

Photokinesis is magnetic field dependent in the multicellular magnetotactic prokaryote *Candidatus Magnetoglobus multicellularis*

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Abstract *Candidatus Magnetoglobus multicellularis* is a spherical, multicellular, magnetotactic prokaryote (MMP) composed of 10–40 genetically-identical, Gram-negative cells. It is known that monochromatic light of low intensity influences its average swimming velocity, being higher for red light (628 nm) and lower for green light (517 nm). In this study, we determined the effect of light of different wavelengths and intensities on the swimming velocity of *Ca. Magnetoglobus multicellularis* under different magnetic field intensities. The swimming velocities of several organisms exposed to blue light (469 nm), green light (517 nm) and red light (628 nm) with intensities ranging from 0.36 to 3.68 Wm⁻² were recorded under magnetic field intensities ranging from 0.26 to 1.47 Oe. Our results showed that MMPs exposed to green light display consistently lower average swimming velocities compared to other wavelengths of light. We also show for the first time that photokinesis in *Ca. Magnetoglobus multicellularis* is dependent on the magnetic field being applied. The relationship between light wavelength and intensity and magnetic field strength and swimming velocity in this MMP is therefore complex. Although the mechanism for the observed behaviour is not completely understood, a flavin-containing chromophore may be involved.

Keywords Multicellular magnetotactic prokaryote · *Candidatus Magnetoglobus multicellularis* · Magnetotaxis · Photokinesis · Average velocity

Introduction

Magnetotactic bacteria are prokaryotes that passively align and actively swim along magnetic field lines (Blakemore 1982; Bazylinsky et al. 1995). These microorganisms contain magnetosomes, organelles consisting of membrane-enveloped magnetic crystals of magnetite (Fe₃O₄) or greigite (Fe₃S₄) (Gorby et al. 1988; Lins and Farina 2001) that cause cells of magnetotactic bacteria to orient along geomagnetic field lines while flagella propel the bacteria (Frankel et al. 1998). ‘*Candidatus Magnetoglobus multicellularis*’ (CMm) is a magnetotactic multicellular prokaryote (MMP) that swims as a unit in helical trajectories along geomagnetic field lines at a speed of 50–150 μm s⁻¹ (Silva et al. 2007; Perantoni et al. 2009; Almedia et al. 2013). CMm forms a sphere of 6.0–9.5 μm in diameter and is composed of several genetically-identical Gram-negative flagellated cells that are organised side by side in a spiral (Abreu et al. 2007). The number of cells is variable, usually between 10 and 40, each containing an average of 52 ± 40 iron sulfide magnetosomes (Martins et al. 2009), distributed in chains near the periphery of the microorganism (Abreu et al. 2008). The spiral distribution of cells creates a unique spatial distribution of magnetic moments that

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determines the magnetic properties of these organisms (Perantoni et al. 2009; Acosta-Avalos et al. 2012).

Photokinetic effects in MMPs were recently described by De Azevedo et al. (2013), showing that CMm exhibits a higher average swimming velocity when exposed to red light ($\lambda = 628$ nm) and lower swimming velocities under green light ($\lambda = 517$ nm), compared to those when the organism was exposed to yellow ($\lambda = 596$ nm) and blue ($\lambda = 469$ nm) light. The magnetic moment was also analyzed through the U-turn curve analysis (Esquivel and Lins de Barros 1986) and it was observed that the U-turn time decreases for green light illumination (De Azevedo et al. 2013) indicating that the magnetic moment must be higher than for the other illumination wavelengths. So far, there is no report relating the magnetic sensibility of MMPs to light wavelength and intensity, and magnetic field. Initially, De Azevedo et al. (2013) proposed that the observed illumination effect on the MMP velocity could be related to a light-dependent magnetotaxis. However, the different wavelengths used in that study do not affect the magnetic alignment behaviour of CMm organisms, maintaining magnetotaxis unchanged. It can be inferred from these results that magnetotaxis is not light-dependent. As a continuation of the study mentioned above, the present report presents an analysis of the effect of light of different wavelengths and intensities on the motion of CMm, measuring its translational velocity in the presence of different magnetic field intensities.

Materials and methods

CMm organisms were collected at Araruama lagoon (22°50'S; 42°13'W), a hypersaline (~ 55 ‰) coastal lagoon of Rio de Janeiro State, Brazil. Samples of water and sediment containing the microorganism were collected at a depth of about 1 m in the lagoon and transferred in the laboratory to aquaria of 12 cm \times 14 cm \times 20 cm with approximately 1/3 of sediment and 2/3 of water. The aquaria were placed near a window to allow sunlight illumination. 1 day after the collection, a portion of that sample, containing water and sediment, was collected at a zone somewhat below the sediment surface with a Pasteur pipette and transferred to a collector of magnetotactic microorganisms, as described by Lins et al. (2003). This glass container has two openings: a large one in the upper region, where water and

sediment are introduced, and a capillary end, where the microorganisms are concentrated, using a permanent magnet. As the MMPs collected are, at first, south-seeking, the north pole of a magnet was placed near and at the level of the capillary end to assemble an optimal amount of microorganisms in this region. After 15 min, a 200 μ L drop of water was collected from the capillary end with a dropper and placed on a coverslip. On the stage of an inverted microscope (Nikon Eclipse TS100, objective lens of 20 \times) was set a pair of coils connected to a DC power supply and, in the middle of these coils, the coverslip with the drop was placed. The pair of coils axis was oriented parallel to the horizontal component of the geomagnetic field. The measured magnetic field in the drop position is the resultant horizontal magnetic field. An electric circuit for changing the voltage polarity (reversal of current) was connected between the power supply and coils, leading to inversion of the magnetic field direction when turned on or off. Three LED lamps (blue—469 nm, green—517 nm and red—628 nm) were fastened to a metal adjustable holder, one by one, as needed. This metal holder puts the lamps exactly on the position of the microscope lamp, illuminating the drop evenly. The north pole of a magnet was placed near and at the level of the drop to assemble an optimal amount of microorganisms at the drop edge. After that, coils were turned on and the magnet was removed, leading the microorganisms to perform movements in a direction toward and away from the edge, in response to inversions of the magnetic field direction. The CMm movement was recorded with a digital camera coupled to the microscope (Infinity 1, Lumenera Corporation). Experiments were carried out in normal atmospheric conditions (pressure at sea level and temperature of about 30 °C in Rio de Janeiro).

Two different experiments were done. In the first experiment the average velocity as a function of the resultant magnetic field intensity (between 0.26 and 1.47 Oe) was measured, maintaining the light power equal for the three wavelengths i.e. 153 μ W (which corresponds to 1.95 W m⁻² in the drop). In this experiment the microscope tungsten lamp was used as a control and denominated as white light. In the second experiment eighty-four different recordings were made, using different drops in each movie, LED lamps, light power (between 28.1 and 289.2 μ W corresponding to

intensities between 0.36 and 3.68 W m^{-2}) and magnetic field intensities (between 0.26 and 1.47 Oe). Twenty-seconds of movement was recorded in each movie. A calibration grid was also recorded to calculate properly distances in μm . The irradiance within the drop was calculated to analyse this parameter and compare the behaviour considering the wavelength. The irradiance was calculated as $I/N_A \cdot E$ ($\mu\text{mol m}^{-2} \text{ s}^{-1}$), where I is the intensity ($\mu\text{W m}^{-2}$) and $N_A \cdot E$ is the amount of energy for 1 mol of quanta for each wavelength. N_A is the Avogadro constant (6.02×10^{23}) and E is the amount of energy per photon, corresponding to $E = h \cdot c/\lambda$, where h is the Planck constant ($6.63 \times 10^{-34} \text{ m}^2 \text{ kg s}^{-1}$), c is the light speed in vacuum ($3 \times 10^8 \text{ m s}^{-1}$) and λ is the wavelength (nm) of each lamp. The movies were analyzed frame by frame with the software AVS Video Converter 6 (AVS4YOU, UK). By using the Adobe Photoshop software it was possible to overlap the frames to identify individual trajectories, and calculate the distance traveled (Δx) (Fig. 1). To calculate the average velocity ($\mu\text{m s}^{-1}$) the relation $V = \Delta x/\Delta t$ was used, where Δt (s) is the time interval between the initial and last frames used to determine the distance. Δt was calculated by $\Delta t = N' \cdot \Delta T$, where N' is the number of frames and ΔT , the temporal spacing between the frames ($1/12 \text{ s}$, as 1 s corresponds to 12 frames). Graphs were made using Microcal Origin 6.0 software and statistical analysis was done with GraphPad InStat software.

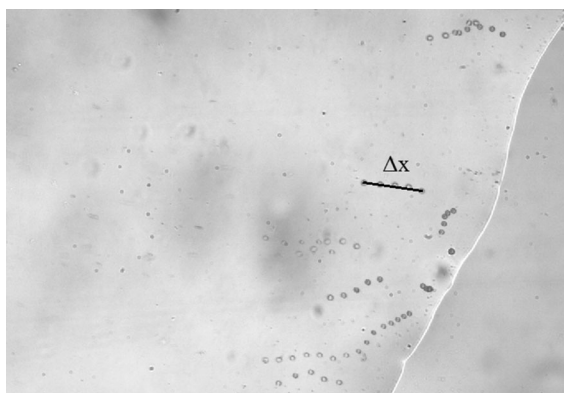


Fig. 1 Example of image used for the measurement of the CMm average velocity under white light and a magnetic field of 1.47 Oe . The black line represents $\Delta x = 150 \mu\text{m}$ at $\Delta t = 1.328 \text{ s}$, resulting in an average velocity of $112.9 \mu\text{m s}^{-1}$

Results and discussion

Figure 2 shows the mean average velocity as a function of the applied magnetic field. As can be observed, for magnetic field intensities higher than 0.4 Oe the average velocities for green light are lower than the velocities associated with the other illumination conditions (ANOVA test, $p < 0.05$). For green light, mean velocities for magnetic fields higher than 0.4 Oe are statistically similar and are different from mean velocities for magnetic fields lower than 0.4 Oe (ANOVA test, $p < 0.05$ and Tukey–Kramer multiple comparison pos-test). The mean average velocities for the magnetic field intensity near to the local geomagnetic value (0.23 Oe total intensity and 0.18 Oe horizontal component) are all similar, not depending on the wavelength (ANOVA test, $p > 0.05$). For magnetic fields higher than 0.4 Oe it was observed that the mean average velocities associated with red light were higher than those associated with blue light, but for some specific magnetic field intensities ($0.89, 1.1, 1.2$ and 1.47 Oe) the mean average velocities for blue light increase until they reach the mean average value for red light (ANOVA test, $p > 0.05$). The mean average velocities for red light were always higher than for white light. The mean average velocity observed in Fig. 2 does not show any recognisable

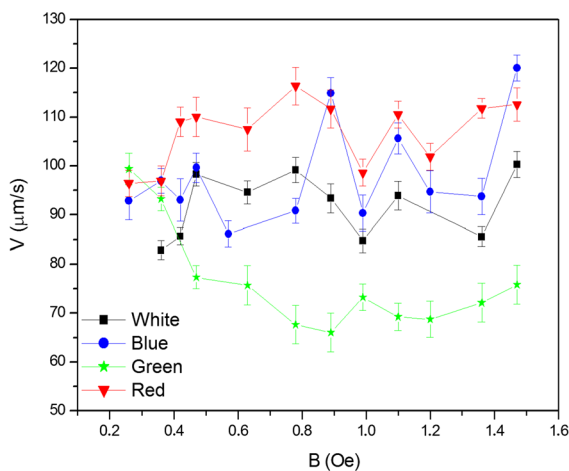


Fig. 2 Mean average velocity (V) as a function of the applied magnetic field (B). Vertical lines in every symbol represents the standard error. The light intensity of 1.95 W m^{-2} was maintained constant for the three wavelengths. White light intensity was measured with a light meter (cal-light 400 L, the Cooke Corp.) and was 237 lx . Each point corresponds to the mean of 40 different measurements

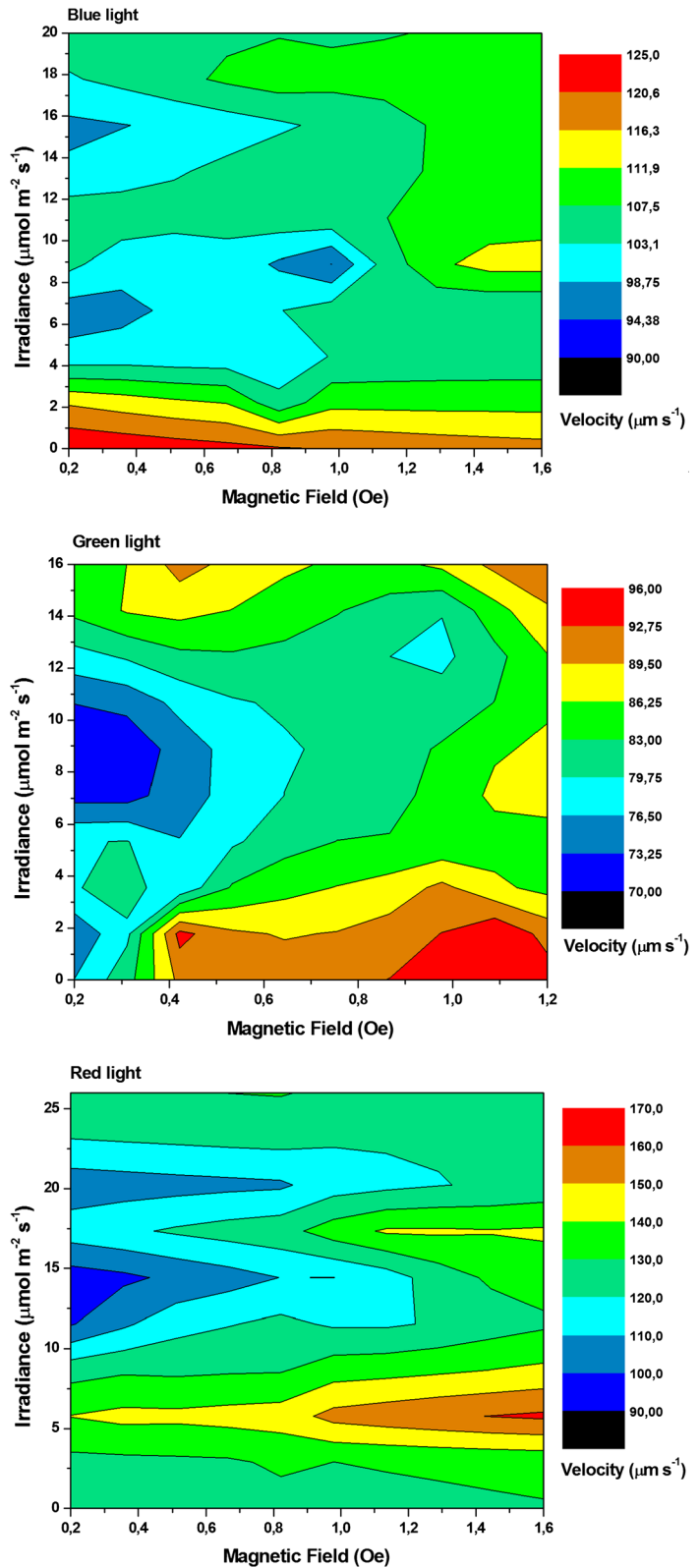
functional behaviour. The parallel component of the velocity to the external magnetic field vector must grow with the magnetic field intensity following a Langevin curve (Kalmijn 1981). In this report the average velocity measured for each CMm corresponds to the projection of the velocity vector in the focal plane, and not to the component of the velocity vector parallel to the magnetic field direction. So, it is not expected that the mean average velocity also follows a Langevin curve. Interestingly, the behaviour observed for the mean average velocity associated with green light illumination is similar to that observed for a magnetotactic coccoid bacteria, but illuminated with white light and observed with magnetic field intensities higher than those used in the present work (Pan et al. 2009). To explain that behaviour in coccoid bacteria, Pan et al. (2009) assumed that the magnetosome chain axis is not collinear with the flagellar propulsion axis, producing an additional torque causing changes in the bacterial helical trajectory. For CMm organisms, the situation is quite different since their magnetic moment is the result of the vectorial addition of individual magnetic moments distributed in a spiral curve (Acosta-Avalos et al. 2012). Perhaps green light influences the flagellar propulsion axis in CMm organisms, changing the relation between the CMm resultant magnetic moment vector and the resultant flagellar propulsion axis. It is interesting to note that as the photon energy depends on the wavelength, the flux of photons impinging on the CMm organisms is different for each wavelength. The irradiance in this experiment was $7.6 \mu\text{mol m}^{-2} \text{s}^{-1}$ for blue light, $8.4 \mu\text{mol m}^{-2} \text{s}^{-1}$ for green light and $10.2 \mu\text{mol m}^{-2} \text{s}^{-1}$ for red light. It is interesting to observe that red light has a higher flux of incident photons and also produced the higher mean average velocities.

The next experiment was analysed using the irradiance instead the light intensity. Figure 3 shows the mean average velocity as function of the magnetic field intensity and the irradiance for each wavelength. As can be seen, the mean average velocity is a complex function of magnetic field and irradiance. Depending on the amount of light energy and magnetic field intensity the average velocity can increase or decrease, as is observed in Fig. 2. Mean average velocities associated with green light were always lower than the velocities associated with the other wavelengths. This result shows that photokinesis is

magnetic field dependent. For red light it is interesting to observe the behaviour at about $6 \mu\text{mol m}^{-2} \text{s}^{-1}$ because at this irradiance the mean average velocity increases monotonically with the magnetic field. For blue light it was observed that the mean average velocity decreased when the irradiance increases up to $4 \mu\text{mol m}^{-2} \text{s}^{-1}$. It is also remarkable that the lower average velocities are in the region with magnetic fields lower than 1 Oe. Observing Figs. 2 and 3, it seems that the main difference between different light wavelengths occurs for magnetic fields higher than the geomagnetic field. This could be associated with the MPPs behaviour in lagoon sediments with magnetic particles, which must increase the local magnetic field. In these microenvironments where MMPs are found, light is filtered by the water column and superficial sediments, leading to a prevalence of blue and green light (De Azevedo et al. 2013) that are the wavelengths producing small velocities, enhancing the chemotaxis of these organisms. The integration of photo-, chemo- and magnetotaxis could result in faster discovery of the proper microenvironments of CMMs. Our results could mean that the local magnetic microenvironment is also important, perhaps increasing the velocity to escape from regions with higher concentrations of magnetic particles and low irradiance (Fig. 3).

The present results show that the average velocity is a complex function of the magnetic field and the light irradiance. It is not clear why the average velocity increases with red light and decreases with green light, having intermediate values with blue light. As the velocity depends on the flagellar activity, its increase or decrease must be related to an interference of magnetic fields and light on the flagellar dynamics. Chromophores in magnetotactic bacteria are little studied. It is known that proteins associated with magnetosome crystal formation and maturation (Mam E, Mam P and Mam T) are *c*-type cytochromes (Siponen et al. 2012). However it is not known if these proteins are sensitive to low intensity magnetic fields. As far as we know, the effect of magnetic fields of about 1 Oe on light absorbing molecules has not been studied. The only molecule known to be sensitive to magnetic fields less than 1 Oe which absorbs light is the cryptochrome, associated with light dependent magnetoreception in animals (Liedvogel and Mouritsen 2010). The chromophores responsible for UV and blue light absorption in that photomolecule are pterin and flavin (FAD), respectively. Interestingly, the

Fig. 3 Mean average velocity as a function of the applied magnetic field and light irradiance. The wavelengths for each monochromatic light were: 469 nm for *blue*, 517 nm for *green* and 628 nm for *red*. The velocity scale is not equal in all level curves because the velocity ranges are different for each case, but the color interpretation is the same: *red* for higher and *black* for lower velocities



flavin semiquinone (neutral radical FADH•) has a light absorption spectrum with peaks at about 500 nm (green) and 630 nm (red) and a minimum at about 540 nm (green) whereas normal FAD has an absorption peak at about 450 nm (blue) (Liu et al. 2010). Also FADH• concentration is affected in the cryptochrome photocycle in the presence of magnetic fields (Ritz et al. 2010). Perhaps FAD and FADH• excited with blue and red light are sensitive to low intensity magnetic fields, modifying the flagellar dynamics in CMm organisms. Flavins have been found in flagella in algae (Dederichs et al. 1999; Fujita et al. 2005) and also in the aerotactic sensor of *Escherichia coli* (Bibikov et al. 2000). CMm organisms are composed of multiple cells (between 10 and 40 individuals) forming spherical organisms. Flagella are located in the part of each cell that faces outside the sphere, with about 30 flagella per cell. The movement of the CMm organism is the result of the coordination of all the flagella in all the bacteria that compose it (Silva et al. 2007). It is unknown if CMm contains flavins bound to its flagella. Perhaps FAD could be also part of a cryptochrome-like sensor protein which could control indirectly or directly flagellar rotation speed. Our results open this possibility and encourage new studies for finding chromophores related to the flagellar apparatus in MMPs.

In conclusion, for the first time it has been shown that photokinesis in CMm organisms is magnetic field dependent. Lower mean velocities are observed consistently when illumination is done with green light (517 nm) and higher velocities with red light (628 nm). It is hypothesised that the chromophore FAD is responsible for this behaviour and related in some way to the CMm flagellar apparatus.

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