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Electromagnetic Signals Are Produced by Aqueous Nanostructures Derived from Bacterial DNA Sequences

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Abstract: A novel property of DNA is described: the capacity of some bacterial DNA sequences to induce electromagnetic waves at high aqueous dilutions. It appears to be a resonance phenomenon triggered by the ambient electromagnetic background of very low frequency waves. The genomic DNA of most pathogenic bacteria contains sequences which are able to generate such signals. This opens the way to the development of highly sensitive detection system for chronic bacterial infections in human and animal diseases.
Key words: DNA, electromagnetic signals, bacteria.

Pathogenic microorganisms in this day of age are not only submitted to high selective pressure by the immune defenses of their hosts but also have to survive under highly active antiviral or antibiotic treatments. Not surprisingly, they have evolved in finding many ways to escape these hostile conditions, such as mutations of resistance, hypervariability of surface antigens, protective biofilms, latency inside cells and tissues.

We initially observed (Montagnier and Lavallee, personal communication) that some filtration procedures aimed at sterilizing biological fluids can yield under some defined conditions the infectious microorganism which was present before the filtration step. Thus, filtration of a culture supernatant of human lymphocytes infected with Mycoplasma pirum, a microorganism of about 300 nM in size, through filters of 100 nM or 20 nM porosities, yielded apparently sterile fluid. The latter however was able to regenerate the original mycoplasma when incubated with a mycoplasma negative culture of human lymphocytes within 2 to 3 weeks.

Similarly, a 20 nM filtration did not retain a minor infective fraction of HIV, the causal agent of AIDS, whose viral particles have a diameter averaging 100-120 nM.

In the course of investigating the nature of such filtering infectious forms, we found another property of the filtrates, which may or may not be related to the former: their capacity to produce some electromagnetic waves of low frequency in a reproducible manner after appropriate dilutions in water. The emission of such waves is likely to represent a resonance phenomenon depending on excitation by the ambient electromagnetic noise. It is associated with the presence in the aqueous dilutions of polymeric nanostructures of defined size. The supernatant of uninfected eukaryotic cells used as controls did not exhibit this property.

In this paper we provide a first characterization of the electromagnetic signals (EMS) and of their underlying nanostructures produced by some purified bacteria.

In addition to *M. pirum*, a more classical bacterium, *E. Col*i, was utilized for the purpose of the analysis. The nanostructures produced by HIV will be the subject of another paper.

M. pirum is a peer-shaped small bacterial cell, ressembling M. pneumoniae, which can be grown in synthetic enriched medium (SP4) (Tully *et al.*, 1977) but also mutiplies at the surface of human T lymphocytes.

The strain (Ber) used in our experiments was isolated from a T lymphocyte culture derived from the blood of an apparently healthy subject (Grau *et al.*, 1993). The strong mycoplasma adherence to lymphocytes is mediated by a specific adhesin, whose gene had been previously cloned and sequenced by the authors (Tham *et al.*, 1994).

We used as primary source of the mycoplasma, supernatants of infected human T lymphocyte cultures or of cultures of the CEM tumor T cell line. All cell cultures were first tested for the lack of *M. pirum* contamination by polymerase chain reaction (PCR) and nested PCR, before starting the experiments. Titers of 10^6-10^7 infec-

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tious Units/ml of *M. pirum* were readily achieved after 5-6 days of incubation following deliberate infection of both types of cultures.

Filtration of the clarified supernatant was first performed on 0.45 μM (450 nM) Millipore filters to remove debris, and subsequently on 0.1 μM (100 nM) Millipore filters or on 0.02 μM (20 nM) Whatman filters, to remove mycoplasma cells. Indeed, the two 100 nM and 20 nM filtrates were confirmed sterile when aliquots were incubated for several weeks in SP4 medium. Repeated search for traces of mycoplasma DNA by PCR and nested PCR using specific primers for the adhesin gene or for the 16S ribosomal gene was consistently negative.

However when the filtrates were incubated for two weeks (100 nM filtrate) or three weeks (20 nM filtrate) with a culture of human activated T lymphocytes, the mycoplasma was recovered in the medium with all its original characteristics as previously observed.

The same filtrates were analyzed just after filtration for production of electromagnetic waves of low frequency. For this purpose we used a device previously designed by Benveniste and Coll (1996; 2003) for the detection of signals produced by isolated molecules endowed with biological activity. The principle of this technology is shown in Fig. 1.

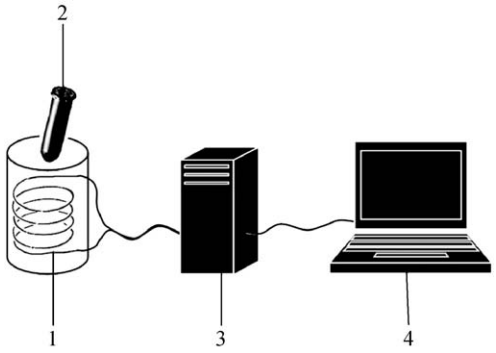

Fig. 1 Device for the capture and analysis of electromagnetic signals (EMS): (1) Coil: a bobbin of copper wire, impedance 300 Ohms; (2) Plastic stoppered tube containing 1 mL of the solution to be analyzed; (3) Amplifier; (4) Computer with softwares.

Briefly, the 100 nM or 20 nM filtrates are serially diluted 1 in 10 (0,1 +0,9 in sterile water (medical grade). The first 2 dilutions (1/10 and 1/100) are done in serum-free RPMI medium, in order to avoid eventual protein precipitation in deionized water.

Each dilution is done in 1.5 mL Eppendorf plastic tubes, which are then tightly stoppered and strongly agitated on a Vortex apparatus for 15 seconds . This step has been found critical for the generation of signals.

After all dilutions have been made (generally 15-20 decimal dilutions), the stoppered tubes are read one by one on an electromagnetic coil, connected to a Sound Blaster Card itself connected to a laptop computer, preferentially powered by its 12 volt battery. Each emission is recorded twice for 6 seconds, amplified 500 times and processed with different softwares for vizualization of the signals on the computer's screen (Fig. 1).

The main harmonics of the complex signals were analyzed by utilizing several softwares of Fourier transformation.

In each experiment, the internal noise generated by the different pieces of the reading system was first recorded (coil alone, coil with a tube filled with water). Fourier analysis shows (Fig. 2(c, d)) that the noise was predominantly composed of very low frequencies, probably generated at least in part by the 50/60 Hz ambient electric current. The use of the 12 V battery for the computer power supply did reduce, but not abolish this noise, which was found to be necessary for the induction of the resonance signals from the specific nanostructures.

When dilutions of the *M. pirum* filtrate were recorded for wave emission, the first obvious phenomenon observed was an increase of the overall amplitude of the signals at certain dilutions over the background noise (Fig. 2(a)) and also an increase in frequencies (Fig. 2(b)). This change was abolished if the tube to be analyzed was placed inside a box sheltered with sheets of copper and mumetal (David, 1998).

Fourier analysis of the *M. pirum* signals showed a shift towards higher frequencies close to 1000 Hz and multiples of it. Profiles were identical for all the dilutions showing an increase in amplitude (Fig. 2(c) and 2(d)).

The first low dilutions were usually negative, showing the background noise only. Positive signals were usually obtained at dilutions ranging from 10^{-5} to 10^{-8} or 10^{-12}. Higher dilutions were again negative (Fig. 3).

The positive dilutions varied according to the type of filtration, the 20 nM filtrate being generally positive at dilutions higher than those of the 100 nM filtrate.

The original unfiltered suspension was negative at all dilutions, a phenomenon observed for all the microorganisms studied.

Size and density of the structures producing the signals in the aqueous dilutions:

An aliquot of the 20 nM filtrate was layered on the top of a 5-20% (w/v) sucrose gradient in water and centrifuged for 2 hours at 35,000 rpm in a swinging bucket rotor. These conditions had previously been used to obtain the density equilibrium of the intact mycoplasma cells wich formed a sharp bound at 1,21 density. Fractions were collected from the bottom of the tubes, pooled 2 by 2 and assayed for signal emission.

Fig. 4 shows that the signal emitting structures were distributed in a large range of densities from 1.15 to 1.25 and also had a high sedimentation coefficient.