Effects of static and time-varying magnetic field exposure on bioluminescence pattern in *Vibrio jasicida*

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Abstract
Static and variable magnetic field radiation, using magnets and radiofrequency sources, is believed to be a stress factor in living biological systems. Here we investigated the effects of the magnetic field by static fields and radiofrequency fields on bioluminescence using as a model organism the bioluminescent bacterium *Vibrio jasicida* belonging to the Harvey clade. This bacterium was cultured either in the absence or in the presence of static (Bo, 0 Hz), very high frequency (VHF, 100 MHz), and ultra high frequency (UHF, 900 MHz) field irradiation in a climate chamber at three different temperatures of 20, 25 and 30 °C. Deconvolution analysis of spectral emission data demonstrated the presence of two major emission peaks centered, respectively, at about 486 nm (peak #1) and 457 nm (peak #2) during the logarithmic phase of growth. The exposure to magnetic fields led to evident changes in the bioluminescence pattern (peak intensity and wavelength) as a function of the incubation time demonstrating measurable effects of the magnetic field in a biological system, whose significance will be discussed.

I. INTRODUCTION

Nowadays, the presence of magnetic fields is continually increasing. This consequence is due to the use of electronic instruments which are feed by electric currents. It is also known variable magnetic fields generate electric fields, called inducted fields different from the electrostatic ones, and they are regularized by the Faraday law. The knowledge of magnetic effects on the live organism has a twofold purpose: the first is to discover the role of magnetism on the behavior of living beings; the second is to understand the result of the biological effects, both positive and negative [1-3].

The importance of the magnetic field in biological matter consists in the fact that it can interact with the moving electric charges, while the electric field interacts with the electric charges, stopped or moving applying the Coulomb force.

Regarding the biological matter, the charges are closely bound to atoms but they are responsible of the magnetic moment of the matter. The exhibited magnetic moment of matter is regulated by quantum mechanics and it is responsible of the ferromagnetism and paramagnetism of molecules, behaviors very evident. Minor evident is the diamagnetism, a characteristic of all molecules, and it is sensitive to the derivative of the magnetic flux

due to the Lentz law. Therefore, in diamagnetism matter, only fields of high frequency can deliver to significant results.

Generally, the biological matter can be considerate paramagnetic, but generally the interaction of the magnetic field with the matter exhibits a mechanical moment corresponding to:

$$\vec{N} = \vec{M} \times \vec{B} \quad (1)$$

where $\vec{M}$ represents the magnetic moment of matter and $\vec{B}$ the magnetic field. Inside the matter the mechanical moment causes a variation of the orientation and of the energy of the same molecule expressed by [4]:

$$E_B = -\vec{M} \cdot \vec{B} \quad (2)$$

The study of living matter exposed to fields is very difficult due to the complexity of the cell membrane structure. In these cases, it is reasonable to suppose that the mechanical moment induced by the magnetic field can influence the charge transport through the membrane and the energetic state. The magnetic field could interact directly with the DNA, but this is to be discovered. Considering the only magnetic moment due to the electron whose spin is ± ½, the energy variation according to Eq. 4 can be positive or negative, that is[4]:

$$E_B = \pm g \mu_B B m \quad (3)$$

where g is the factor of Landé which is close to 2.00 for free electrons and for most organic radicals, μ_B is the magnet of Bohr, B is the magnetic field and $m$is the quantum constant[5].

It is known that on the earth a magnetic field intensity is present and it is very relevant for many living organisms even if its intensity is very low, about $50\ \mu T$ as well as its frequency. In general, more intense fields are used in laboratory experiments and they are expressed by the term of moderate field and range from a few 10^{-3} to 1 Tesla.

II. EXPERIMENTAL APPARATUS

II:1**Bacterial strains and growth conditions**

For bioluminescence monitoring, the bacterium *Vibrio jasicida* PS1 was cultured on luminescent agar (LA) medium (30 g/l NaCl, 5 g/l Yeast extract, 10 g/l Bacto Peptone, 15 g/l Bacto Agar). Single luminescent colonies were picked, inoculated into 5 ml luminescent broth (30 g/L NaCl, 5 g/L Yeast extract, 10 g/L Bacto Peptone), grown to 0.3 O.D. at 550 nm. Ten µl of broth cultures were spotted at the centre of LA agar plates. Plates were incubated at 20, 25 and 30 °C, either in presence or in the absence of stresses as detailed below.

II:2 **Static magnetic field (Bo) irradiation**

The static magnetic field, from now on named Bo, is easily obtained by a simple magnet. In this case we used a magnetic disco by $4\ cm$ in diameter. To irradiation the sample contained in Petri cells by $5.5\ cm$ in diameter, we placed it on the magnet on the North late. The magnetic field intensity was about $40\ mT$ at about $0.5\ cm$ from magnet center.

Figure 1: Photo of the static magnetic field (Bo)

During the exposure, the samples were not moved allowing to magnetic field to operate with the same configuration. Therefore, all eventual magnetic moments present after to have be orientated stayed stop. *Fig. 1* shows the experimental set up.

II:3 **Very high frequency (VHF) magnetic field irradiation**

Our goal was to carry out measurements in the range of hundred MHz and for this reason we have created a transmission line. It was designed with a height $h = 1.4\ cm$, width $a = 9\ cm$ and with a lengthy of $20\ cm$. The length of the line does not affect the characteristic impedance but allows to treat more samples. The RF generator was a RHODE & SCHWARZ SM 300. Its output power is $20\ mW$. The expression of the characteristic impedance of the flat line, excluding the external radiation and pointing out with L and C, inductance and capacitance for length unit, respectively, is looked at the following formula[6]:

$$\sqrt{\frac{L}{C}} = \sqrt{\frac{\mu_o}{\varepsilon_o}}\frac{h}{a} \qquad (4)$$

where ε_o and μ_o are the electrical permittivity and magnetic permeability, respectively. From Eq. 4 it is deduced that our line has an impedance of about $50\ \Omega$ like the one of the generator output impedance. As a consequence, one line connector (input) was connected to the generator via a high frequency $50\ \Omega$, while the output was connected to a $50\ \Omega$ load utilizing 4, $200\ \Omega$ resistors, *Fig. 2*.

The wavelength at $100\ MHz$ in vacuum is about $3\ m$, while makes secure the transversal uniformity of the fields.

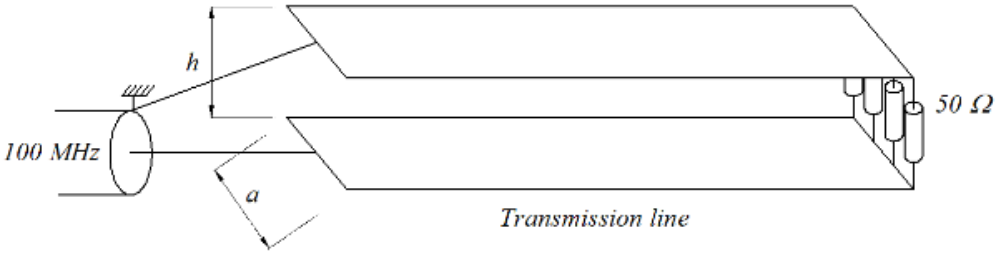

Figure 2: Plane transmission line for Very High Frequency (VHF)

The applied input signal was $1\ V$ at $100\ MHz$ and the correspondent electric field was about $71\ V/m$, while the magnetic one was about $240\ nT$.

All measurements were made with a Le Croy Wavepro 7100 fast oscilloscope, 200GS/s with 1 GHz band limitation.

II:4 **Ultra high frequency (UHF) magnetic field irradiation**

To perform treatments at $900\ MHz$ we utilize a short transmission line in order to limit the effects of stray irradiations. Therefore, to preserve the characteristic impedance of the system, the dimensions of the line were again $h = 1.4\ cm$, $a = 9\ cm$, while the lengthy was of $12\ cm$. The RF generator was a RHODE & SCHWARZ SMF 100A having an output power of 20 W. The expression of the characteristic impedance of the flat line is express by $Eq.5$ excluding the external radiation.

The wavelength at $900\ MHz$ in vacuum is about $0.33\ m$. So, to limit the irradiation, it is necessary to modify the conductors bending the lateral outline and the final line width is about $0.10\ m$

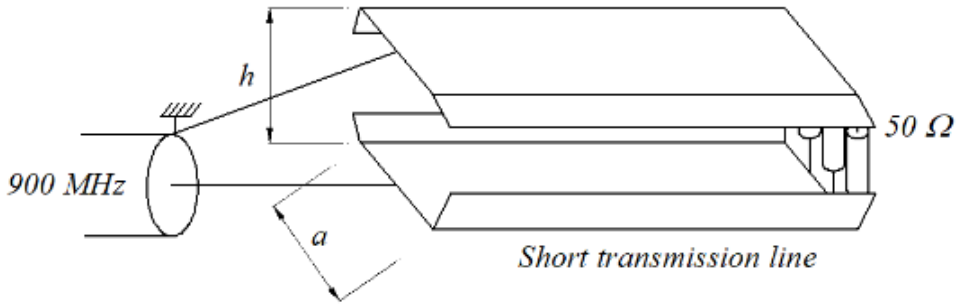

Figure 3: Short transmission line for Ultra High Frequency (UHF)

The applied input signal was again $1\ V$ and the correspondent electric field was about $71\ V/m$, while the magnetic one was again about $240\ nT$.

All measurements were made with a Le Croy Wavepro 7100 fast oscilloscope, 200GS/s with 1 GHz band limitation.

II:5 **Experimental arrangement for the bioluminescence measurements.**

Measurements of bioluminescence were performed in a climate chamber sketched in *Fig. 4*. Inside the chamber it is possible to arrange simultaneously the control plate (CTR), the static magnetic field plate Bo (0 Hz); the transmission line for the irradiation of bacteria by VHF (100 MHz) and the one for the irradiation by UHF (900 MHz).

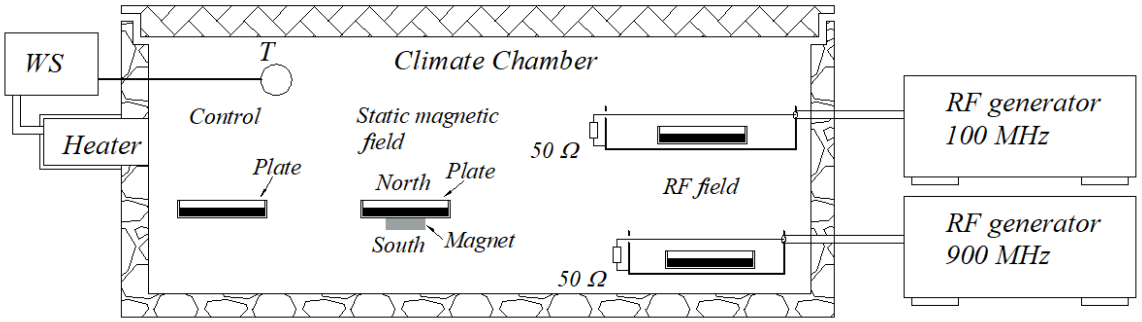

Figure 4: Climate chamber for the bacteria incubation and for the treatment with magnetic field: WS: Work Station; T: Temperature probe. Inside the chamber from the left side: Control plate (CTR); Static(Bo) magnetic field plate (0 Hz); Very high frequency (VHF) magnetic field irradiation (100 MHz) and Ultra high frequency (UHF) magnetic field irradiation (900 MHz).

Figure 5 shows all the equipments necessary for the bioluminescence measurements: a photomultiplier (PMT) to record the light, an optical fiber to deliver the light to the monochromator, and an oscilloscope to measure the light signal intensity. The PMT was a 1P28 capable to record light of low intensity emitted by our samples. The gain factor of PMT was of 5×10^6. The sensibility range of PMT ranged from 185 to 700 nm.

The monochromator was a SP-308, 0.300 meter focal length. The plates containing the samples were exposed to the entrance of the optical fiber which leaded the emitted light to the monochromator entrance. The output of the monochromator was coupled to the PMT. The signal intensity of the PMT was interfaced to the oscilloscope and a PC capable to draw the spectra. The measurements were performed by an UV grating of 1200 g/mm and the working range of the apparatus was between 400 and 660 nm.

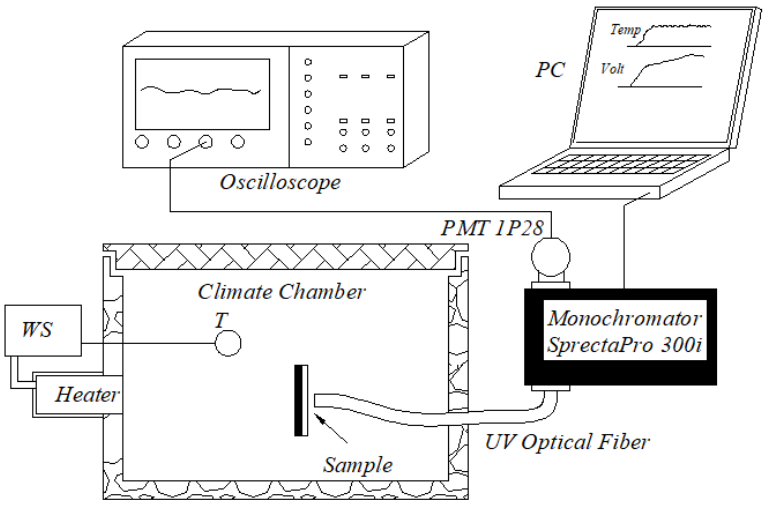

Figure 5: Climate chamber for spectrum emission measurements.

Almost all output signals were very low due to the low photon concentration emitted from the samples and the acceptance of the fiber. For this reason, it was necessary to use the detecting method termed single photon counting. To estimate the value of the intensity, we operated the overlapping of the output pulses up to 500 sampling obtaining a continuum value. Recording the signals on wavelength, we determined the spectra. Measurements were performed after $24\ h$, $48\ h$ and $72\ h$ of exposure.

III. RESULTS

III: 1 Effects of stationary phase on bioluminescence emission spectra (CTR)

In this study we used the luminescent bacterium *Vibrio jasicida* PS1 belonging to the Harvey clade, which was described previously [7], and also used in a previous study describing the effects of exposure to static magnetic field on quorum sensing circuit [8]. Bioluminescence intensity and emission spectra were analyzed at different times, during the logarithmic ($24\ h$), exponential ($48\ h$) or late exponential ($72\ h$) phase of growth.

Deconvolution analysis of spectral emission data with an incubation temperature of 30°C demonstrated the presence of two major emission peaks centered, respectively, at $457.38\ nm$ (blue line, peak #2) and $486.47\ nm$ (green line, peak #1) in the non-exposed control (CTR) bacteria during the logarithmic phase of growth ($1\ day, 24\ h$) (*Fig. 5* and *Fig. 6*). These two peaks were consistent with previous findings [2], and may be due to primary emission at 460 nm by bacterial luciferase, and a secondary emission at 490 nm by fluorescent pigment(s). Indeed, endogenous fluorescent proteins that shift the primary emission by bacterial luciferase have been described in luminescent *Vibrio* spp., including the yellow fluorescent protein (YFP) of *Vibrio fischeri* Y1 carrying the riboflavin 5'-phosphate (FMN), which is responsible for yellow shift, and is sensitive to the redox state [9]. Progression of exponentially growing bacteria towards the stationary phase was associated with slight changes in the bioluminescence pattern (*Fig. 6* and *Fig. 7*). In particular, the peak #1 shifted toward higher wavelengths, i.e., 490.34 nm at $48\ h$, and 492.89 nm at $72\ h$. Minor changes were observed for the peak #2 (459.6 nm at $48\ h$, and 457.32 nm at $72\ h$).

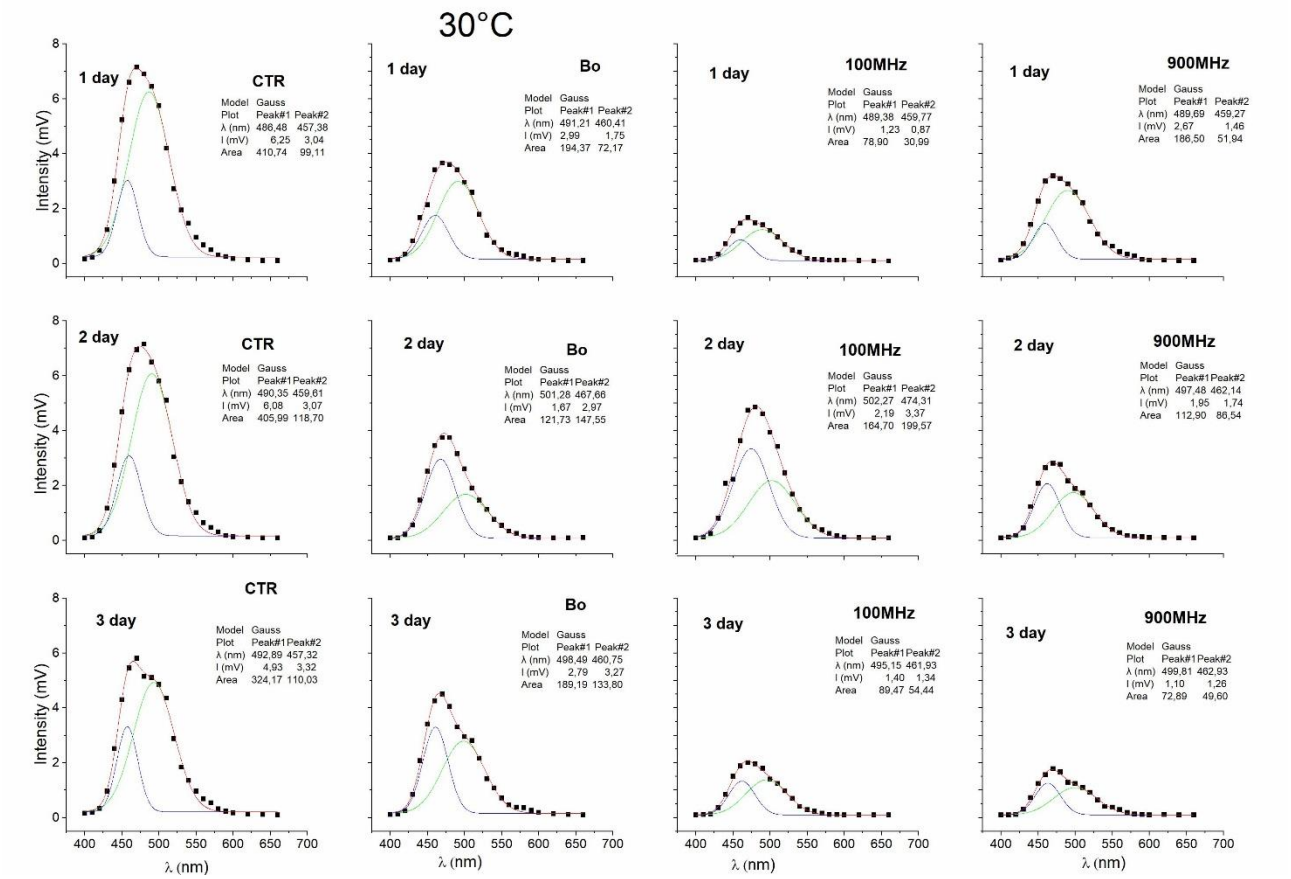

Figure 6: Effects of stationary phase and magnetic field exposure on *V. jasicida* bioluminescence pattern. *V. jasicida* PS1 was cultured on LA agar at 30 °C, either in the absence (CTR) or in the presence of static (Bo, $0\,Hz$), very high frequency (VHF, $100\,MHz$), or ultra high frequency (UHF, $900\,MHz$) magnetic field irradiation. At different time points (1 day-24 h: top panels; 2 day-48 h: middle panels, 3 day-72 h, bottom panels), light emission spectra were recorded and analyzed. Experimental results are represented by the squared points; the Gaussian curves (green lines, peak #1; blue lines, peak #2) are the deconvolution results while the red lines are the sum of the two curves.

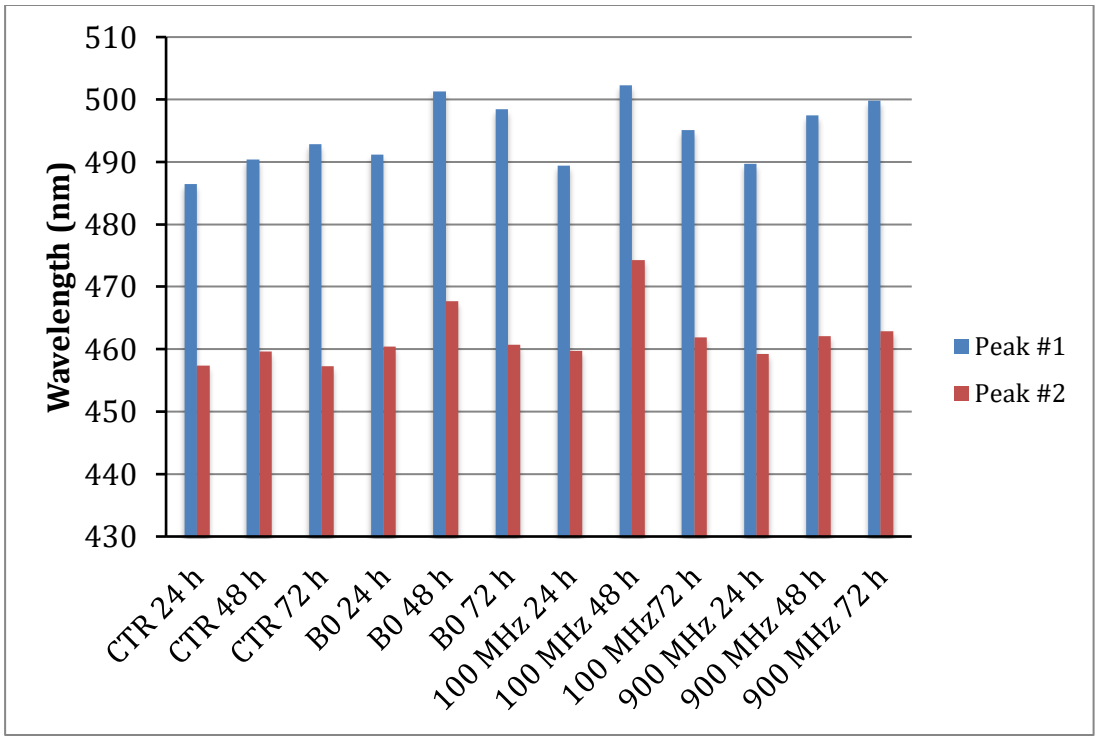

Figure 7: Effects of stationary phase and magnetic field exposure on *V. jasicida* bioluminescence emission peaks. *V. jasicida* PS1 was cultured on LA agar at 30 °C, either in the absence (CTR) or in the presence of static (Bo, 0 Hz), very high frequency (VHF, 100 MHz), or ultra high frequency (UHF, 900 MHz) magnetic field irradiation. At different time points (24 h, 48 h, 72 h), light emission spectra were recorded, and bioluminescence emission peaks, as deduced by deconvolution analysis, were plotted in this graph.

III: 2 Effects of magnetic field irradiation on bioluminescence emission spectra

Analysis of light emission spectra of bacteria exposed to Bo, VHF and UHF magnetic field irradiation demonstrated more pronounced changes in the bioluminescence pattern as compared those observed during the progression toward the stationary phase (*Fig. 6* and *Fig. 7*). Effects were specific to the type of magnetic field exposure.

In bacteria exposed to Bo (*Fig. 6* and *Fig. 7*), both peaks (peak #1 and peak #2) were shifted toward higher wavelengths after 48 h with respect to non-exposed control bacteria (CTR 24-72 h) or bacteria exposed to Bo after 24 h or 72 h (*Fig. 7*). In particular, the peak #1 shifted from 491.21 nm to 501.28 nm, and then decreased to 498.49 nm at 72 h. The peak #2 shifted from 460.41 nm to 467.65 nm, and then decreased to 460.75 nm at 72 h.

A similar effect was detected in bacteria exposed to VHF (*Fig. 7*). In particular, the peak #1 shifted from 489.37 nm to 502.26 nm, and then decreased to 495.15 nm at 72 h. The peak #2 shifted from 459.76 nm to 474,30 nm, and then decreased to 461.92 nm at 72 h.

A different behavior was observed in bacteria exposed to UHF. In this case, the shift was more progressive, with a slight increase toward higher wavelengths during the time course. In particular, the peak #1 shifted from 489.69 nm to 497.4 nm, and then increased further to 499.81 nm at 72 h. The peak #2 shifted from 459.27 nm to 462.14 nm., and then increased further to 462.93 nm at 72 h.

In addition to peak shifts, the exposure to either static Bo, or VHF or UHF magnetic

fields produced marked effects on the relative intensity of peak #1 and peak #2 as reported in Table 1.

Table 1: Ratio between the two emission peaks, as deduced by deconvolution analysis.

$I_{peak\#1}/I_{peak\#2}$				
	CTR	**Bo (0 Hz)**	**VHF (100 MHz)**	**UHF (900 MHz)**
24h	2.06	1.71	1.41	1.83
48h	1.98	0.56	0.65	1.12
72h	1.48	0.85	1.04	0.87

While the relative intensity of peak #1 was higher than that of peak #2 in non-exposed control bacteria at all time points (CTR 24-72 h), and in exposed Bo, or VHF or UHF magnetic field-exposed bacteria at 24 h, the relative intensity of peak #2 exceeded or it is comparable with that one of peak #1 for all the magnetic field-exposed bacteria at 48 h or 72 h (see Fig.5) with a ratio below or slight higher than.

In order to assess the actual impact of the treatments through electro-magnetic fields on the bacteria colonies, a statistical analysis, based on the Kolmogorov-Srmirnov test, has been carried out on their emission spectra. Such a test allows to determine if a set of data is likely to come from one continuos distribution or, as in our case, if two sets are likely to belong to the same distribution. The main result of this test is a *p-value*, ranging from 0 to 1, providing a quantitative estimation of the probability that the samples follow the same distribution (*i.e.* a small *p-value* states that it is less likely that the two sets of data come from the same parent distribution). Thus, under the null hypothesis that the treated colonies spectra and those of the control ones belong to the same distribution, with a significativity level set at 0.05, one finds significant changes if the *p-value* associated with one spectrum is lesser than or equal to 0.05. In this case, it is quantitatively shown that the treatment had a significant effect on the mechanism of bioluminescence of the bacteria.

As can be seen in Tables 2 and 3, the static magnetic field does not affect the bacteria luminescence in any case. The major effects can be seen only at 30°C with high frequency electromagnetic fields, starting from the first day of treatment. For these samples, whichever change in the behavior of the luminescence spectra is really likely to be amenable to their exposure to the chosen fields. We analyzed the data at 25 and 30 °C.

Table 2: Table listing the *p*-values coming from significativity Kolmogorov-Smirnov tests on the treated samples at 25°C for each day of treatment without interruption. Highlighted values mark the samples whose emission spectrum has to be considered significantly different from those of the

	Bo	100 MHz	900 MHz
1 day	0.889	0.443	0.250
2 day	0.825	0.571	0.571
3 day	0.895	0.258	0.444
4 day	0.848	**0.039**	**0.041**

At 25 °C we performed experiments up to 4 days and only two cases exhibited significativity.

Table 3: Table listing the *p*-values coming from significativity Kolmogorov-Smirnov tests on the treated samples at 30°C for each day of treatment without interruption. Highlighted values mark the samples whose emission spectrum has to be considered significantly different from those of the

	Bo	100 MHz	900 MHz
1 day	0.263	0.029*	0.064
2 day	0.259	0.027*	0.029*
3 day	0.253	0.010*	0.029*

At 30 °C we performed experiments up to 3 days and almost all the radiofrequency exhibited significativity.

IV. Conclusions

In conclusion, the data here presented demonstrate the suitability of the experimental apparatus and tester microorganism in demonstrating measurable effects of the magnetic field in a biological system. Evidence is provided that light emission by luminescent *V. jasicida* is subject to modulation both in peak intensity and wavelength, a phenomenon that is expected to occur also in other luminescent *Vibrio* spp. Modulation in light emission wavelength in response to stationary phase and environmental stresses including oxidative stress may have important biological significance. Indeed, *Vibrio* spp. bioluminescence, which is a coordinated population response that is subject to quorum sensing regulation, play many roles in marine environment, where these bacteria colonize a number of specific hosts establishing mutualistic symbioses [7]. In addition, it has been also proposed that bioluminescence provides several *Vibrio* spp. with an internal light ensuring effective DNA repair by DNA photoreactivation [2, 10]. As in biological systems magnetic field exposure induces oxidative stress leading to DNA damage [11], it is reasonable that the blue/green to blue/violet shift is implicated in the DNA photoreactivation. In fact, the photoreactivation process, which relies on photolyase enzyme, is mostly activated by blue/violet light, in accordance with the emission shifting observed in magnetic field-exposed bacteria.

The bioluminescence of the bacteria incubated with a temperature of 20°C and 25°C didn't show such evident modulation both in peak intensity and wavelength suggesting that the temperature associated with magnetic field exposure play together as environmental stress for the modification of bioluminescence of *V. jasicida*.

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