJ, Brazil
Tel. +55-2125626738. Fax +55-2125608344
E-mail: ulins@micro.ufrj.br

Several morphologies of magnetotactic bacteria, including cocci, rod-shaped cells, spirilla, and multicellular microorganisms, have been reported. Spherical magnetotactic microorganisms formed by an assemblage of gram-negative bacterial cells have been described in sulfur-rich environments [5,8,9,16,18,21,25,27]. High numbers of a many-celled magnetotactic bacterium belonging to the Proteobacteria phylum in the Deltaproteobacteria class [1,8,15] have been detected in the hypersaline Araruama Lagoon, near the city of Rio de Janeiro. Due to its unique feature, this microorganism has been named '*Candidatus* Magnetoglobus multicellularis' [1]. In lagoon sediments, *Ca.* M. multicellularis occurs in the anoxic zone [1]. The morphology, ultrastructure, and crystallography of magnetosomes in *Ca.* M. multicellularis have been described in several studies [2,16]. Each *Ca.* M. multicellularis individual is a highly organized spherical assemblage of about 10–40 gram-negative bacteria (average 17.4 ± 3.59) [14], distributed radially around an internal acellular compartment [1]. Each cell is asymmetrically multiflagellated and contains about 60–100 greigite magnetosomes [1,27]. The microorganism aligns along magnetic field lines and moves in straight or helicoidal trajectories that change direction and speed, resulting in complex coordinated swimming patterns [16,27].

Very little is known about the ecological niches and potential roles of *Ca.* M. multicellularis in biogeochemical cycles, particularly in the iron cycle. The composition and abundance of its greigite magnetosomes and the fact that the magnetosomes can be digested by their ciliate predators, which graze on the microorganisms [22], suggest a role for *Ca.* M. multicellularis in the biogeochemical cycles of iron and sulfur. Simmons et al. [30] described the quantitative distribution of the many-celled magnetotactic prokaryote and of other magnetotactic bacteria in the water column of a brackish seasonally stratified pond. The average iron content of this prokaryote has been estimated based on magnetosome sizes and numbers per cell. A recent report of many-celled magnetotactic bacteria that produce both magnetite and greigite in the magnetosomes further supports their contribution to the biogeochemical cycles of iron [21].

The life cycle of *Ca.* M. multicellularis is unique among bacteria because all stages are believed to be multicellular, and cell organization is required for coordinated and synchronized cell division [15]. Initially, each microorganism grows by enlarging the volume of its cells, not their number; the cells then divide until their number in the microorganism roughly doubles. Next, the microorganism elongates, becomes figure-eight-shaped, and finally splits into two equally spherical microorganisms with the same magnetic polarity as the single microorganism from which they originated. Since no swimming cell detaching from a multicellular assemblage has been observed, *Ca.* M. multicellularis is thought to have a completely multicellular life cycle. The highly ordered multicellular cell organization is also crucial for survival because a cell that is naturally separated from the microorganism loses its viability, and the whole microorganism disaggregates when several cells are lost [2,3].

The uniqueness of the *Ca.* M. multicellularis life cycle has prompted the idea that it is regulated by variables different than those regulating the life cycles of other magnetotactic bacteria. However, variation of the osmostic pressure or treatment with different chemicals [2] leads to the same behavior observed in naturally detaching cells. In this work, we report that the distribution of *Ca.* M. multicellularis was related to the salinity level in the lagoon. Understanding the influence of salinity on the viability and population distribution of *Ca.* M. multicellularis may provide insights into its ecological niches and, specifically, into the importance of this organism in the microbiota of Araruama Lagoon.

Materials and methods

Site description and sampling. Araruama Lagoon, in the state of Rio de Janeiro, Brazil (22°50′–22°57′ S, 42°00′–42°30′ W) is a hypersaline coastal lagoon formed as a result of semi-arid climate conditions. It consists of a small drainage basin and a choked entrance channel. The unbalanced annual precipitation (900 mm) and evaporation (1400 mm) contribute to the lagoon's permanent salinity, which has been continuous for at least four or five centuries [31]. Araruama Lagoon is a stable environment, and its large area (about 220 km^2) contributes to the formation of different microenvironments in which population behaviors can be assessed.

To determine the distribution and seasonal variation of *Ca.* M. multicellularis, samples consisting of the upper sediment layer (0–10 cm) and water were collected in 500-ml Plexiglas tubes (9 cm diameter). Sediment cores were immediately sub-sampled by removing and homogenizing the top 5 cm of the sediment, the preferred location for *Ca.* M. multicellularis [1]. This sediment layer was immediately used to count *Ca.* M. multicellularis. For microcosm experiments, the samples were collected at Baleia Beach (São Pedro d'Aldeia city, RJ, Brazil; 22°52′ S, 42°07′ W), a highly urbanized area, with waters having an average salinity of 50‰. Samples consisting of water and sediment (1:2) were stored in 10-l containers. To determine seasonal variation, monthly samples were collected at Baleia Beach during periods without precipitation to ensure that salinity differences were not influenced by recent rainfall. Unlike many other bacteria (magnetotactic or not), *Ca.* M. multicellularis can be easily detected by direct microscopy because of its unusual morphology [2,3] and magnetotactic behavior. In addition, *Ca.* M. multicellularis was the only magnetotactic microorganism found at Araruama Lagoon, which facilitated its study in this environment. Salinity was measured in pre-filtered water (through a 0.22-μm Millipore filter) from each sampling site with a refractometer. The sediment water content was measured as the difference between the wet and dry (60°C, 72 h) weights of the sediment.

Magnetic concentration and counting. The concentration of magnetotactic bacteria was determined as previously described [2,19]. The bacteria were counted in a Neubauer chamber and the counts adjusted to the fixed sediment volume used for magnetic concentration (about 50 ml). The

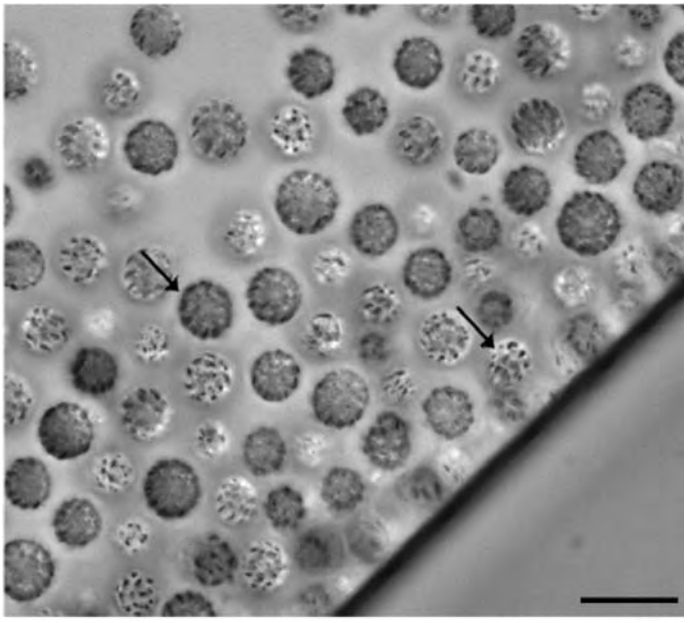

Fig. 1. Morphology of *Candidatus* Magnetoglobus multicellularis. Bright field light microscopy micrographs of *Ca.* M. multicellularis magnetically concentrated showing their unique morphology and magnetosome chains (arrows). Scale bar = 10 μm.

detection and counting of *Ca.* M. multicellularis by light microscopy were greatly simplified by the organism's unique morphology (Fig. 1) [2,3].

Salinity microcosm experiments. Microcosms were adjusted to three salinities other than the average salinity of the lagoon (55‰) by adding distilled water (30 or 45‰) or NaCl (65‰). Fixed volumes of sediments were used for magnetic concentration and counting of *Ca.* M. multicellularis. For light microscopy analysis, magnetically isolated *Ca.* M. multicellularis from freshly collected samples were exposed to different salinities. The natural salinity was 46‰ at the time of sample collection. The salinities were adjusted by adding distilled water or NaCl to the lagoon water. Both the microorganisms treated with different salinities (25, 30, 40, 55, 60, 65, and 70‰) and those that were untreated were stained using the LIVE/DEAD B*ac*light Bacterial viability kit (Molecular Probes, USA) for determination of cell viability.

For viability tests, *Ca.* M. multicellularis were stained according to the protocols provided by the kit's manufacturer and by a previously described method [2]. The samples were periodically observed using bright-field microscopy, and when the microorganisms stopped swimming under fluorescence microscopy. This was done to avoid possible damage caused by fluorescent-light energy. After a loss of viability was confirmed, the salinity exposure time that resulted in cell death was recorded.

For transmission electron microscopy (TEM), magnetically concentrated *Ca.* M. multicellularis were exposed to a salinity of 30‰ for 10 min and 65‰ for 25 min. These times were sufficient to detect the effects observed in the viability tests. Both treated and untreated *Ca.* M. multicellularis were processed for TEM as described below.

Microscopy. Magnetically concentrated samples, under the influence of the magnetic field from a NdFeB magnet, were imaged with bright-field light microscopy using an Axioplan 2 microscope (Carl Zeiss, Germany) attached to a digital camera. For TEM, the samples were fixed with 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2) prepared in lagoon water, washed in the same buffer, dehydrated in an acetone series, embedded in PolyBed 812 resin, and ultrathin-sectioned with a Reichert Ultramicrotome. Grids were stained with 5% uranyl acetate for 30 min and with 0.4% lead citrate for 5 min. Alternatively, the samples were deposited on Formvar-carbon coated copper grids and air-dried. All samples were observed with a FEI Morgagni transmission electron microscope at 80 kV. Whole-mount TEM images were used for magnetosome measurements on magnification-calibrated negatives. The average diameter of magnetosomes was measured using AnalySIS software (Soft Imaging System).

Total bacteria counts. Sediment samples (1 g) were fixed in 4% paraformaldehyde for 2 h, centrifuged at 10,000 ×*g*, washed in PBS, centrifuged again, and stored in a 1:1 mixture of PBS and 100% ethanol at –20°C. To separate bacteria from particles, the samples were sonicated (three times 30 s, with pulse mode) with a 5-mm microtip probe (Sonifier 250, Branson, output control 3; duty cycle 20%) and then left still for 3 min to precipitate sediment particles. A volume of the sonicated sample was then mixed with 10 ml of PBS and filtered through a polycarbonate membrane (0.2-μm pore size, Millipore) and then through a second filter (0.45-μm pore size, Millipore). The filters were stained with DAPI (1 μg/ml) for 3 min, washed briefly with distilled water, air dried, mounted on slides with n-propyl gallate (0.2 M in glycerol:PBS, 9:1) as mounting medium, and observed as described for light microscopy. For each membrane, 25 fields were photographed randomly along two perpendicular transects. Bacteria counts were added and then adjusted to the filtration area, sample volume, and dilution. Three filters were counted for each sample. The total bacteria counts were expressed per gram of dry weight of sediment (i.e., g sed).

Results

The magnetic concentration of Araruama Lagoon sediments contained only *Ca.* M. multicellularis; as previously described [3,14]. Virtually all *Ca.* M. multicellularis individ-

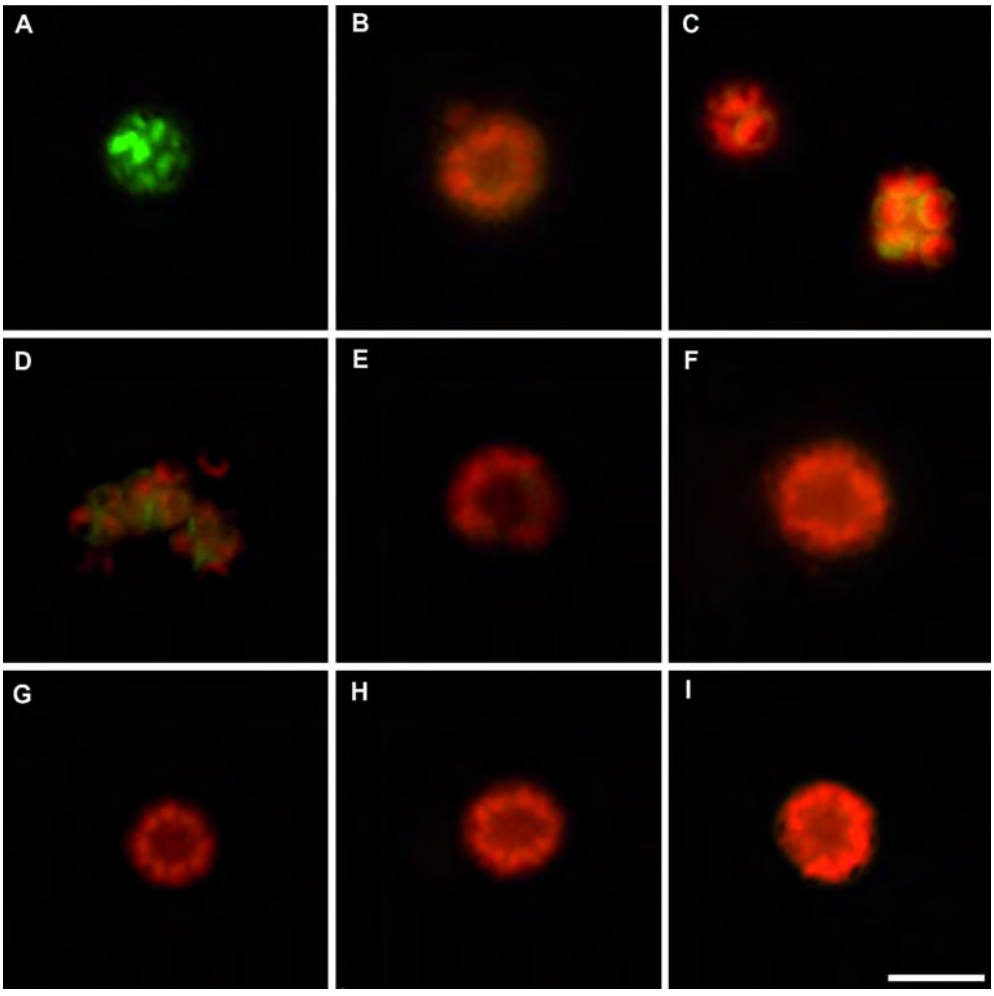

Fig. 2. *Candidatus* Magnetoglobus multicellularis untreated and exposed to various salinities stained with BacLight™ kit. Untreated *Ca*. M. multicellularis immediately after magnetic concentration (**A**); and 1 h 45 min after magnetic concentration (**B**). Note the spherical morphology and integrity of the microorganisms (A) and its natural disaggregation and death (B). (**C**) Five minutes after exposure to 25‰ salinity. The cells are large and rounded and the microorganisms lost viability and are loose and not spherical. (**D**) Ten minutes after exposure to 30‰ salinity. Note that the microorganism lost completely its morphology and the cells collapsed. One hour and forty five minutes after the exposure to 40 (**E**) and 55‰ salinity (**F**). Note that the microorganisms preserve the spherical morphology but lost its viability and there is an increase in the internal compartment. *Ca*. M. multicellularis exposed for twenty five minutes to 60‰ salinity (**G**), twenty minutes to 65‰ salinity (**H**) and ten minutes to 70‰ salinity (**I**). Note that the cells remained attached but lost viability and there is an increase in the internal compartment. Scale bar = 10 μm.

uals are south-seeking magnetotactic microorganisms, and swim actively for up to 2 h after magnetic concentration. The disaggregation of *Ca.* M. multicellularis into non-motile individual and non-viable microorganisms following a decrease in salinity (after the addition of distilled water) has been previously reported [2]. Moreover, multicellular magnetotactic bacteria are usually found in hypersaline to brackish environments [16]. In this work, *Ca.* M. multicellularis was exposed in vitro to low and high salinities and bacterial viability observed, as described by Abreu et al. [2] (Fig. 2). Untreated *Ca*. M. multicellularis showed active movement and remained viable, retaining its spherical morphology and integrity, for up to 1 h and 30 min before gradually losing motility and viability. Some cells were observed to separate from the microorganism, accompanied by a slight increase in the size of the internal compartment. *Ca.* M. multicellularis exposed to low salinities (25 and 30‰) quickly lost its viability and its spherical shape (5 and 10 min after exposure, respectively) as well as its organized arrangement. The separated cells were large and round. When the microorganism was treated with salinities closer to the natural level (40 and 55‰), its behavior was similar to that of untreated *Ca.* M. mul-

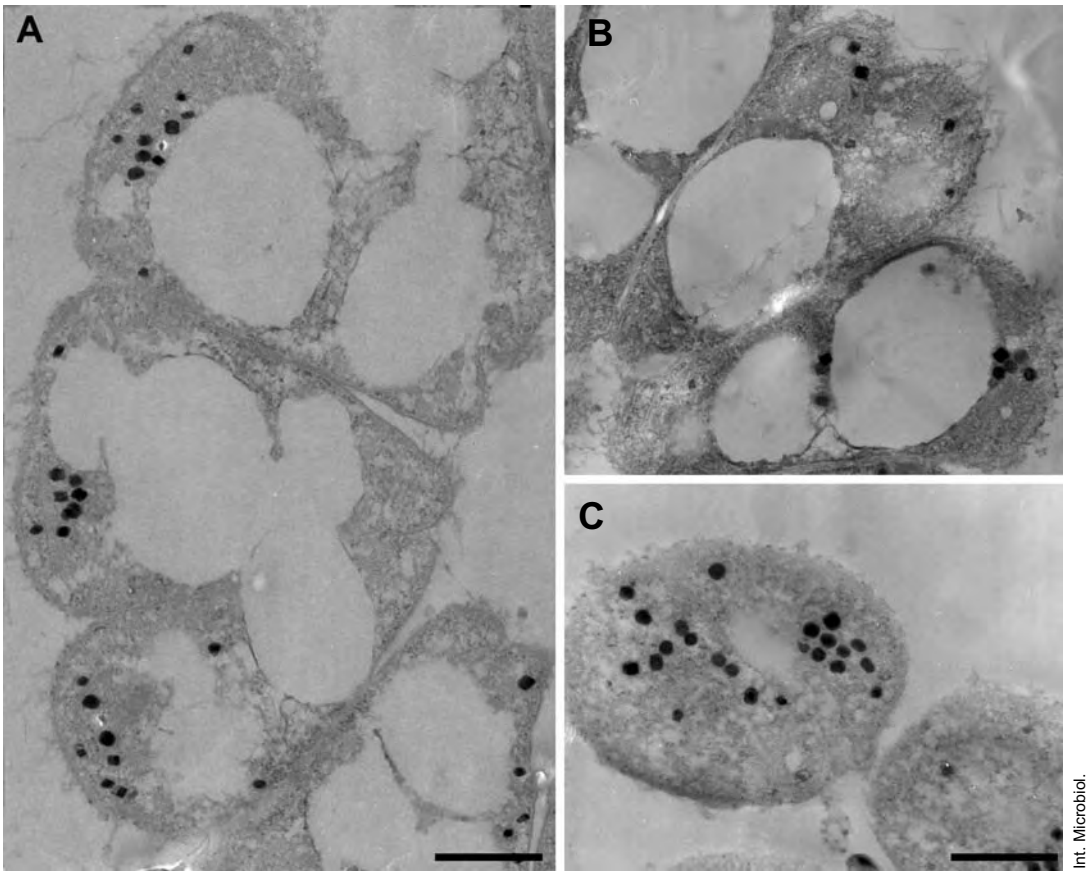

Fig. 3. Effect of salinity in the ultrastructure of *Candidatus* Magnetoglobus multicellularis. Transmission electron micrographs of the microorganism treated with (**A**) untreated; (**B**) 65‰ salinity; or (**C**) 30‰ salinity. Note that at low salinity the cells were large and round while at high salinities they maintain their pyramidal shape, as in untreated microorganisms. Scale bars = 500 nm.

ticellularis, i.e., it lost viability 1 h and 45 min after exposure. The microorganism preserved its spherical morphology but the internal compartment increased in size. The highest salinities (60, 65, and 70‰) did not cause disaggregation, but the microorganism survived for at most 25 min after exposure.

Transmission electron microscopy confirmed the effects observed with fluorescence microscopy. In *Ca.* M. multicellularis exposed to low salinities (30‰), the cells were large and round (Fig. 3C). At high salinities, however, the cells kept their pyramidal shape (Fig. 3B), as in the untreated microorganisms (Fig. 3A). The arrangement of the magnetosome chains did not change after any of the treatments.

To determine whether either low or high salinity downregulated populations of *Ca.* M. multicellularis in the lagoon sediment, we observed and counted this microorganism in microcosms at the average salinity of the lagoon (55‰) and at three different salinities: 30, 45, and 65‰. Under conditions of intermediate salinity (45 and 55‰), the size of the population slightly increased 3 days after sampling. The low counts obtained at intermediate salinities on the first day were probably because the sediment was disturbed during sampling. After the first day, the population recovered and increased; however, 7 days after sampling, the population again decreased in size. This behavior was observed in other microcosm experiments without any salinity adjustment (data not shown) and was thus considered as the standard behavior of *Ca.* M. multicellularis in microcosms. At the lowest and highest salinities (30 and 65‰), the population did not recover after the first day, implying that *Ca.* M. multicellularis is unable to grow under these conditions.

The concentrations of *Ca.* M. multicellularis and salinities at Baleia Beach over the year confirmed the results observed experimentally (Fig. 4). *Ca.* M. multicellularis was detected throughout the year, with populations reaching their maximal abundances (10^3/ml or 10^2/g sed) at the beginning of the Brazilian summer (December, 2005 and January, 2006). Between October 2005 and January 2006, the population greatly increased, reaching a peak during January, when

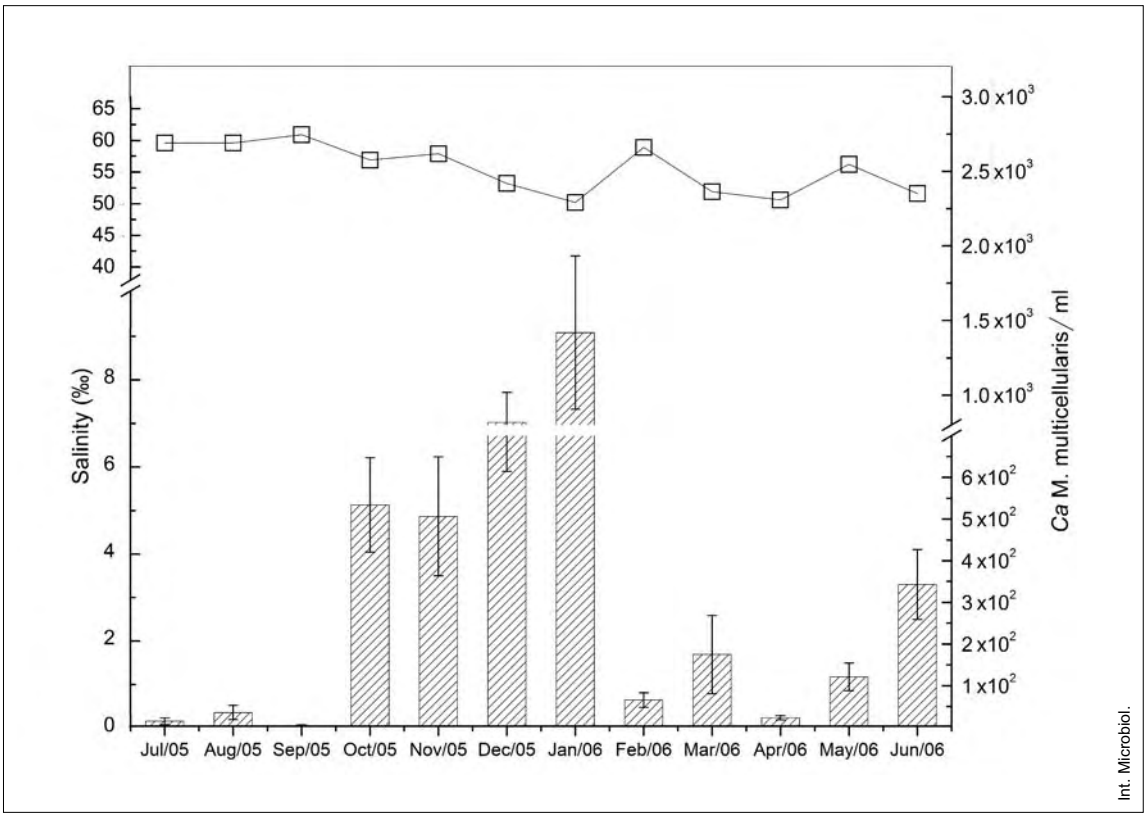

Fig. 4. Annual variation of *Candidatus* Magnetoglobus multicellularis population at Baleia beach in Araruama lagoon (hatched bars) and the effects of salinity (line and squares). The hatched bars are the average of six replicates (±SD).

the salinity dropped from 61 to 55.7‰. In February 2006, *Ca.* M. multicellularis counts decreased abruptly in parallel with an increase in the salinity, from 50 to 59‰. These results suggest that both low and high salinities down-regulate *Ca.* M. multicellularis populations; the extreme salinities alter the organism's morphology and possibly halt its life cycle. For comparison, we quantified the total microbial community at Baleia Beach during the period when *Ca.* M. multicellularis counts reached their highest density (January 2006). Counts of total bacteria in the top 5 cm of sediment were $6.9 \times 10^8 \pm 1.6 \times 10^8$ cells/g sed.

The *Ca.* M. multicellularis concentrations in the Araruama Lagoon waterside were also examined and the results confirmed their relationship to the salinity levels (Fig. 5). *Ca.* M. multicellularis could not be detected at the Rio das Moças estuary when the salinity was 23.7‰, the lowest measured in this study. Large populations of *Ca.* M. multicellularis were observed at Baleia, Iguabinha, and Rebolo Beaches, with salinities of 56, 60, and 54.6‰, respectively. Note that *Ca.* M. multicellularis was not detected in sediments collected at most sites within the lagoon, although the salinities were similar to those at sites with high numbers of

Table 1. Magnetosome distribution in *Candidatus* Magnetoglobus multicellularis

	Average	Minimum	Maximum	SD[a]	Number
Magnetosome diameter (nm)	83	56	163	10.1	746[b]
Number of magnetosomes/cell	52	0	168	39.9	289[c]

[a]Standard deviation.
[b]Number of magnetosomes counted.
[c]Number cells counted.

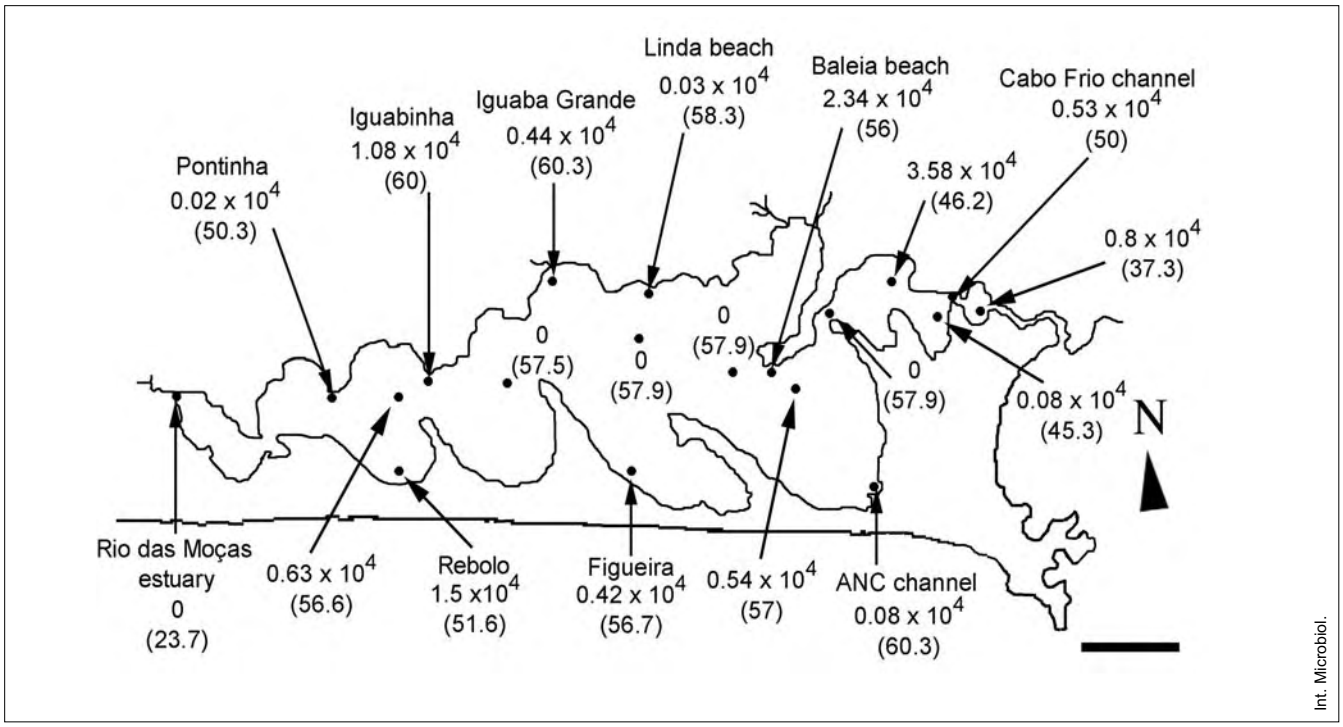

Fig. 5. Distribution of *Candidatus* Magnetoglobus multicellularis per ml in the sediment of Araruama lagoon. Numbers in parentheses represent the salinity at the time of sampling. No microorganism was observed in the water column. Scale bar = 2.5 km.

Ca. M. multicellularis. The distribution of *Ca.* M. multicellularis from the water margin up to 6.5 m within the lagoon showed that there was a preferred zone, between 2.5 and 4.5 m, where 59.9% of the *Ca.* M. multicellularis population was found. This fact may explain why *Ca.* M. multicellularis were not detected in most samples within the lagoon.

To estimate the amount of iron retained in magnetosomes, TEM images of magnetically concentrated *Ca.* M. multicellularis were used to measure and count the magnetic particles. The average diameter and magnetosome distribution in cells are shown in Table 1. The number of magnetosomes per cell varied greatly. Few cells had intact magnetosome chains, which explains the discrepancy between the number of cells analyzed to determine the number of magnetosomes per cell and the number of magnetosome chains per cell.

Discussion

Candidatus M. multicellularis and other morphologically similar multicellular forms of magnetotactic bacteria have not been described in freshwater environments. The susceptibility of this microorganism to low osmotic pressure, which causes loss of morphological integrity and death, is well documented [2]. We observed a lower *Ca.* M. multicellularis density during high and low salinity months. In microcosm experiments, the microorganism preferred intermediate salinity for growth, while in extreme salinities there was no population increase over time. The extreme salinity levels observed and tested probably altered the integrity of the cell membrane, leading to the microorganism's death and thus resulting in the decrease of the population in the environment. Although in the in vitro viability tests *Ca*. M. multicellularis was exposed to other stress conditions besides salinity, such as light damage and oxygen, the results are valid because they clearly showed that extreme salinities strongly affected the survival time of microorganisms under the intrinsic stress conditions of the experiment. In the environment, the effect of extreme salinities on the microorganisms might be slower, but viability is nonetheless affected. The sensibility of *Ca*. M. multicellularis to osmolarity and the microorganism's unique multicellular cell cycle together indicate that disruption of its highly organized cell architecture, caused by salinity variations, affects its distribution in the lagoon. Depending on its relative abundance, *Ca.* M. multicellularis is likely to play a significant role in the Araruama Lagoon because of the biomineralization function of its magnetosomes, analogous to other magnetotactic bacteria which are recognized as significantly contributing to the geomicrobiology of sediments [29].

Morphological alterations caused by strong osmotic changes have been shown to convert *Ca.* M. multicellularis into non-motile or non-magnetotactic microorganisms [2]. In this work, we showed that at low salinities *Ca.* M. multicellularis quickly lost its spherical shape and disaggregated. In samples directly removed from the lagoon (no previous magnetic concentration), flagellates grazing on partially disaggregated *Ca.* M. multicellularis were noted following a decrease in the salinity of the lagoon waters after heavy rainfall (unpublished observations). At high salinities, the microorganism did not undergo any major morphological alterations but died relatively quickly. In the environment, *Ca.* M. multicellularis was not detectable at a salinity ≥60‰, which confirms the results obtained in vitro.

In another study, quantitative PCR was used to determine the distribution and abundance of the magnetotactic bacteria community in the sediment of Salt Pond (Falmouth, MA, USA); the results indicated that the population of multicellular magnetotactic prokaryotes accounts for ~1.9% of all bacteria [30]. In this work, we found that *Ca.* M. multicellularis corresponded to approximately 0.001% of total bacteria. Our data were obtained by direct cell counting using light microscopy, as employed in previous studies [3,10,23,28]. This method avoids certain biases that can be introduced by molecular biology techniques, such as incomplete DNA extraction and cell number estimates based on an arbitrary number of gene copies [30]. The use of magnetic concentration may underestimate the abundance of magnetotactic bacteria counts in the environment since this approach is based on the organism's ability to swim in a magnetic field at an arbitrary time. Greater phylogenetic diversity among magnetotactic cocci in environmental samples was detected by a metagenome-based PCR approach [17] than by the capillary-racetrack-based PCR method. Enrichment achieved with the capillary racetrack approach is similar to the enrichment method used by our group [19], as both are based on magnetic concentration of the bacteria. With this method, bacterial cells that are slow swimmers or are attached to the sediment particles are not counted, which may explain the discrepancies between our values and estimates of multicellular magnetotactic prokaryotes in other places and by other techniques.

The *Ca.* M. multicellularis population corresponds to a very small percentage of all bacteria in the lagoon environment, but their role in the biogeochemical cycle of iron may be significant. Each microorganism contains about 17.4 cells [14] with up to 52 magnetosomes each, i.e., up to 905 magnetosomes per microorganism. The number of magnetosomes per microorganism obtained in our analysis is twice as high as reported for multicellular magnetotactic prokaryotes in the Salt Pond sediments [30]. This higher number of magnetic crystals in *Ca.* M. multicellularis may be required to compensate for the lower geomagnetic field intensity in Rio de Janeiro, as has been suggested for cocci [20]. For an average magnetosome size of 82 nm, resulting in a cubic volume of 5.5×10^5 nm^3, we estimated a total amount of 2.05×10^{-12} g of greigite per microorganism (greigite density = 4.1 g/cm^3 [13]), corresponding to 1.16×10^{-12} g Fe per *Ca.* M. multicellularis. The total amount of iron trapped in a *Ca.* M. multicellularis population in Baleia Beach in January, 2006, 6.3×10^2 microorganisms/g of sediment (the highest abundance), corresponded to 7.3×10^{-10} g Fe/g sed. The trapped iron in heterotrophic bacteria can be quantified by estimating biomass and iron requirements, resulting in a value referred to as the iron quota [33]. The iron quota for heterotrophic marine bacteria has been reported to be 7.5 μmol Fe mol/C [32]. Using the biomass factor of 30.2 fg C/cell, determined for bacteria from coastal environments [12], and the total bacteria cell counts in Baleia Beach in January, 2006 (6.9×10^8 cells/g sed), we estimated the total trapped iron in heterotrophic bacteria to be 7.3×10^{-7} g Fe/g sed. Thus, iron accumulation in *Ca.* M. multicellularis corresponded to 0.1% of the total bacteria biogenic iron in the sediment of Araruama Lagoon. The total iron in Baleia Beach sediment is 2.7×10^{-3} g Fe/g sed (unpublished results). Although compared with the total amount of iron in the environment the amount of iron stored by *Ca.* M. multicellularis seems to be negligible, it is worth noting that it was trapped by a single bacterial species. We have shown that ciliates graze on *Ca.* M. multicellularis and that magnetosomes can be dissolved within their acidic vacuoles [22]. Thus, depending on the rate of *Ca.* M. multicellularis consumption by protozoa, the significant amounts of iron stored in the magnetosomes may be recycled into the environment in a more soluble form.

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