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'*Candidatus* Magnetoglobus multicellularis', a multicellular, magnetotactic prokaryote from a hypersaline environment

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Phylogenetic analysis and phenotypic characterization were used to assign a multicellular magnetotactic prokaryote the name '*Candidatus* Magnetoglobus multicellularis'. '*Candidatus* Magnetoglobus multicellularis' lives in a large hypersaline coastal lagoon from Brazil and has properties that are unique among prokaryotes. It consists of a compact assembly or aggregate of flagellated bacterial cells, highly organized in a sphere, that swim in either helical or straight trajectories. The life cycle of '*Candidatus* Magnetoglobus multicellularis' is completely multicellular, in which one aggregate grows by enlarging the size of its cells and approximately doubling the volume of the whole organism. Cells then divide synchronously, maintaining the spherical arrangement; finally the cells separate into two identical aggregates. Phylogenetic 16S rRNA gene sequence analysis showed that '*Candidatus* Magnetoglobus multicellularis' is related to the dissimilatory sulfate-reducing bacteria within the *Deltaproteobacteria* and to other previously described, but not yet well characterized, multicellular magnetotactic prokaryotes.

Magnetotactic bacteria inhabit freshwater and marine environments. Despite the large morphological variety and widespread distribution of magnetotactic bacteria, only three cultivated strains have been assigned to species with validly published names (Sakaguchi *et al.*, 2002; Schleifer *et al.*, 1991). Multicellular forms of magnetotactic bacteria have been described in different brackish, marine and hypersaline sulfur-rich environments of the northern and southern hemispheres as spherical organisms capable of aligning themselves and swimming as a unit along magnetic field lines. They consist of 10–40 Gram-negative cells, containing numerous magnetite (Lins *et al.*, 2007) or greigite magnetosomes, organized in a sphere that exhibits an unusual movement called 'ping-pong' or escape motility (for a recent review, see Keim *et al.*, 2007). So far, no validly published name has been proposed for these very similar magnetotactic bacteria. Instead, confusing and imprecise terminology has been used: magnetotactic multicellular aggregates (MMAs; Farina *et al.*, 1983), many-celled magnetotactic prokaryotes (MMPs; Rodgers *et al.*, 1990), multicellular magnetotactic prokaryotes (Greenberg *et al.*, 2005) and magnetotactic multicellular organisms (MMOs; Keim *et al.*, 2004a). The phenotypically best characterized of all magnetotactic multicellular bacteria is the MMO, which is found in Araruama lagoon, a hypersaline coastal environment in Brazil (Keim *et al.*, 2004a, b, 2007; Silva *et al.*, 2007; Winklhofer *et al.*, 2007). All multicellular magnetotactic bacteria are affiliated to the *Deltaproteobacteria* (DeLong *et al.*, 1993; Keim *et al.*, 2004b; Simmons & Edwards, 2007). However, the phylogenetic position of MMO was not known, despite its partial phenotypic characterization. The cell architecture, unique life cycle, magnetic properties, coordinated motility and habitat are distinctive enough to warrant assigning these organisms a name in the *Candidatus* category. Here, we present a phylogenetic description of the MMO based on

Abbreviations: FISH, fluorescent *in situ* hybridization; MMO, magnetotactic multicellular organism; MMP, many-celled magnetotactic prokaryote.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence obtained from '*Candidatus* Magnetoglobus multicellularis' in this study is EF014726.

FISH results, fluorescence micrographs and flow cytometry results are available as supplementary material with the online version of this paper.

16S rRNA gene sequences as well as additional phenotypic characterization of multicellular magnetotactic bacteria detected in a hypersaline lagoon in Brazil.

Samples were collected at Araruama lagoon (22° 50′ S 42° 13′ W), Rio de Janeiro state, Brazil, stored and magnetically concentrated as described in Lins *et al.* (2003). Further purification was done using a small magnet attached to the side of a polypropylene tube in the proper orientation and washing the pellet with sterile lagoon water. Electron microscopy techniques were done as described previously (Keim *et al.*, 2004b; Silva *et al.*, 2007).

The motility and magnetic properties of '*Candidatus* Magnetoglobus multicellularis' were studied using a pair of coils coupled to a DC source that was adapted to the microscope stage. With this device, it is possible to generate a maximum magnetic field of the order of 15 G (1.5×10^{-3} T) parallel to the glass slide. A special coil was used to obtain magnetic fields perpendicular to the glass slide. In this case, the maximum magnetic field obtained was about 1×10^{-3} T. The field is homogeneous in the region of observation, which means that no net magnetic force acts on the aggregate.

For flow cytometric analysis, magnetically concentrated and purified aggregates were analysed with a Becton-Dickinson FacsCalibur flow cytometer. The purity of the samples was verified using light microscopy. The concentration of '*Candidatus* Magnetoglobus multicellularis', determined using a Neubauer chamber, was 3.6×10^6 aggregates ml^{-1}.

The 16S rRNA gene was amplified and PCR products were cloned with the Inst/Aclone PCR product cloning kit (Fermentas). Extracted plasmids containing inserts were sequenced in an ABI 377 DNA Sequencer (PE Applied Biosystems) with the ABI Prism BigDye terminator cycle sequencing ready reaction kit (Perkin Elmer).

For 16S rRNA gene phylogenetic analysis, sequences were obtained from GenBank for sequence alignment using the CLUSTAL W multiple alignment accessory application in the BioEdit sequence alignment editor (Hall, 1999). Phylogenetic and molecular evolutionary analyses were conducted with MEGA version 3.0 (Kumar *et al.*, 2004) applying the neighbour-joining method (Saitou & Nei, 1987). Bootstrap values were calculated with 1000 replicates. A specific rhodamine-labelled probe for '*Candidatus* Magnetoglobus multicellularis' was designed (5′-GAT-TTATACTCTTATAAGT-3′) based on the 16S rRNA gene sequence obtained from the clones and obtained from BioSynthesis Inc. (http://www.biosyn.com). *In situ* hybridization was done as described by Spring *et al.* (1998).

To analyse the spatial distribution of '*Candidatus* Magnetoglobus multicellularis', the top (1 cm) layer of the sediment was repeatedly removed from a hand-held 500 ml Plexiglas core sampler, diluted immediately with sterile lagoon water and placed onto a microscope slide. The slide was exposed to the magnetic field of an ordinary magnet for 5 min, at which point the aggregates that had reached the edge of the drop were counted directly with a Zeiss Axiostar Plus microscope. Oxygen profiles were measured with a 25 μm tip oxygen microsensor coupled to a micromanipulator (Unisense; http://www.unisense.-com). The microsensor was inserted in steps of 50 μm until anoxic conditions were recorded.

Light and scanning electron microscopy showed that '*Candidatus* Magnetoglobus multicellularis' is spherical and composed of many flagellated bacteria, tightly linked and organized in a spiral (Fig. 1). All cells face both the outer environment and an acellular internal compartment found at the centre of the aggregate. Each cell of '*Candidatus* Magnetoglobus multicellularis' contains about 60–100 pleomorphic magnetosomes (Fig. 1b), which averaged 88 nm in length and 71 nm in width ($n = 112$). The aspect ratio and shape factor distribution showed that the magnetosomes are not very elongated and follow a Gaussian size distribution (not shown).

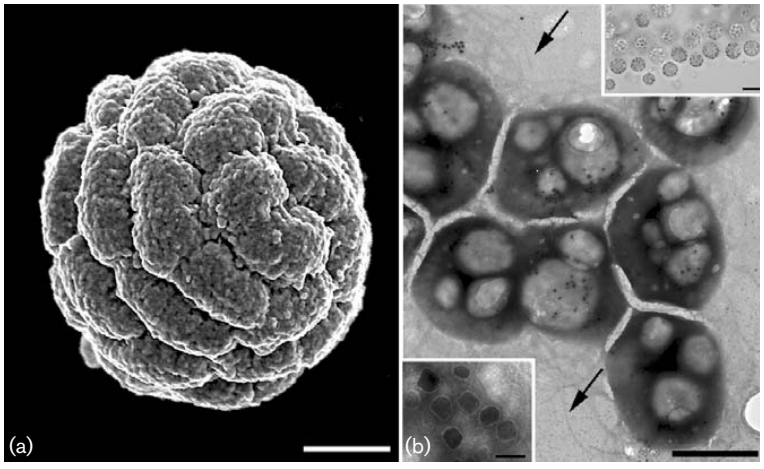

Fig. 1. (a) Scanning electron microscopy of '*Candidatus* Magnetoglobus multicellularis' showing its spherical morphology and the tightly bound cells. Note the highly organized disposition of the cells. Bar, 2 μm. (b) Light (upper right inset; bar, 10 μm) and transmission electron microscopy of the micro-organism deposited on nickel grids and stained in a 2% phosphotungstic acid solution in water. Note the presence of magnetosomes (lower left inset; bar, 100 nm) and many flagella (arrows). Bar, 1 μm.

Flow cytometry showed two populations in freshly collected '*Candidatus* Magnetoglobus multicellularis' samples. One population is composed of smaller aggregates, containing cells with fewer granules, that could represent daughter-aggregates that have divided recently, and the other population comprises bigger aggregates with higher granularity, possibly representing the stage just before constriction and separation (Supplementary Fig. S1, available in IJSEM Online). Accordingly, our flow cytometer results suggest that '*Candidatus* Magnetoglobus multicellularis' has its internal components (like magnetosomes and granules) multiplied or enlarged before cell division, which is in agreement with the life cycle proposed by Keim *et al.* (2004b). Furthermore, the flow cytometric results confirm the exclusively multicellular life cycle and characterize the size variation in a population of '*Candidatus* Magnetoglobus multicellularis'.

Four different types of motility are observed in '*Candidatus* Magnetoglobus multicellularis': free motion, rotation, walking and escape. In free motion, they swim in either straight or helical trajectories in a uniform magnetic field (Keim *et al.*, 2004a). Free trajectory velocities reach 90 ± 20 μm s^{-1} (Silva *et al.*, 2007), where the migration velocity is aligned to the field lines. The aggregates swim in a helical trajectory with the symmetry axis aligned to the magnetic field with a typical radius of several tens of micrometres and pitch varying from one sample to another. The helix rotates clockwise, while the cell body rotates in the same sense relative to the trajectory. In one pitch, the body rotates 2π radians (360°). All aggregates observed ($n > 200$) had the same sense of rotation of the body and the trajectory. At the edge of the drop, we observed rotational movement, where aggregates spin around an axis that passes through its centre. The sense of rotation varies from one individual to another. In walking, they walk freely in a complex trajectory when they reach the air–water interface at the top of the drop, while maintaining the same sense of body rotation. The most peculiar behaviour of '*Candidatus* Magnetoglobus multicellularis' is the escape motility, also called 'ping-pong' or excursion. It consists of a backward movement (north-seeking in the southern hemisphere) for some tens or hundreds of micrometres, followed by a forward movement. The backward movement decelerates continuously with time, whereas the forward movement that follows it shows uniform acceleration (Greenberg *et al.*, 2005). This movement is very rapid, and the aggregate can reach distances of 100–150 μm. They maintain the same orientation with respect to the magnetic field lines when the sense of rotation of the body is inverted.

Aggregates demagnetized after a few seconds of exposure to a commercial tape recorder demagnetizer (60 Hz, 0.06 T) and showed no response to an applied magnetic field. When demagnetized samples were again exposed to an intense magnetic field produced by a SmCo magnet, we observed the remagnetization of the aggregates, predominantly of the original polarity. The alignment of the aggregates exposed to a 1×10^{-3} T magnetic field and then submitted to a short (1 ms) 0.1 T applied pulse showed that their net magnetization increased by only about 20 % relative to natural magnetization. The distribution of the magnetic moments of the magnetosomes is therefore optimized in the whole organism (Winklhofer *et al.*, 2007). We conclude that the disposition of magnetosome chains in the cells and the whole assembly is a highly controlled process that leads to efficient magnetotactic behaviour.

The vertical distribution of '*Candidatus* Magnetoglobus multicellularis' in core samples of undisturbed sediment located them within the anaerobic zone, preferentially at the 4.0 cm layer of the sediment, where sulfide is abundant because of dissimilatory sulfate reduction. In this environment, measurable dissolved oxygen occurs at a depth of no more than 0.3 cm into the sediment (Fig. 2). The distribution of '*Candidatus* Magnetoglobus multicellularis' seems to follow the same trend of magnetotactic bacteria that synthesize iron sulfide crystals, which are detected at the base of the oxycline or in the anoxic zone of aquatic environments (Simmons *et al.*, 2004).

After a standard PCR protocol for amplification and cloning, 16S rRNA gene fragments were characterized by restriction endonuclease digestion. The clone sequenced was

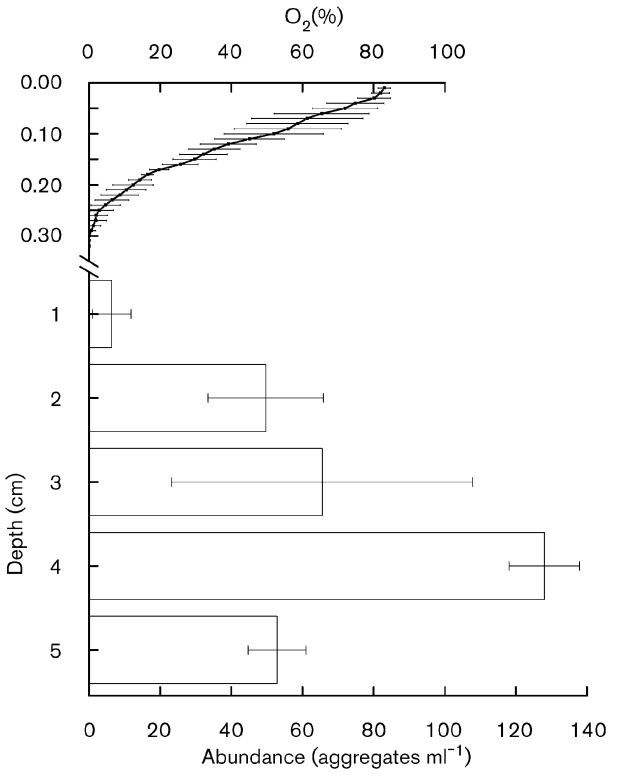

Fig. 2. Oxygen profile and density gradient of '*Candidatus* Magnetoglobus multicellularis' in the sediment of Araruama lagoon. The preferential location is within the anoxic zone of the sediment.

96 % similar to the deposited sequence of clone MMP1991 (GenBank accession no. L06457; DeLong *et al.*, 1993). Fluorescent *in situ* hybridization (FISH) showed that this sequence belongs to '*Candidatus* Magnetoglobus multicellularis' (Supplementary Fig. S2).

Phylogenetic analysis based on the 16S rRNA gene showed that '*Candidatus* Magnetoglobus multicellularis' belongs to the phylum *Proteobacteria*, class *Deltaproteobacteria*, order *Desulfobacterales*, family *Desulfobacteraceae*. '*Candidatus* Magnetoglobus multicellularis' groups together with the MMP collected in Little Sippewissett salt marsh (Simmons & Edwards, 2007), which was the closest affiliation (Fig. 3). Members of the genera *Desulfosarcina*, *Desulfonema* and *Desulfococcus* were the closest named organisms to which '*Candidatus* Magnetoglobus multicellularis' is related. The similarity value is 91 % for *Desulfococcus biacutus* and 92 % for *Desulfococcus multivorans*, *Desulfonema magnum* and *Desulfosarcina variabilis* strains. Recently, an unexpected phylogenetic diversity was found in populations of MMPs, suggesting that they should be considered a separate genus in the *Deltaproteobacteria* rather than a single species (Simmons & Edwards, 2007). In contrast, we did not observe sequence divergence that could imply the presence of different species in our samples.

As these aggregates are an uncultured, well-characterized bacterium (Keim *et al.*, 2004a, b; Silva *et al.*, 2007; Winklhofer *et al.*, 2007), we propose the name '*Candidatus* Magnetoglobus multicellularis', referring to the spherical morphology and magnetotactic behaviour of the aggregates from Araruama lagoon.

Species description

'*Candidatus* Magnetoglobus multicellularis' is a multicellular magnetotactic prokaryote or aggregate that moves as a unit in straight or helicoidal trajectories aligned to magnetic field lines, at a velocity of 90 ± 20 μm s^{-1}. Each aggregate is composed of 10–40 genetically identical Gram-negative bacteria containing magnetosomes and large lipid or polyhydroxyalkanoate inclusions. The cells are trapezoidal and organized precisely, side by side, in a sphere, 6.0–9.5 μm in diameter, and are polarized in the aggregate. '*Candidatus* Magnetoglobus multicellularis' disaggregates under low osmotic pressure or during extended light microscopy observation into individual cells, which are not viable, emphasizing its exclusively multicellular nature (Abreu *et al.*, 2006) (Supplementary Fig. S3). On the surface of the aggregate, bacteria are covered by a capsule, composed of radially arranged filaments and flagella. The high density of flagella (30 per cell on average) promotes its coordinated complex movements, including rotation and the so-called escape motility. Both the cell and outer membranes of adjacent cells are tightly apposed, indicating specific mechanisms for cell-to-cell binding. Each cell faces both the external environment and an acellular compartment in the middle of the organism. This acellular compartment is called the internal compartment and it is delimited by a belt of filaments between the apices of the cells. During its life cycle, the aggregate grows, approximately doubling its volume and the number of constituent cells. An increase in the total magnetic moment of the compact aggregate is also observed. After the increases in volume, number of cells and total magnetic moment, the compact aggregate divides into two new, identical aggregates. The whole process is multicellular and prevents any contact of the internal compartment with the exterior. '*Candidatus* Magnetoglobus multicellularis' contains pleomorphic crystals (length 88 nm; width 71 nm) composed of iron sulfide, comprising organelles named magnetosomes. The magnetosomes are distributed in planar groups near the periphery of each cell of the aggregate. Some characteristics of the aggregate are unique among prokaryotes, such as a multicellular life cycle, atypical cell division and requirement for cell organization in a multicellular form for survival. Furthermore, cell division in '*Candidatus* Magnetoglobus multicellularis' seems to be coordinated and synchronized. Phylogenetic analysis showed that this aggregate belongs to the phylum *Proteobacteria*, class *Deltaproteobacteria*, order *Desulfobacterales*, family

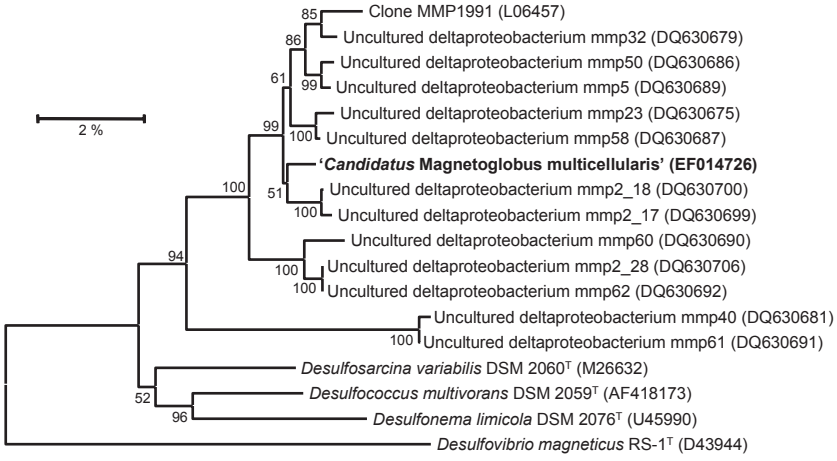

Fig. 3. Dendrogram of 16S rRNA gene sequences displaying the phylogenetic position of '*Candidatus* Magnetoglobus multicellularis'. Bootstrap values at nodes are percentages of 1000 replicates. GenBank accession numbers are given in parentheses. Bar, 2 % sequence divergence.

Desulfobacteraceae, and is related to the genera *Desulfonema*, *Desulfosarcina* and *Desulfococcus*.

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References

Abreu, F., Silva, K. T., Martins, J. L. & Lins, U. (2006). Cell viability in magnetotactic multicellular prokaryotes. *Int Microbiol* **9**, 267–272.

DeLong, E. F., Frankel, R. B. & Bazylinski, D. A. (1993). Multiple evolutionary origins of magnetotaxis in bacteria. *Science* **259**, 803–806.

Farina, M., Lins de Barros, H. G. P., Esquivel, D. M. S. & Danon, J. (1983). Ultrastructure of a magnetotactic microorganism. *Biol Cell* **48**, 85–88.

Greenberg, M., Canter, K., Mahler, I. & Tornheim, A. (2005). Observation of magnetoreceptive behavior in a multicellular magnetotactic prokaryote in higher than geomagnetic fields. *Biophys J* **88**, 1496–1499.

Hall, T. A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NY. *Nucleic Acids Symp Ser* **41**, 95–98.

Keim, C. N., Abreu, F., Lins, U., Barros, H. G. L. & Farina, M. (2004a). Cell organization and ultrastructure of a magnetotactic multicellular organism. *J Struct Biol* **145**, 254–262.

Keim, C. N., Martins, J. L., Abreu, F., Rosado, A. S., Lins de Barros, H. P., Borojevic, R., Lins, U. & Farina, M. (2004b). Multicellular life cycle of magnetotactic prokaryotes. *FEMS Microbiol Lett* **240**, 203–208.

Keim, C. N., Martins, J. L., Lins de Barros, H., Lins, U. & Farina, M. (2007). Structure, behavior, ecology and diversity of multicellular magnetotactic prokaryotes. In *Magnetoreception and Magnetosomes in Bacteria*, pp. 103–132. Edited by D. Schüler. Berlin & Heidelberg: Springer.

Kumar, S., Tamura, K. & Nei, M. (2004). MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. *Brief Bioinform* **5**, 150–163.

Lins, U., Freitas, F., Keim, C. N., Lins de Barros, H., Esquivel, D. M. S. & Farina, M. (2003). Simple homemade apparatus for harvesting uncultured magnetotactic microorganisms. *Braz J Microbiol* **34**, 111–116.

Lins, U., Keim, C. N., Evans, F. F., Farina, M. & Buseck, P. R. (2007). Magnetite (Fe_3O_4) and greigite (Fe_3S_4) crystals in multicellular magnetotactic prokaryotes. *Geomicrobiol J* **24**, 43–50.

Rodgers, F. G., Blakemore, R. P., Blakemore, N. A., Frankel, R. B., Bazylinski, D. A., Maratea, D. & Rodgers, N. (1990). Intercellular structure in a many-celled magnetotactic prokaryote. *Arch Microbiol* **154**, 18–22.

Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.

Sakaguchi, T., Arakaki, A. & Matsunaga, T. (2002). *Desulfovibrio magneticus* sp. nov., a novel sulfate-reducing bacterium that produces intracellular single-domain magnetite particles. *Int J Syst Evol Microbiol* **52**, 215–221.

Schleifer, K. H., Schüler, D., Spring, S., Weizenegger, M., Amann, R., Ludwig, W. & Köhler, M. (1991). The genus *Magnetospirillum* gen. nov. – description of *Magnetospirillum gryphiswaldense* sp. nov. and transfer of *Aquaspirillum magnetotacticum* to *Magnetospirillum magnetotacticum* comb. nov. *Syst Appl Microbiol* **14**, 379–385.

Silva, K. T., Abreu, F., Almeida, F. P., Keim, C. N., Farina, M. & Lins, U. (2007). Flagellar apparatus of south-seeking many-celled magnetotactic prokaryotes. *Microsc Res Tech* **70**, 10–17.

Simmons, S. L. & Edwards, K. J. (2007). Unexpected diversity in populations of the many-celled magnetotactic prokaryote. *Environ Microbiol* **9**, 206–215.

Simmons, S. L., Sievert, S. M., Frankel, R. B., Bazylinski, D. A. & Edwards, K. J. (2004). Spatiotemporal distribution of marine magnetotactic bacteria in a seasonally stratified coastal salt pond. *Appl Environ Microbiol* **70**, 6230–6239.

Spring, S., Lins, U., Amann, R., Schleifer, K., Ferreira, L. C. S., Esquivel, D. M. S. & Farina, M. (1998). Phylogenetic affiliation and ultrastructure of uncultured magnetic bacteria with unusually large magnetosomes. *Arch Microbiol* **169**, 136–147.

Winklhofer, M., Abraçado, L. G., Davila, A. F., Keim, C. N. & Lins de Barros, H. G. P. (2007). Magnetic optimization in a multicellular magnetotactic organism. *Biophys J* **92**, 661–670.

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