Bioluminescence of marine vibrios is sensitive to magnetic field

A. Talà,[1] M.V. Siciliano[2], G.Buccolieri[3], S.M. Tredici[1] F.Paladini[2], M.De Stefano[4], V.Nassisi[2] and P.Alifano[1*]

[1]*Dipartimento di Scienze e Tecnologie Biologiche ed Ambientali, Università del Salento, via Provinciale Lecce-Monteroni, 73100 Lecce, Italy;*

[2]*Dipartimento di Fisica, Università del Salento INFN - Lecce, via Provinciale Lecce-Monteroni, 73100 Lecce, Italy;*

[3]*Dipartimento di Scienza dei Materiali, University of Salento, via Provinciale Lecce-Monteroni, 73100 Lecce, Italy;*

[4]*Dipartimento di Scienze Ambientali. Seconda Università di Napoli, via A. Vivaldi 43, 81100 Caserta, Italy*.

* Tel: +39 (0) 832 298856, Fax: +39 (0) 832 29898626, e-mail: pietro.alifano@unisalento.it

Abstract

In *Vibrio harveyi*, a bacterium living in symbiosis with marine organisms, bioluminescence and the expression of several virulence factors are regulated by quorum sensing (QS), the communication circuit that many bacteria use to sense population density and regulate a diverse array of physiological activities. In this study, the evidence of magnetic crystals in *V. harveyi*-related strain PS1 led us to investigate the behaviour of this bacterium under exposure to magnetic field (MF). We found that MF stimulated bioluminescence and the physiological significance of the observed magnetic responses of *Vibrio* sp. PS1 has been discussed with reference to its symbiotic life.

INTRODUCTION

The magnetic-field sensory perception called magnetoreception is one of the most intriguing phenomena in Nature. It influences the activities of many living organisms, including the migratory behavior of birds [1], homing orientation of pigeons [2], navigation of sharks, rays and sea turtles [3, 4], quick directionalization to the nearest shore by salamanders, frogs and sea turtles when they sense danger [3-6], comb building and homing orientation by honeybees [7-9]. Magnetoreception has been also reported in plants [10] and protists [11], and magnetite biomineralization has been demonstrated in human brain [12].

Magnetic responses are fairly common among marine bacteria. The best-known examples are the magnetotactic bacteria that use highly ordered and chemically pure crystals of magnetite (Fe_3O_4) and greigite (Fe_3SO_4), arranged in one or more chains, to form magnetosomes. These magnetic organelles, which are surrounded by an electron dense lipid layer, act like a compass needle to orient magnetotactic bacteria in Earth's magnetic field, thereby simplifying their search for their preferred microaerophilic environments [13-16].

In this study, the discovery of magnetic crystals in the cytoplasm of *V. harveyi*, a common inhabitant of tropical and temperate marine environments, either in a free-living state or in symbiosis with marine life [17-21] led us to investigate the behaviour of this luminescent microorganism under exposure to static MF. We found that bioluminescence is sensitive to MF. When exposed to a static MF an enhancement of the luminous intensity emitted by the cultures growing on solid medium was observed as a function of dose.

RESULTS

Vibrio sp. PS1 contains unusual magnetic crystals

PS1 is a luminescent bacterium, taxonomically related to the species *V. harveyi*, which was recently isolated from a marine hydrozoon [21]). In the course of a study dealing with the ultra-structure of this bacterial isolate, transmission electron microscopy (TEM) images showed intracellular electron dense inclusions about 10–50 nm in size (Fig.1A-D). These inclusions exhibited a polyhedral shape and were typically located in the nucleoid area. They did not appear to be surrounded by membrane or membrane-like structure and were never arranged in a chain. The crystals (or some of them) were magnetic as they could be purified by magnetic separation and observed by TEM and scanning electron microscopy (SEM) (Fig. 2A-B).

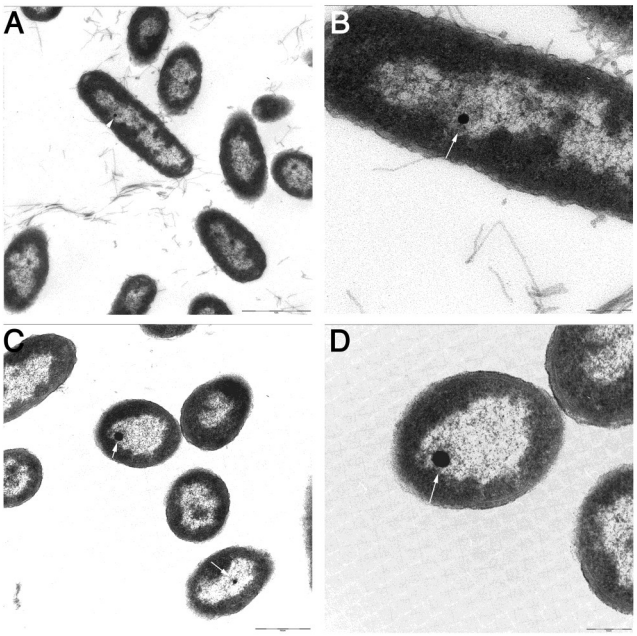

Fig. 1. Transmission electron microscopy (TEM) analysis of *Vibrio* sp. PS1. Note the presence, in the nucleoid area, of the electron dense inclusions about 10–50 nm in size exhibiting a polyhedral shape (arrows). Panels B and D are enlargement of regions of panels A and C, respectively. Bars represent 1 mm in A, 0.5 mm in C and 0.2 mm in B and D.

Bioluminescence is affected by static MF exposure

The presence of magnetic crystals in the cytoplasm of Vibrio sp. PS1 led us to investigate the behaviour of this microorganism under exposure to MF. The presence of magnetic crystals in the cytoplasm of Vibrio sp.

PS1 led us to investigate the behaviour of this microorganism under exposure to MF. We started analysing possible magnetotactic responses without success. Then we explored the effect of the exposure to static MF on bioluminescence. When spotted onto agar plates, Vibrio sp. PS1 exhibited an intense luminescence showing a peak at 470 nm and a shoulder near 500 nm (Fig. 3).

Bacteria were exposed or sham-exposed to a static MF of 20, 200 and 2000 Gauss during their growth in a climate chamber under nearly constant temperature and humidity conditions, in the dark. Luminescence was monitored over a period of 350 h by using the apparatus showed in Fig. 4. When compared to sham-exposed bacteria, the light emission of MF-exposed bacteria growing on solid medium was not affected at 20 Gauss, but it enhanced progressively at 200 and 2000 Gauss, protracting much longer during the stationary phase when an enhancement of light emission, barely detectable in sham-exposed bacteria and in bacteria exposed to 20 and 200 Gauss, was ob-

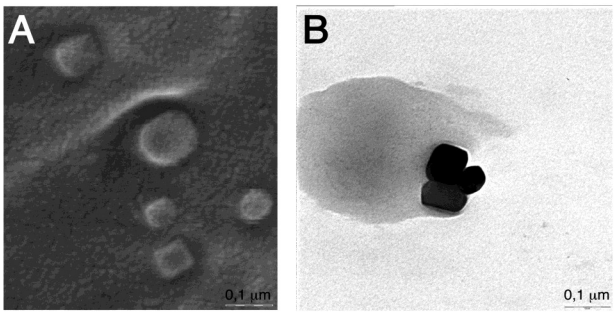

Fig. 2. Images of purified magnetic particles. (A) Purified magnetic particles were analysed by scanning electron microscopy (SEM). (B) Purified magnetic particles were analysed by transmission electron microscopy (TEM). Note the tendency of the particles to stick together confirming their magnetic nature. Bars represent 0.1 mm in A and B.

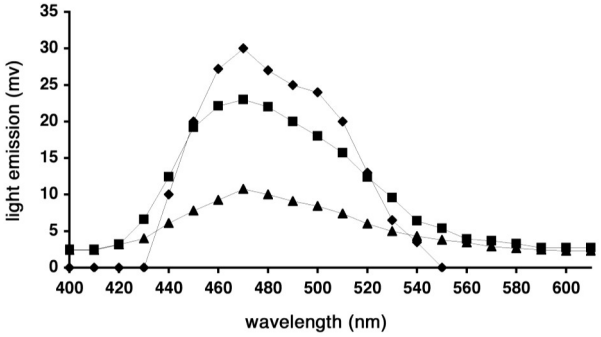

Fig. 3. Emission spectra of Vibrio sp. PS1after 8 (triangles), 16 (squares) and 24 (diamonds) h of growth on nutrient brtoth containing 3% NaCl at 20°C.

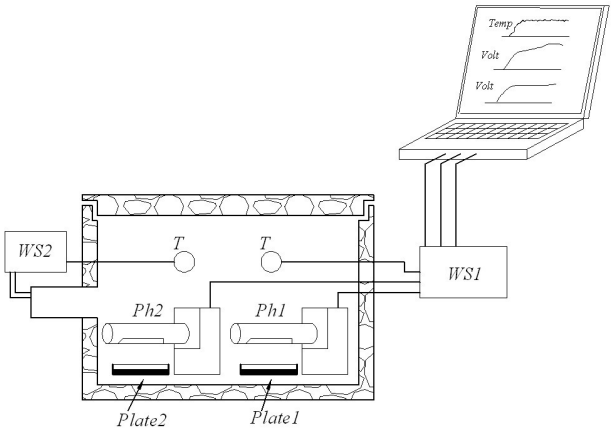

Fig. 4. Light emission monitoring during growth of *Vibrio* sp. PS1. Light emission of MF-exposed (20, 200 or 2000 Gauss) or sham-exposed *Vibrio* sp. PS1 was monitored during growth on nutrient agar 1.5% containing

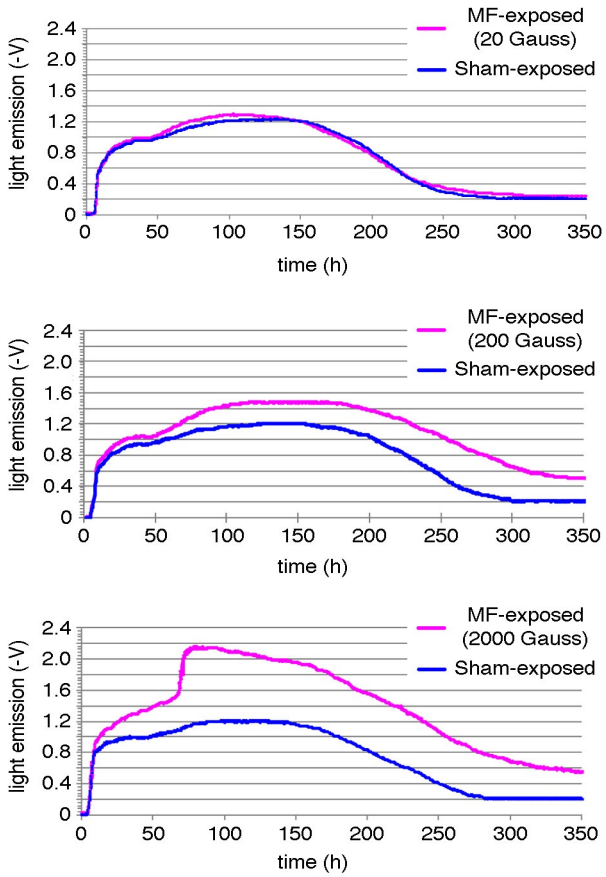

Fig. 5. Light emission monitoring during growth of *Vibrio* sp. PS1. Light emission of MF-exposed (20, 200 or 2000 Gauss) or sham-exposed *Vibrio* sp. PS1 was monitored during growth on nutrient agar 1.5% containing 3% NaCl. These measurements were repeated five times with comparable results.

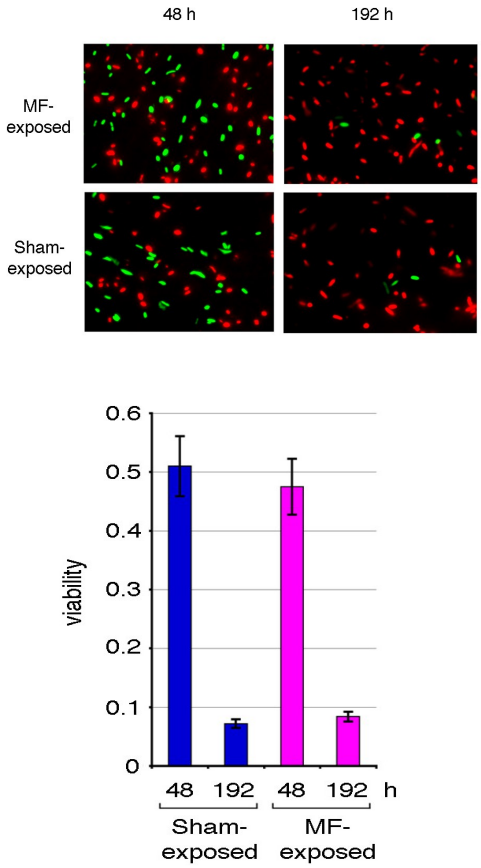

Fig. 6. Viability assessment during growth of *Vibrio* sp. PS1. Viability of MF-exposed (200 Gauss) or sham-exposed *Vibrio* sp. PS1 was determined by using the Live/Dead Bac*Light* method as detailed in the Materials and Methods section. Data are shown as mean ± standard deviation from five independent experiments.

This phenomenon was not due to an effect of MF on bacterial growth or viability. In fact, viability assessment by using either the CFU method (data not shown) or a dead/live staining (Fig. 6 and data not shown) demonstrated the absence of any significant differences between MF-exposed (20, 200 or 2000 Gauss) and sham-exposed bacteria.

The enhancement was not even due to a direct effect of MF on the photochemical reaction catalyzed by the bacterial luciferase because the luminous intensity of the bacteria grown for 48 h without MF (20, 200 or 2000 Gauss) did not change in a short time (as expected if a direct effect of MF on the photochemical reaction were involved) following exposure to MF (data not shown). We thus believed that gene regulation could be involved, as demonstrated in *Shewanella oneidensis* in which whole genome microarray data showing that a *luxR* family gene was among the 21 genes of *Shewanella oneidensis*, whose expression was significantly up-regulated following exposure to a strong static MF [22].

DISCUSSION

In this study, evidence is provided that *Vibrio* sp. PS1, a luminescent bacterium, taxonomically related to the species *V. harveyi*, is responsive to static MF. When compared to sham-exposed bacteria, the light emission of MF-exposed bacteria growing on solid medium was significantly enhanced, with a dose-response relationship, and protracted much longer during the stationary phase (Fig. 5).

The magnetic crystals observed in the cytoplasm of *Vibrio* sp. PS1 (Figg. 1,2) might also be involved in the mechanism of magneto-reception. At variance with the magnetite and greigite crystals of the magne-

tosomes occurring in magnetotactic bacteria, these crystals are not arranged in chains. Thus it is unlikely that they can act like a compass needle to orient the bacteria during their movement. Their size up to 50 nm is big enough to have a magnetic field and at the same time small enough to remain a single magnetic domain [23]. However, the precise mechanism by which they may act as MF sensors is currently unknown, as well as the chemical composition and biogenesis of these crystals.

The most intriguing question concerns the physiological significance of the presence of magnetic crystals and the observed magnetic responses of *Vibrio* sp. PS1. As previously mentioned, *V. harveyi* is often found in symbiosis with marine organisms that use bioluminescence as a form of optical communication for attracting mates or prey, or for defence against predation in dark environments [24]. Since it seems unlikely that these bacteria use the magnetic crystals for magnetotaxis, it is possible that the observed magnetic responses may play a role during the host colonization when bacteria form a well-structured biofilm and activate the QS circuit. In this regard, it is worthy of note the study of Simmons and colleagues [25], which emphasizes the need of new models to address the role of magnetoreception in bacteria. host colonization when bacteria form a well-structured biofilm and activate the QS circuit. In this regard, it is worthy of note the study of Simmons and colleagues [25], which emphasizes the need of new models to address the role of magnetoreception in bacteriastructured biofilm and activate the QS circuit. In this regard, it is worthy of note the study of Simmons and colleagues [25], which emphasizes the need of new models to address the role of magnetoreception in bacteria.

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MATERIALS AND METHODS

Bacterial strains and growth media

Vibrio sp. PS1 was described previously (20). For bioluminescence monitoring *Vibrio* sp. PS1 was cultured on nutrient broth (Difco) containing 3% NaCl at 20°C to an optical density of 1.0 at 550 nm. Ten microliters of the suspension was spotted at the centre of 3% NaCl nutrient agar plates.

Bioluminescence monitoring

To perform measurements of bacteria luminescence we prepared two identical experimental set up inserted inside the climate chamber under nearly constant temperature and humidity conditions (Fig. 4). Absolute dark inside was operated. Each experimental set up contained a very sensible photomultiplier (PMT) 1IP28 capable to record light of low intensity emitted by our samples. Indeed the gain factor was of $5x10^6$. The sensibility range of photomultiplier ranged form 185 to 700 nm. Its active window was 24 mm height and 8 mm width that we utilized to pick up the whole light emitted from samples. The photomultiplier signals were leaded to a workstation interfaced to a personal computer utilized like storage. A channel of the workstation was utilized to record the temperature.

Wavelength monitoring

The wavelength monitoring was performed by a 0.300 meter focal length monochromator SP-308 interfacing with a PC capable to control the wavelength value (Fig. 7). The grating utilised had f 1200 g/mm. and the whole system was sensible for the range from 350 to 800 nm. The plates contained the nutrient agar were exposed to the entrance of a UV optical fiber which leaded the emitted light to the monochromator. The output of the monochromator was connected to a 1IP28 photomultiplier The signal intensity of the PMT was very low and in this case a number of photons enter the photomultiplier tube and create an out-

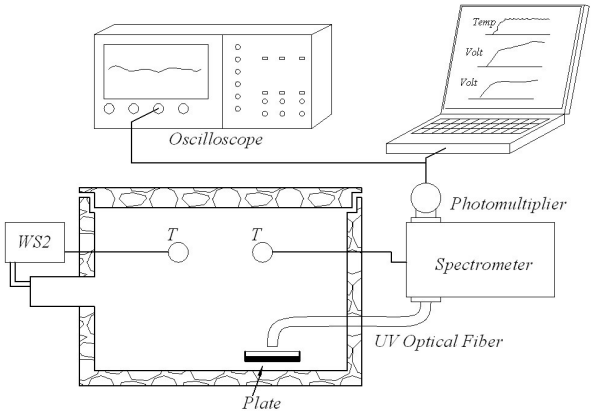

Fig. 7. Sketch of the apparatus utilized to record the emission spectrum. WS2: workstation; T: thermometer.

put pulse train. Thee oscilloscope shows flash signals of different intensity and repletion rate. Therefore to estimate the value of the intensity we operated the overlapping of the output pulses till up 300 samples. Recording these results on wavelength the response was a constant value by it we determined the wavelength spectra.

Exposure of bacteria to static MF

Exposure of bacteria to static MF was achieved by using magnets of circular geometry, which were applied on the back of Petri dishes as shown in Fig. 8. The magnetic flux density (20 Gauss [= 2 mT], 200 Gauss [= 20 mT] and 2000 Gauss [= 200 mT]) was modulated by using magnets of different strength. Magnetotactic responses were determined microscopically using the method of Blakemore and collegues [26].

Electron microscopy

For transmission electron microscopy (TEM) samples were fixed with 2% glutaraldehyde and 1% formaldehyde in 0.04 M piperazine-N, N'-bis (2-ethansulfonic acid) (PIPES) buffer at pH 7.0 for 2 h at room temperature. The samples were rinsed in 0.08 M PIPES buffer and twice in 0.08 M Na-cacodylate buffer and post-fixed in 1% OsO_4 in 0.08 M Na-cacodylate buffer, pH 6.7, overnight at 4°C. Following dehydration in a step gradient of ethanol with three changes of anhydrous ethanol and one of propylene oxide incubation step at 4°C, the samples were slowly infiltrated with Epon 912 resin at 4°C, transferred to polypropylene dishes and incubated at 70°C for 24 h. Thin sections were stained with 3% uranyl acetate in 50% methanol for 15 min and in Reynold's lead citrate for 10 min and then examined with a Leo 912AB electron microscope.

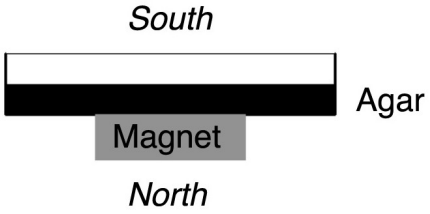

Fig. 8. Example of magnet position.

For scanning electron microscopy (SEM) observations, samples were fixed with 1% glutaraldehyde, washed three times with distilled water by centrifugation, dehydrated in a graded alcohol series and critical-point dried. The sample was then mounted on Aluminum stubs, sputter-coated with gold and examined at an accelerating voltage of 20 kV with a Jeol 6060LV Scanning Electron microscope.

Separation of magnetic crystals

Magnetic nanoparticles of *Vibrio* sp. PS1 were purified from broken cells by a magnetic separation technique. Approximately 2 x 10^{11} bacteria cells suspended in 5 ml of buffer A (50 mM Tris-Cl pH 7.5, 0.1 mM phenylmethylsulfonyl fluoride) were disrupted by two passes through a French pressure cell at 750 p.s.i. (1 p.s.i. = 6.89 kPa). Unbroken cells and cell debris were removed from samples by centrifugation at 10.000 *g* for 15 min. The cell extract (1 ml) was poured into a 1 cm spectrophotometric cuvette and covered on top. Two magnets generating a magnetic field gradient were applied on opposte sides of the cuvette. Control cuvettes without magnets were used. After 12 h incubation a blackish magnetic fraction accumulated at the sides of the cuvette nearest the magnets. The nonmagnetic fluid fraction was removed by aspiration, and the magnetic phase was suspended in 500 µl of buffer A and again subjected to magnetic separation. This procedure was repeated at least three times. Finally, the magnetic particles were fixed for TEM or SEM analysis as described above.

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