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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.

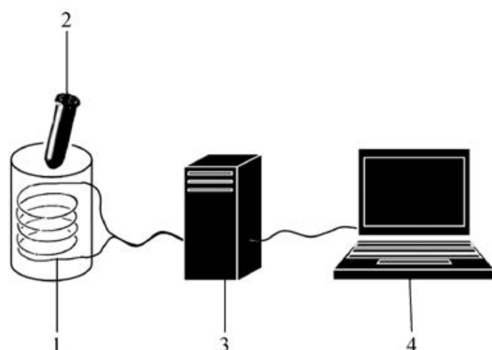


Fig. 1 Device for the capture and analysis of electromagnetic signals (EMS): (1) Coil: a bobbin of copper wire, impedance 300 Ohms; (2) Plastic stoppered tube containing 1 mL of the solution to be analyzed; (3) Amplifier; (4) Computer with softwares.

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.

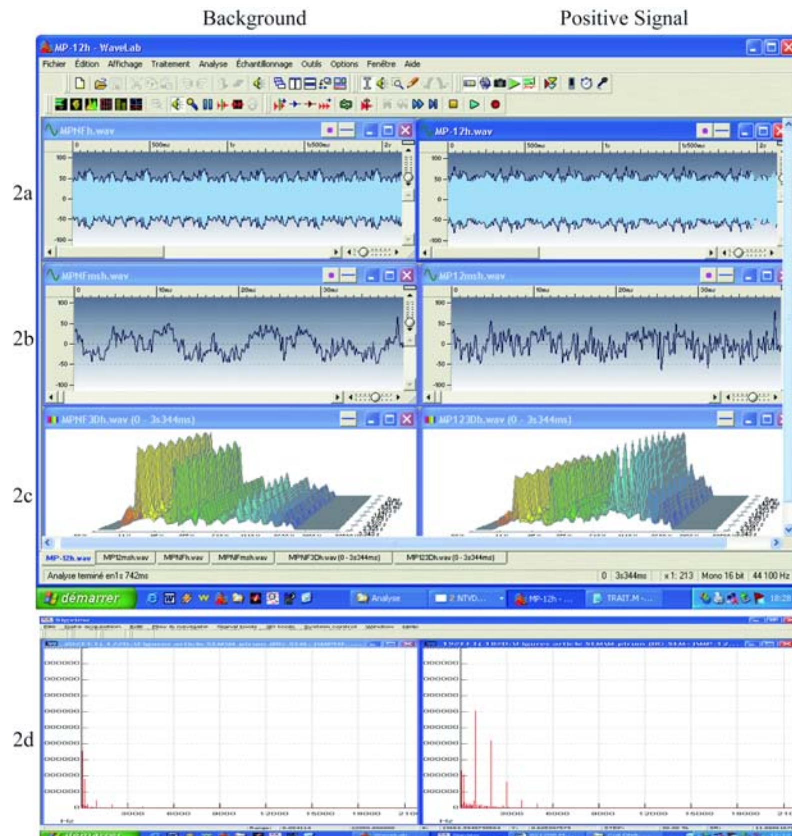


Fig. 2 Detection of EMS from a suspension of Mycoplasma pirum: Left: background noise (from an unfiltered suspension or a negative low dilution). Right: positive signal (from a high dilution D-7 (10-7)). (a) actual recording (2 seconds from a 6 second recording) after WaveLab (Steinberg) treatment; (b) detailed analysis of the signal (scale in millisecondes); (c) Matlab 3D Fourier transform analyzis (abcissa: 0-20 kHz, ordinate: relative intensity, 3D dimension: recording at different times); Frequencies are visualized in different colors; (d) Sigview Fourier transform: note the new harmonics in the range of 1 000-3 000 Hz.

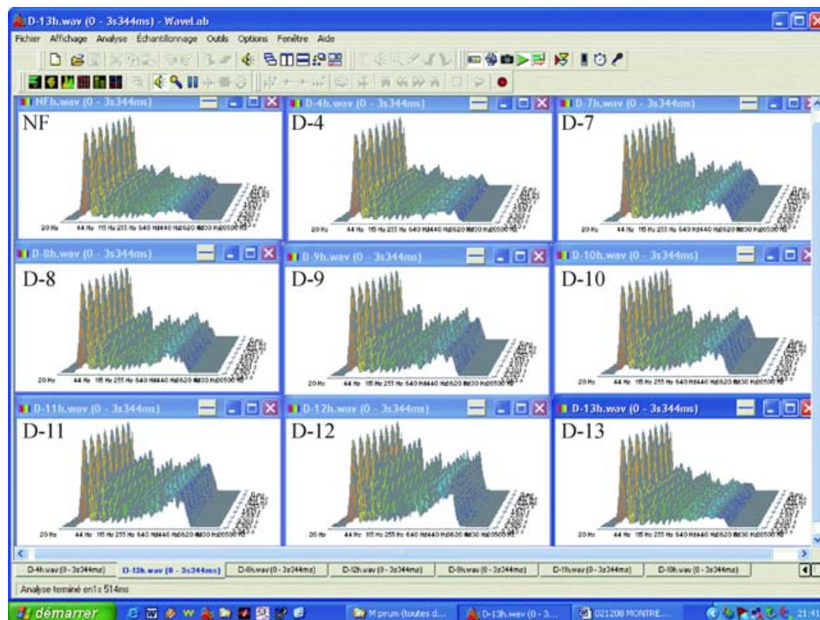


Fig. 3 A typical recording of signals from aqueous dilutions of *M. Pirum* (Matlab software): note the positive signals from D-7 to D-12 dilutions.

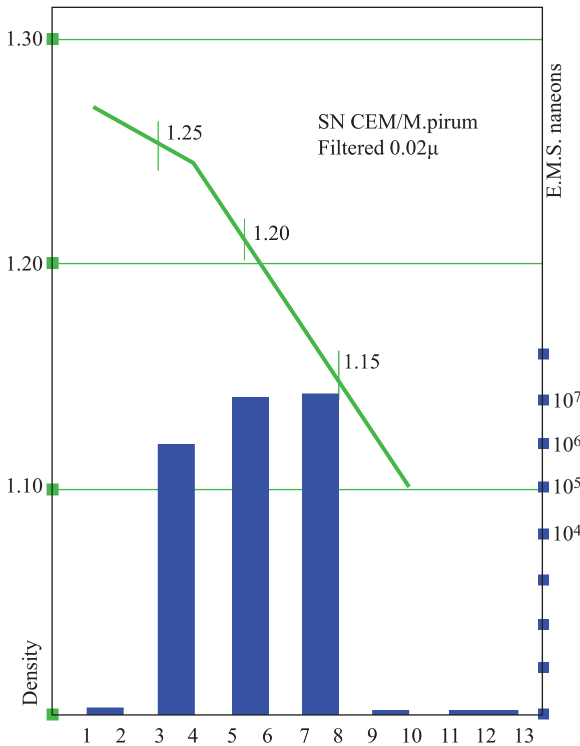


Fig. 4 Sucrose density centrifugation (35 000 rpm, 2 Hr) of a 0.02μ filtrate of Mycoplasma pirum suspension. The collected fractions were pooled 2 by 2 and diluted up to D-15 and tested for EMS. The bars indicate the fractions positive for EMS.

We then turned to a more classical bacterium, *E. Coli*, using the laboratory strain K1.

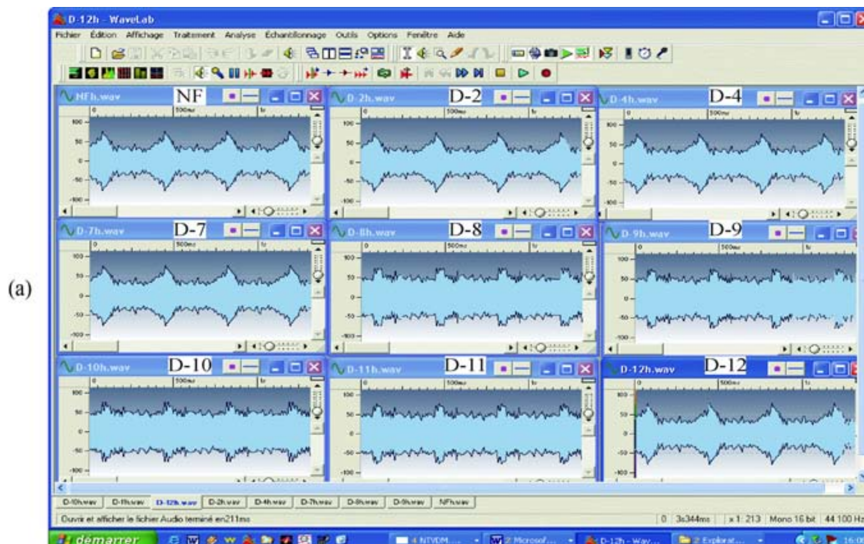
A culture of *E. Coli* in agitated (oxygenated) conditions, yielded 10^9 bacterial units/mL, measured by spectrometry. The suspension was then centrifuged at 10,000 rpm for 15 minutes, the supernatant was filtered on 450 nM filter and the resulting filtrate was filtered again on a 100 nM filter. The final filtrate was found sterile, when plated on nutrient agar medium and was analyzed for electromagnetic wave emission, as described above for *M. pirum*. Signal producing dilutions usually range from 10^{-8} to 10^{-12}, with profiles upon Fourier transformation, similar to those of *M. Pirum* (Fig. 5). In one experiment, some very high dilutions were found positive, ranging from 10^{-9} to 10^{-18}. An aliquot of the unfiltered supernatant did not show any signals above background up to the 10^{-38} dilution, indicating again the critical importance of the filtration step for the generation of specific signals.

The only difference with *M. pirum* was that no signal appeared after filtration on 20 nM filters, suggesting that the structures associated with the signals were retained by these filters and, therefore, had a size greater than 20 nM and lower than 100 nM.

We then asked why the lower dilutions, which logically should contain a larger number of signal-producing structures, were "silent". When we added 0.1 mL of a negative low dilution (*e.g.* 10^{-3}) to 0.4 mL or 0.9 mL of a positive dilution (10^{-8}), the latter became negative. This indicate that the "silent" low dilutions are self-inhibitory, probably by interference of the multiple sources emitting in the same wave length or slightly out of phase, like a radio jamming. Alternatively, the abundance of nanostructures can form a gel in water and therefore are prevented to vibrate.

-Evidence for homologous "cross talk" between dilutions

We then wonder whether or not it was possible to generate new signal-emitting structures from tube to tube by using wave transfer. The following experiment



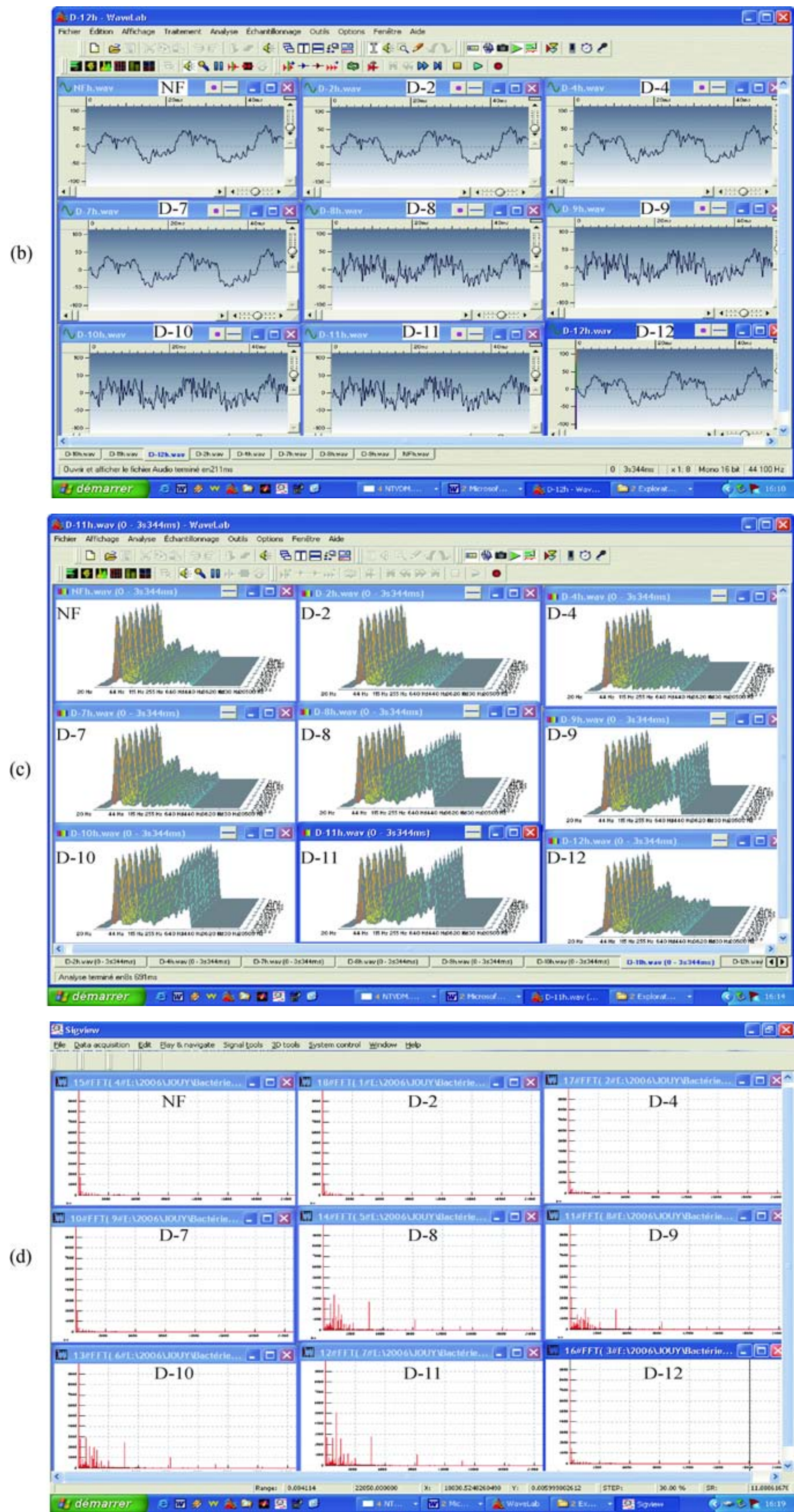


Fig. 5 EMS from *E. Coli* 0.1 μ filtrate. EMS positive from dilution D-8 to D-11: (a) Actual recording; (b) millisecond analysis; (c) Fourier transform analysis Matlab; (d) Fourier transform analysis SigView. NF: not filtered.

which was repeated several times showed that indeed this was the case.

A donor tube of a low "silent" dilution of E. Coli (10^{-3}) was placed side by side close to a receiver tube of the positive "loud" highest dilution of the same preparation (10^{-9}). Both tubes were placed in a mumetal box for 24 hours at room temperature, so that the tubes were not exposed to external electromagnetic noise, and only exposed to the signals generated by the structures present in the tubes themselves.

The tubes were then read again by the signal detecting device: the donor tube was still silent, however the receiver tube became also silent.

Moreover, when further dilutions were made from the receiver tube (10^{-10}, 10^{-11}, 10^{-12}), these dilutions had became positive (Fig. 6). These results suggest that the receiver tube was made silent by formation of an excess of new nanostructures, which could emit signals upon further dilution.

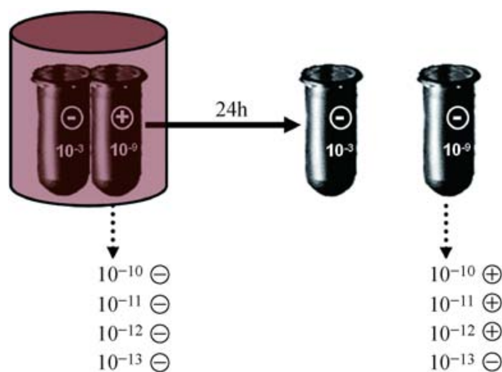


Fig. 6 Cross-talk between dilutions (from an *E. Coli* 0.1 µ filtrate), see explanation in the text.

This effect was suppressed by interposing a sheat of mumetal between the two tubes during the 24 hours contact period, pointing to a role of low frequency waves in the phenomenon.

Emission of similar electromagnetic signals was also observed with some other bacterial species such as: Streptococcus B, Staphylococcus aureus, Pseudomonas aeroginosa, Proteus mirabilis, Bacillus subtilis, Salmonella, Clostridium perfringens, all in the same range of dilutions observed for *E. Coli*, and only after filtration at 100 nM (and not at 20 nM).

Importantly, the transfer effect between two tubes, one silent, one loud, was only observed if both contained dilutions of the same bacterial species. In other words, a Staphylococcus donor tube could only "talk" with a receiver tube containing a Staphylococcus dilution, and not with a tube of Streptococcus or *E. Coli*, and reciprocally.

These results indicate that the transfer effect is mediated by species-specific signals, the frequencies of which remain to be analyzed.

Finally, two others problems were investigated in the *E. Coli* system: the first was the role of the initial number of bacterial cells in the induction of the filterable signal-producing structures. For this a stationary culture of *E. Coli* was counted and adjusted to 10^9 cells/mL and serial dilutions from 100 to 100 were done down to 1 cell/mL. Each dilution was filtered at 100 nM and then analyzed for signal emission. Surprisingly, the range of positive dilutions were not strictly dependent on the initial concentration of *E. Coli* cells, being roughly the same from 10^9 cells down to 10 cells, suggesting that the same final number of nanostructures was reached at all concentrations. Thus, paradoxically, 10 cells are giving the same signals than 10^9 cells.

We were also concerned by the possible personal influence of the operator in the reading.

To address this point, two healthy operators were asked to measure independantly the same dilutions of *E. Coli*, each one unknowing the results of the other. The results of their readings were identical.

In addition, the results were independant of the order in which the samples were read, whether in descending dilutions from to the lowest to the highest or in ascending the dilutions from the highest to the lowest.

Finally an other laboratory worker placed the diluted samples in a random order, the labels being unknown from the person reading the samples. The same range of positive dilutions was detected again provided each tube was well separated from the other, to avoid their "cross talk".

We also found that the results were also independant of the location of the reading site: starting from the same unfiltered preparation of *E. Coli*, positive dilutions of the filtrates were found to be the same in two different locations in France (Paris center and suburb), one in Canada (Montreal), and one in Cameroun (Yaoundé).

As shown in the figures, the background noise was variable, according to the location and time of recording. It was generally higher in large cities than in isolated aeras. However, positive signals always clearly differenciate over the background by higher frequency peaks.

Nature of the aqueous nanostructures:

Treatments by RNAseA (Promega, 1 µg/ml, 37℃ 1 h), Dnase I (Invitrogen, 10 U/µg DNA, 37℃, 18 h), Lysozyme (Fisher, 1 mg/mL, 37℃ 10 min), Proteinase K (Promega, 0.12 mg/mL, in 1% sodium dodecyl sulphate, 56℃ 1 h) did not suppress the EMS producing activity of the "loud" dilutions nor did activate the "silent" dilutions.

However, heating at 70℃ for 30 min suppressed irreversiblly the activity, as well as did freezing for 1 hour at −20℃ or −60℃. DMSO (10%), and formamide (10%) had no effect.

Treatment with lithium cations, known to affect the

hydrogen bonding of water molecules, was able to reduce the intensity of the signals, while the range of the positive dilutions remained unchanged.

Nature of the bacterial molecules at the origin of the nanostructures:

In preliminary experiments, we had observed that a pretreatment of a suspension of *E. Coli* by 1% formaldehyde did not alter its capacity to induce the electromagnetic signals, while killing the bacteria. This treatment alters the surface proteins of the bacterial cells without attacking their genetic material, *i.e.* double- helical DNA. This suggested that the source of the signals may be the DNA itself.

Indeed, DNA extracted from the bacterial suspension by the classical phenol: chloroform technique was able upon filtration and appropriate dilutions in water to emit EMS similar to those produced by intact bacteria under the same conditions. DNAse treatment of the extracted DNA solution abolishes its capacity to emit signals, at the condition that the nanostructures previously induced by the DNA are destroyed. A typical experiment is described as follows:

E. Coli DNA was treated by Proteinase K in the presence of SDS (sodium dodecyl sulfate) and further deproteinized by phenol-chloroform mixture. The pellet obtained by ethanol precipitation was resuspended in Tris 10-2 M, pH 7,6 and an aliquot was diluted 1/100 in water. The dilution (10^{-2}) was filtered first through a 450 nM filter and the resulting filtrate was then filtered again on a 100 nM filter. The filtrate was further diluted in serial decimal dilutions in water as previously described.

As for the intact microorganisms, the filtration step was found to be essential for detection of the EMS in the DNA dilutions. In its absence, no signals could be detected at any dilutions.

In contrast to the microorganism suspension, where the filtration was supposed to retain the intact cells, the filtration at 100 nM did not retain the DNA, which was still present in the filtrate, as measured by optical density. However, filtration with a 20 nM Whatman filter retained the nanostructures emitting the EMS, suggesting that they have the same range of sizes than those originating from intact bacteria.

In the case of DNA, the role of the 100 nM filtration is probably to dissociate the network of nanostructures organized in a gel-like liquid crystal at high concentrations in water, allowing their dispersion in further dilutions. As shown in Fig. 7, the dilutions positive for EMS were in the same range that those observed for the intact bacteria, generally between 10^{-7} to 10^{-13}.

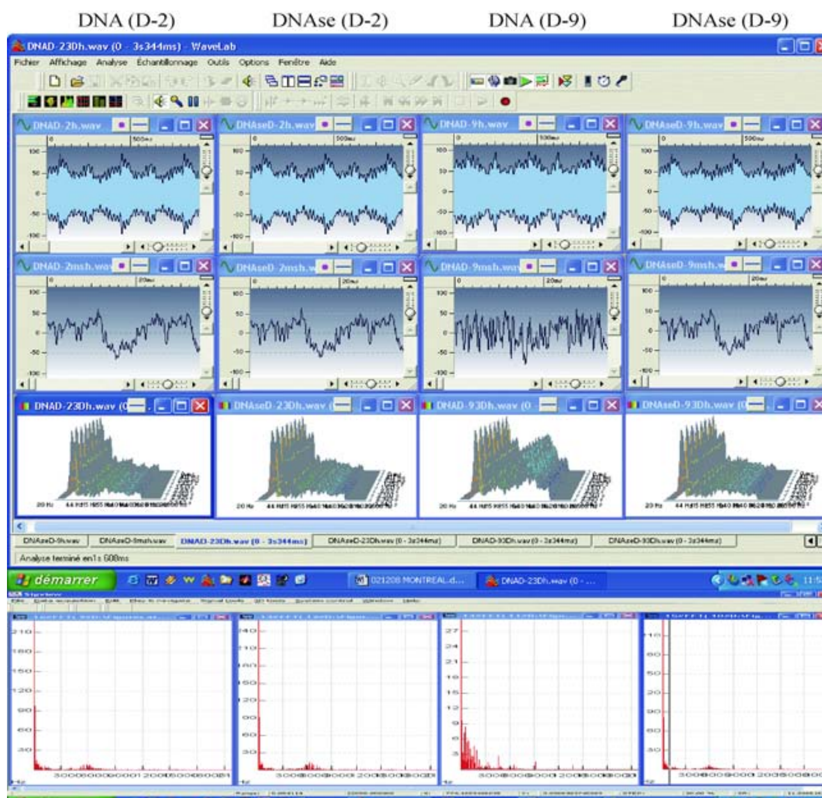


Fig. 7 DNAse effect on EMS production. The DNAse treated *E. Coli* DNA solution and the untreated DNA are diluted from D-2 to D-15. Analyzis of the EMS as described in Fig. 5. D-2 dilution (negative for EMS) is shown as control. D-9 is positive for EMS (from a range of positive dilutions D-8 to D-11). Note the signal disappearance in the DNAse treated DNA.

At the high dilution of 10^{-13}, calculations indicate that there is no DNA molecule of MW larger than 10^5 in the solution, making it unlikely that the EMS are produced directely by the DNA itself, but rather by the self-sustained nanostructures induced by the DNA.

Generally, all the bacterial species shown to be positive for EMS yielded also DNA preparations positive for EMS. Further demonstration that the EMS produced by bacteria come from their DNA was shown by their disappearance after DNAse treatement.

This inactivation was however only complete when the nanostructures induced in the DNA solution which are themselves resistant to DNAse were previously fully destroyed.

This destruction was obtained either by freezing the DNA solution at −20 °C for 1 hour or heating it at 90 °C for 30 minutes.

After slow cooling to allow the heated DNA to re-anneal, DNAse 1 at a final concentration of 10 U/μg of DNA was added and the mixture was incubated at 37 °C for 18 hours in the presence of 5 mM of $MgCl_2$. An aliquot of the untreated DNA solution was kept as a positive control.

The DNAse-treated preparation was found completely devoid of EMS emission at any dilution (Fig. 7).

Treatment of the DNA solution by a restriction enzyme acting at many sites of *E. Coli* DNA (EcoRV) did not suppress the production of EMS, suggesting that this emission is linked to rather short sequences or is associated with rare sequences.

Nature of the DNA sequences at the origin of the EMS:

A non exhaustive survey of the bacterial species and of their DNA able to display EMS suggests that most of bacteria pathogenic for humans are in this category.

By contrast, probiotic "good" bacteria as Lactobacillus and their DNA are negative for EMS emission.

In the case of *E. Coli*, we found that some strains used to carry plasmids for gene cloning were also negative (Fig. 8).

This suggested that only some sequences of DNA are at the origin of the EMS.

As pathogenicity is often associated with the capacity of the microorganism to bind eukaryotic cells, particulary mucosal cells, we focussed our analysis again to *M. pirum* DNA, where a single gene (adhesin: 126-kDa protein) is responsible for the adhesion of the mycoplasma to human cells.

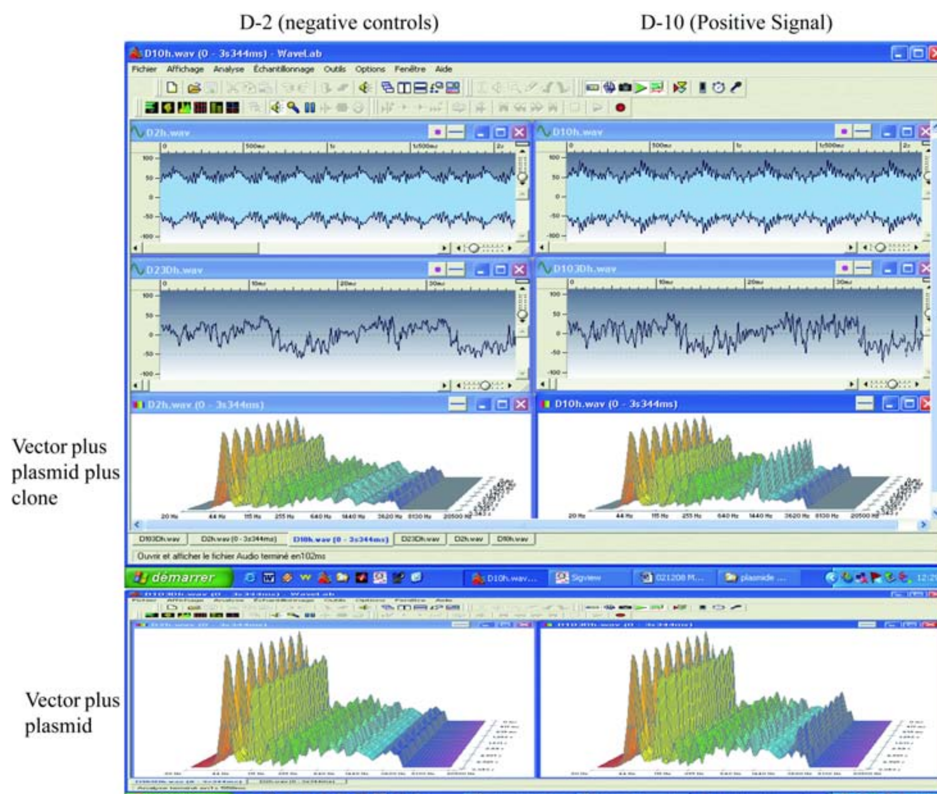


Fig. 8 EMS produced by the 1.5 kb fragment of the adhesin DNA of *M. Pirum.* The plasmid DNA containing the 1.5 Kb fragment was used to transform an *E. Coli* vector, XL1blue. The whole DNA was extracted and diluted for EMS analysis. Left: control background noise of a negative dilution (D-2). Right: positive signal at D-10 (range from D-9 to D-12). Bottom: Note the lack of EMS produced by the DNA extracted from the strain transformed by the plasmid alone.

This gene had previously been cloned and sequenced in our laboratory (Tham *et al.*, 1994). The cloned DNA existed as two fragments in two plasmids, corresponding respectively to the N terminal (1.5 Kbp) and the C terminal (5 Kbp) of the protein.

The two plasmids (pBluescript SK, Stratagene) containing the DNA fragments were amplified in a *E. Coli* strain, XL1blue.

The DNA of the *E. Coli* strain (with or without the plasmid) alone did not yield EMS at any dilutions.

By contrast when the strain was transformed with either plasmids carrying an adhesin gene fragment, EMS were produced (Fig. 8).

The two adhesin DNA fragments were then cut by specific restriction enzymes (N Terminal: 1.5 kbp/SpeI-EcoRI) (C Terminal: 5 kbp/HindIII-XbaI) and isolated by electrophoresis in 0.8% agarose gel. Each DNA fragment was able to induce EMS (not shown).

We also purified a large fraction of the adhesin DNA from the whole mycoplasma genomic DNA using specific primers and amplification by PCR.

Again this fragment induced EMS, thus indicating that no contaminant DNA coming from the plasmid carried by *E. Coli* was involved (not shown).

Discussion

We have discovered a novel property of DNA, that is the capacity of some sequences to emit electromagnetic waves in resonance after excitation by the ambient electromagnetic background.

Owing to the low sensitivity and specificity of our signal capture and analysis, the frequencies emitted are all alike, regardless of the bacterial species involved.

However, the experiments of transfer of information through plastic tubes suggest that, by refining the analysis and eliminating the variability of the exciting signals, we might detect specific differences between species, and even between sequences. Indeed, this property may be a general one shared by all double-helical DNAs, including human DNA.

But in our conditions of detection, it seems to be associated with only certain bacterial sequences.

It remains to be seen whether they are restricted to some genes involved in diseases.

Experiments to be reported elsewhere indeed indicate that this detection applies also at the scale of the human body: we have detected the same EMS in the plasma and in the DNA extracted from the plasma of patients suffering of Alzheimer, Parkinson disease, multiple Sclerosis and Rheumatoid Arthritis. This would suggest that bacterial infections are present in these diseases.

Morever, EMS can be detected also from RNA viruses, such as HIV, influenza virus A, Hepatitis C Virus. In these cases, optimal filtration for detection of EMS requires prior 20 nM filtration suggesting that the nanostructures produced are smaller that those produced by bacterial DNA.

In patients infected with HIV, EMS can be detected mostly in patients treated by antiretroviral therapy and having a very low viral load in their plasma. Such nanostructures persisting in the plasma may contribute to the viral reservoir which escapes the antiviral treatment, assuming that they carry genetic information of the virus.

The physical nature of the nanostructures which support the EMS resonance remains to be determined.

It is known from the very early X-ray diffraction studies of DNA, that water molecules are tightly associated with the double helix, and any beginner in molecular biology knows that DNA in water solution forms gels associating a larger number of water molecules.

Moreover, a number of physical studies have reported that water molecules can form long polymers of dipoles associated by hydrogen bonds (Ruan *et al.*, 2004; Wernet *et al.*, 2004).

However these associations appear to be very short-lived (Cowan *et al.*, 2005). Could they live longer, being self-maintained by the electromagnetic radiations they are emitting as previously postulated by Del Guidice, Preparata and Vitielo (1988)?

We have studied the decay with time of the capacity of dilutions for emitting EMS, after they have been removed (in mumetal boxes) from exposure to the excitation by the background. This capacity lasts at least several hours, some time up to 48 hours, indicating the relative stability of the nanostructures.

Are the latter sufficiently specific of DNA sequences to be able to carry some genetic information?

If so, what could be their role in pathogenicity, particularly in the genesis of chronic diseases?

Further studies involving close collaboration between physicists and biologists are obviously needed to resolve these problems.

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