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**Bactericidal effects of low-intensity
extremely high frequency electromagnetic
field: overview with phenomenon, cellular
mechanisms, targets and applications**



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Bactericidal effects of low-intensity extremely high frequency electromagnetic field

The bacterial effects of the extremely high frequencies electromagnetic field (EMF, ν of 30 to 300 GHz), with low (low-energetic) intensity and with non-thermal action, are of interest because of two reasons at least:

- bacteria and other cells can interact with each other through EMF (for reviews, see: Kaprelyants A., Kell D.B *Trends Microbiol.* 1996. 4:237-242; Trushin M.V *J. Microbiol. Immunol. Infect.* 2003. 36:153-160; Belyaev I, *Microwave Rev.* 2005. 11:13-29; Reguera G. *Trends Microbiol.* 2011. 19:105-111).

It has been shown, for instance, that bacteria possess the ultrasonic radiation or reemission of secondary photons in **sub-millimeter** frequency range. However extremely high frequency radiation is not clearly registered in spite of the fact that the fine methods have been developed and enough sensitive instruments have been already constructed for last years.



Bactericidal effects of low-intensity extremely high frequency electromagnetic field



Extremely high frequency EMF is widely used in telecommunication technology, therapeutic practice, soil and agricultural wastewater disinfection, and in food (fresh products, rice, raw meat and juices) protection technologies although low-orbital systems of cosmic communication and different elements of mobile one, and in addition, low-energetic devices used in therapeutic practice radiate EMI of this frequency, small and very small doses of which (low flux capacity of 0.005 mW/cm^2) might affect cells.

Bactericidal effects of low-intensity extremely high frequency electromagnetic field

EMF with low intensity have been shown to cause different, including bactericidal effects on *Escherichia coli* and the other bacteria (Lukashevsky K.V., Belyaev I.Ya. *Med. Sci. Res.* 1990. 18:955-957; Belyaev I.Ya. et al. *Bioelectromagnetics*. 1996. 17:312-321; Gub N.M. et al., *Millimeter waves in medicine and biology*. 10th Russian Symp. with int. part. M., 1995. P. 96; Bulgakova V.G. et al. *Biophysics*. 1996. 41:1289-1293; Trchounian A. et al. *Biophysics*. 2001. 46:69-76; Isakhanyan V., Trchounian A. *Biophysics*. 2005. 50:604-606; Tadevosyan A. et al., *Biophysics*, 2007. 52:893-898; *Cell Biochem. Biophys.* 2008, 51:97-103; Ohanyan V. et al., *Biophysics*, 2008, 53:406-408; Zand N. et al. *Afr. J. Microbiol. Res.* 2010. 4:2011-2012; Torgomyan H. et al., *Cell Biochem. Biophys.* 2011. 60:275-281; *FEMS Microbiol. Lett.* 2012. 329:131-137; Torgomyan H., Trchounian A. *Biochem. Biophys. Res. Commun.* 2011. 414:265-269)

Such effects depend on

- phase of the bacterial growth,
- anaerobic or aerobic conditions of the bacterial growth,
- composition of growth media,
- genetic features and peculiarities of the metabolism in bacterial strains.

Together with these, coherent and noise MMW can render the different bacterial effects depending on

- wavelength and intensity of MMW,
- duration of the irradiation and the other parameters.

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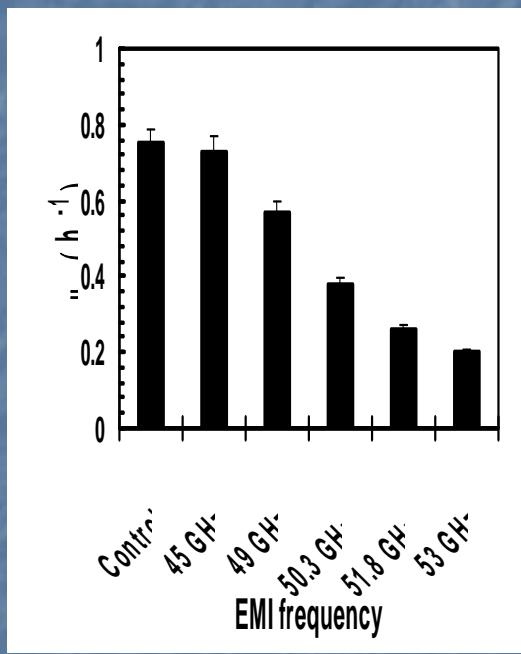
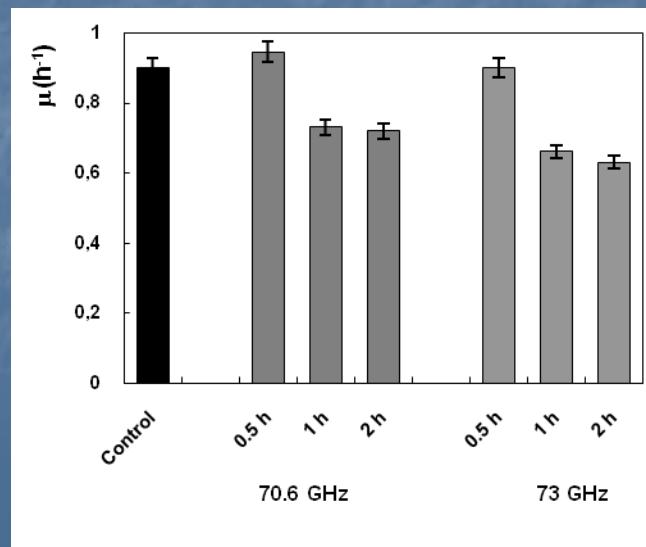
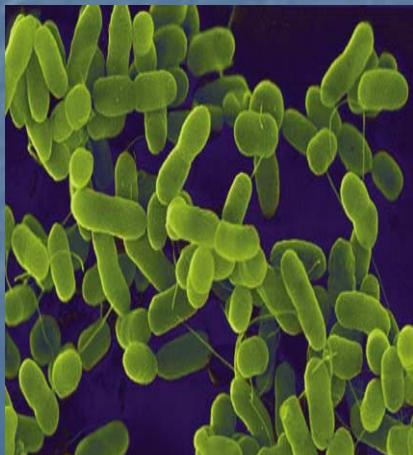


Inhibitory effect of coherent EMF on bacterial growth

The changes in the *E coli*K12 growth rate after direct irradiation with coherent extremely high frequency EMI of 45 to 53 GHz.

The specific growth rate (μ) was defined as $0,693/\text{time}$ of the reduplication to absorbance (OD) in bacterial suspension (when logarithm of OD linearly increased at time); irradiation time was 1 h; in checking control, bacteria without irradiation.

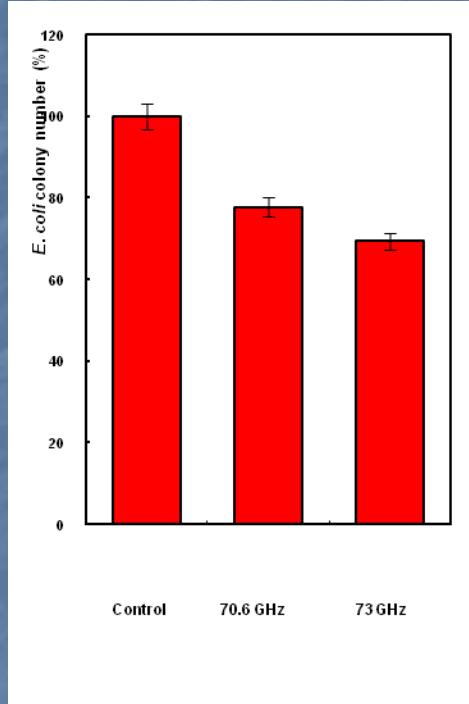
(Tadevosyan et al., *Cell Biochem. Biophys.* 51 (2008) 97-103)



The changes in the *E coli*K12 growth rate after direct irradiation with coherent extremely high frequency EMI at 70.6 and 73 GHz depending on radiation duration.

(Torgomyan et al., *Cell Biochem. Biophys.* 60 (2011) 275-281)

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The changes in *E. coli* colony-forming units number of spread on solid growth medium irradiated with the frequencies of 70.6 and 73 GHz.

In control, bacteria without irradiation (100 %).

Inhibitory effect of coherent EMF on bacterial growth

(Torgomyan, Trchounian, *Biochem. Biophys. Res. Commun.* 414 (2011) 265-269; Torgomyan et al., *Cell Biochem. Biophys.* 60 (2011) 275-281)

The results with direct irradiation of bacteria on solid growth medium with the frequencies of 70.6 and 73 GHz show the decreases of the settled colonies numbers compared with non irradiated control:

with 70.6 GHz that was ~22.2 % and with 73 GHz ~30.6 %.

Interestingly, the EMF influenced not only on decreases of the colonies numbers, but also on their dimension.

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Inhibitory effect of coherent EMF on bacterial growth at different pH

The specific growth rate of *E. coli* K12 irradiated by EMI of extremely high frequency at different pH.

pH*	Growth rate (h^{-1})	Control	51.8 GHz	53.0 GHz
6.0	0.86 ± 0.05		0.63 ± 0.03 ($p < 0.05$)**	0.60 ± 0.03 ($p < 0.05$)
7.5	0.82 ± 0.03		0.52 ± 0.02 ($p < 0.01$)	0.34 ± 0.02 ($p < 0.002$)
8.0	0.77 ± 0.03		0.70 ± 0.05 ($p < 0.05$)	0.50 ± 0.02 ($p < 0.05$)

* irradiation time was 1 h, for the other conditions, see the legends to Fig.

** p is calculated for the difference between the values in checking control and obtained ones.

Since the specific growth rate practically does not depend on medium pH in noted range, the change in this rate after irradiation can be indicative of some process as in cells themselves, so and in water medium with determined reaction (pH), in which bacteria grow.

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Similar inhibitory effects by coherent EMF at 49 to 53 GHz have been observed with *Enterococcus hirae*:

the *E. hirae* ATCC9390 specific growth rate (pH 8.0) was decreased maximally ~1.4-fold by irradiation with EMI at 53 GHz:

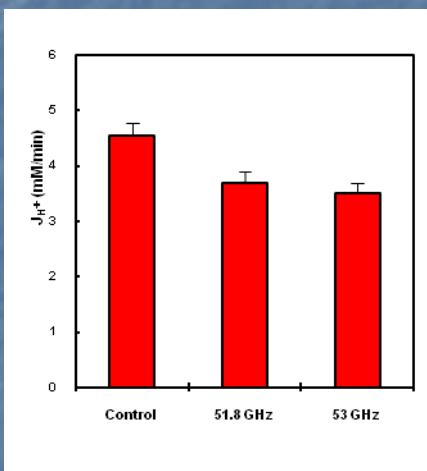
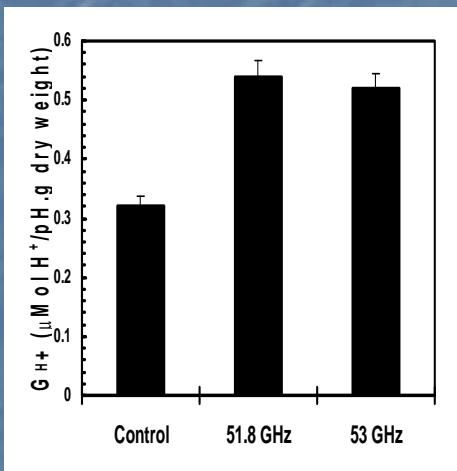
- this effect was increased with irradiation time of 0.5 h to 2 h
- the effect did not depend on medium pH (pH 6.0 and 8.0)

(Ohanyan V. et al., *Biophysics*, 2008, 53:822-825;
Torgomyan H. et al., *FEMS Microbiol. Lett.* 2012, 329:131-137)



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On mechanisms of EMF bacterial effects

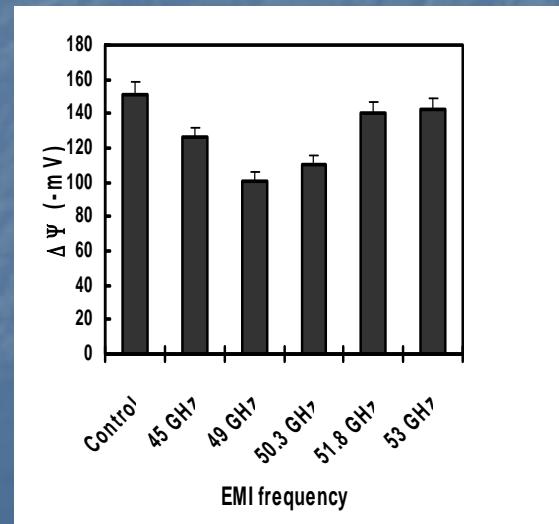


Changes in membrane proton conductance ($G_{m\text{H}^+}$, A) and energy-dependent proton fluxes (J_{H^+} , B) of *E coli* K12 after direct irradiation with coherent EMF.

The irradiation time was 1 h, the frequency was shown. $G_{m\text{H}^+}$ was determined by an acid-pulse technique used. Energy-dependent ion fluxes were determined by using selective (pH) electrode upon glucose (22 mM) adding; the values represented are for bacterial count of 10^{12} cells.

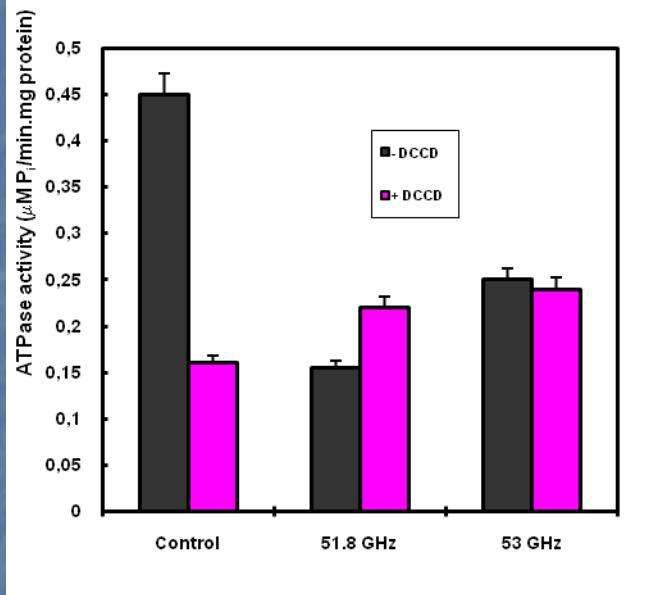
E. coli K12 membrane potential ($\Delta\Psi$) values after irradiation with coherent EMF.

The irradiation time was 1 h. $\Delta\Psi$ was calculated from distribution of tetraphenyl-phosphonium cation (initial external concentration of 1 mM) between the cytoplasm and the external medium determined with appropriate selective electrode.



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On mechanisms of EMF bacterial effects

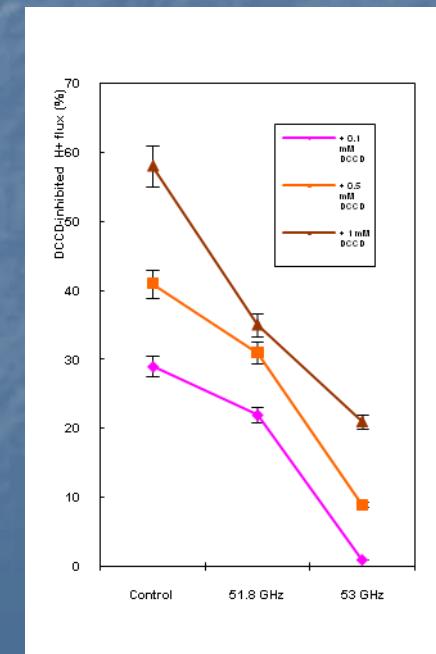
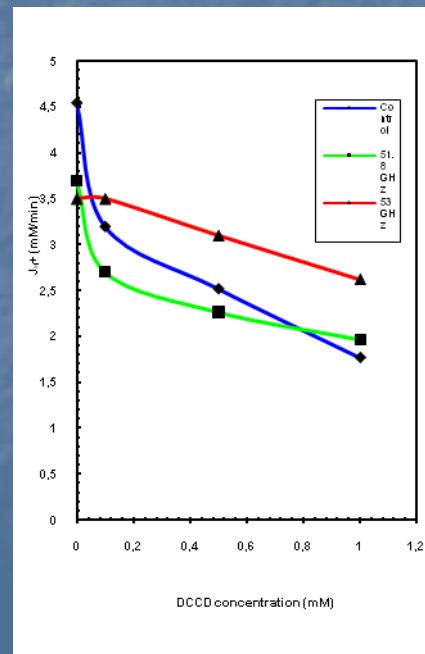


The ATPase activity of membrane vesicles isolated from *E. coli* K12 after irradiation with coherent EMF.

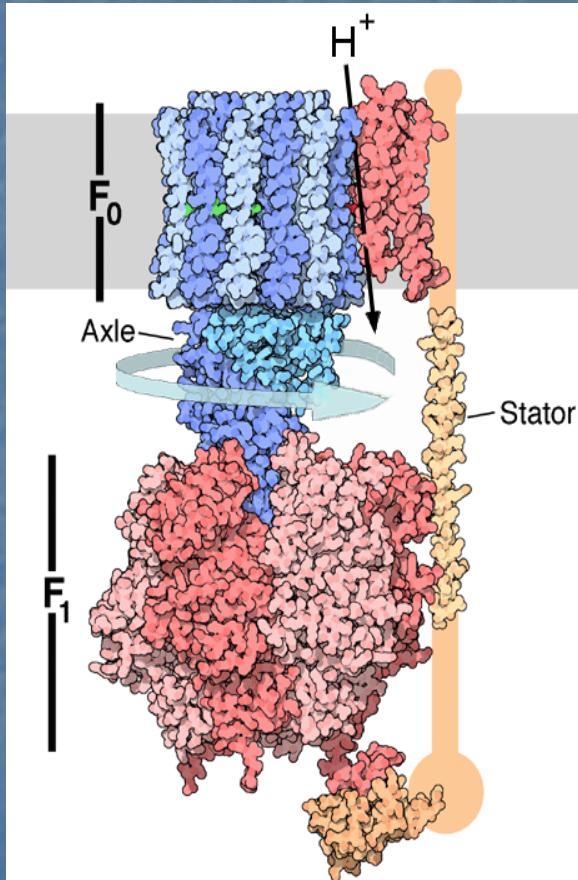
The irradiation time was 1 h. ATPase activity was calculated by colorimetric determination of liberation of inorganic phosphate (Pi) per time and protein upon ATP (3 mM) adding. DCCD was added in final concentration of 0.05 mM in parallel assays.

The changes in inhibitory effect of DCCD on JH+ from *E. coli* K12 after direct irradiation with coherent MMW.

The irradiation time was 1 h.



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Proposal:

The H^+ -translocating F_0F_1 -ATPase, the main membrane-associated enzyme of bioenergetic relevance, could be a main target for EMF

(Torgomyan H., Trchounian A., *Cell Biochem. Biophys.* 2011, 62:451-461; *Crit. Rev. Microbiol.* 2012, Epub Jun 6)

Bactericidal effects of low-intensity extremely high frequency electromagnetic field

Mediated action of EMF on bacteria

Mediated action of EMF on the following growth rate of *E. coli* K12.

Conditions*	Growth rate (h ⁻¹)	Control	51.8 GHz	53.0 GHz
Doubly distilled water	0.82±0.03 (100 %)	0.52±0.02 (63.4 %) (p<0.01)	0.34±0.02 (41.5 %) (p<0.002)	
Assay medium	0.78±0.03 (100 %)	1.22±0.05 (156.4 %) (p<0.01)	1.41±0.08 (180.8 %) (p<0.01)	

* irradiation time was 1 h .

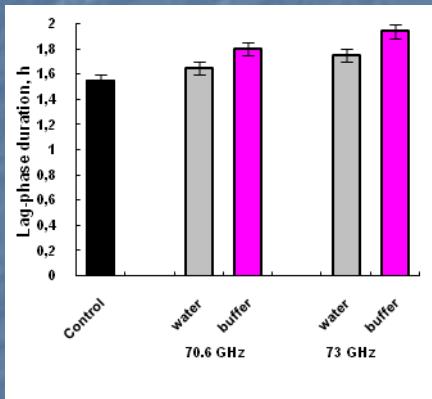
** p is calculated for the difference between the values in checking control and obtained ones.

The results point out a role of water and medium composition in effects of EMF on bacteria.

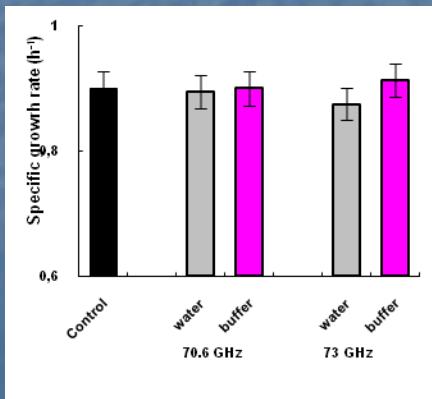
Note that water has acidic reaction and transferring cells from slightly acidic medium or slightly alkaline medium could affect growing bacteria. In addition, osmotic shock should be also taken into consideration, since cells are transferring into the medium with greater osmolarity. However, such procedures were shown do not affect growing *E. coli*.

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Mediated action of EMF on bacteria

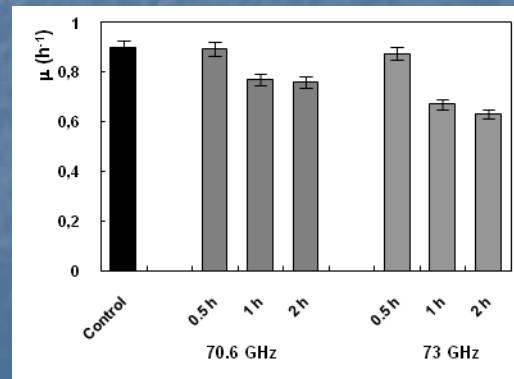
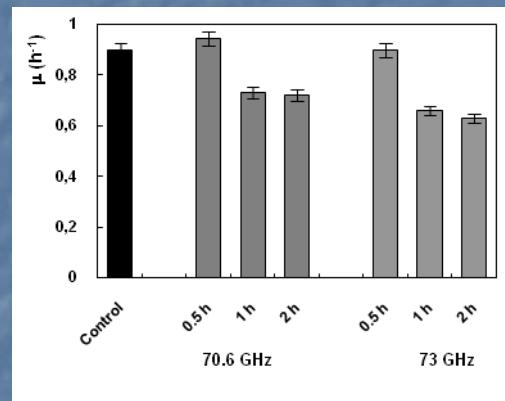


The changes of *E. coli* K12 growth lag-phase duration and specific growth rate after transfer into irradiated with the frequencies of 70.6 and 73 GHz (during 1 h) water and the assay buffer solution.



Compare with:

The changes of *E. coli* K12 growth lag-phase duration and specific growth rate after irradiation with the frequencies of 70.6 and 73 GHz in water and in the buffer solution during 1 h.



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EMF effects on water properties

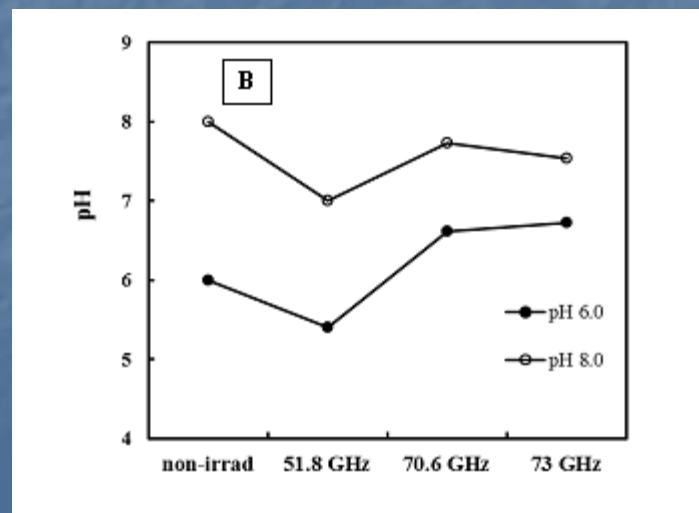
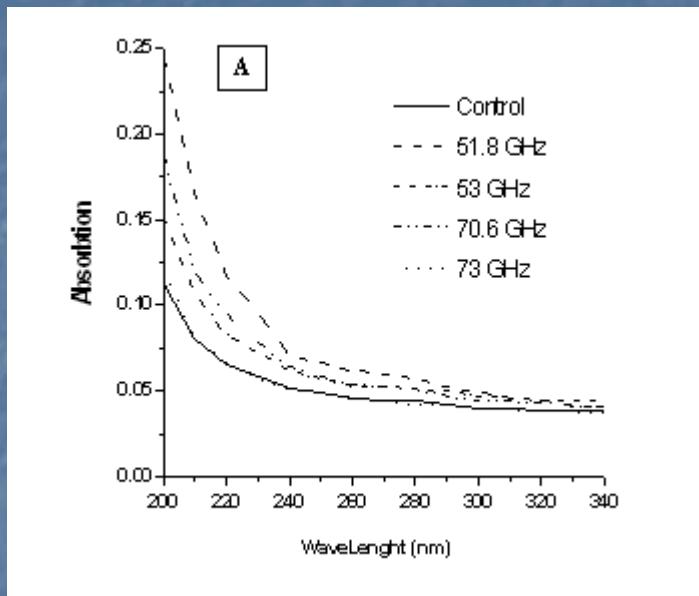
Control		70.6 GHz		73 GHz	
pH	conductivity (μS)	pH	conductivity (μS)	pH	conductivity (μS)
4.0	47.20 ± 0.10 $p < 0.05$	4.26 ± 0.05 $p < 0.05$	48.60 ± 0.20 $p < 0.025$	4.50 ± 0.10 $p < 0.05$	49.10 ± 0.15 $p < 0.01$
6.0	1.54 ± 0.2 $p < 0.05$	6.62 ± 0.15 $p < 0.05$	2.32 ± 0.1 $p < 0.05$	6.72 ± 0.05 $p < 0.01$	3.30 ± 0.30 $p < 0.025$
8.0	4.17 ± 0.20 $p < 0.05$	7.73 ± 0.07 $p < 0.05$	4.84 ± 0.10 $p < 0.05$	7.53 ± 0.10 $p < 0.05$	5.46 ± 0.15 $p < 0.025$

The water pH and conductivity without and after EMI of 70.6 and 73 GHz frequencies

The oscillating and fluctuating character of pH values in bi-distilled water depending on time, spontaneous fluctuations of geomagnetic field and environmental conditions, and other types of intra correlation between physical and chemical parameters of water can occur (Fesenko E. et al., *FEBS Lett.* 1995, 366, 49-52; Drokina T. et al. *Phys. Metals Metallograph.* 2006, 102, S96-S97). And water conductivity changes might result by pH changes.

After irradiation with the frequencies of 70.6 and 73 GHz the water conductance more increased in the case of pH 6.0.

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EMF effects on water properties

Bi-distilled water absorbance spectra (A) in near ultraviolet region (200-340 nm of wavelength) and pH value (B) after by low-intensity EMF. In the control non-irradiated H_2O was used.

The data were adapted from Tadevosyan et al. (2007) and Torgomyan et al. (2011).

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The results with water properties changes might be explained

by suggestion that the energy of EMF could accumulate into the structures of water till critical values and increase of free H⁺ and OH⁻ dispersions or local super-saturation of air (Yemets B. *Int. J. Infrared Millimeter Waves* 2001, 22, 639-643) by modification of molecular cluster structuring (Sinitsyn N. et al. , *Crit. Rev. Biomed. Eng.* 2000, 28, 269-305).

But

the results with no mediated effects on bacteria and in the same time with the changed properties (pH, electroconductivity, absorption spectra, surface tension) of water irradiated with 70.6 and 73 GHz, which known as no resonant frequencies for it (Sinitsyn, N. et al. *Crit. Rev. Biomed. Eng.* 2000, 28, 269-305.), are contradictory.

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Repeated action of EMF on bacteria

The repeated action of coherent EMF with the frequencies in 51.8 or 53.0 GHz on *E. coli* K12 reduced considerably an inhibitory effect (in 1.7- or 1.9-fold for the frequency of 51.8 or 53.0 GHz, correspondingly; p<0.01).

Similar effects have been obtained with coherent EMF with the frequencies in 70.6 or 73 GHz on *E. coli* K12.

(irradiation during 1 h with interruption of 2 h, in checking control repeated irradiation was absent but bacteria were kept with the same time)

(!) Certainly, compensatory mechanisms in bacteria have not been studied, although miscellaneous defensive proteins like those discovered in animal cells (Novoselova E.G. et al. *Dokl. Russ. Acad. Sci.* 2005. 401:117-119) might play a role.

Bactericidal effects of low-intensity extremely high frequency electromagnetic field

Concluding remarks (for anaerobic fermentative conditions):

- ✓ the coherent EMF of such frequency (the range of frequencies from 45 to 53 GHz) with low intensity (the flux capacity of 0.06 mW/cm²), upon direct irradiation (during 30 min or 1 h), affected *E. coli* K12, wild-type, or *E. hirae* ATCC9390, wild-type, grown under anaerobic conditions with fermentation of sugar (glucose):
 - ✓ it caused a decrease in *E. coli* bacterial growth rate, maximal inhibitory effect was achieved at the frequency of 51.8 or 53 GHz. This effect depended on medium pH, the maximal one was at pH 7.5.
- ✓ Similar effects have been obtained by direct irradiation of *E. coli* during 1 h with the coherent EMF of the frequency 70.6 and 73 GHz.
- ✓ the noticeable changes in membrane proton conductance, membrane potential, proton fluxes through the membrane of whole cells and overall and *N,N'*-dicyclohexylcarbodiimide (DCCD)-inhibited ATPase activity of membrane vesicles were determined with 51.8 and 53 GHz.

Bactericidal effects of low-intensity extremely high frequency electromagnetic field

Concluding remarks (for anaerobic fermentative conditions):

- ✓ A decrease in sensitivity to reagents (DCCD) by cells was also determined.
- ✓ It had mediated effects on bacteria: separate irradiation (the frequency of 51.8 and 53 GHz) of bi-distilled water or some inorganic ions containing Tris-phosphate buffer (pH 7.5), into which grown cells were transferred, during 30 min or 1 h, had changed further growth of these bacteria in different, opposite directions; irradiation of water only caused inhibitory effect.
 - ✓ No mediated effects have been obtained with 70.6 and 73 GHz.
- ✓ A significant action disappeared upon repeated irradiation at the frequency of 51.8 and 53 GHz, or 70.6 and 73 GHz during 1 h and with interruption for 2 h. This result indicates some compensatory mechanisms within bacteria.

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- Among cellular mechanisms of EMF bacterial effects the membranous changes are of significance (Gub N.M. et al. *Millimeter waves in medicine and biology*. 10th Russian Symp. with int. part. M., 1995. P. 96; Bulgakova V.G. et al. *Biophysics*. 1996. 41:1289-1293; Trchounian A. et al. *Biophysics*. 2001. 46:69-76; Isakhanyan V., Trchounian A. *Biophysics*. 2005. 50:604-606; Tadevosyan A., Trchounian A., *Biophysics*, 2009, 54:1055-1059; Torgomyan H., Trchounian A. *Biochem. Biophys. Res. Commun.* 2011, 414:265-269; Torgomyan H. et al., *FEMS Microbiol. Lett.* 2012, 329:131-137; etc) those are connecting with
 - surface characteristics of plasma membrane,
 - ion and other substances transport across the membrane,
 - energy-conversing processes.
- Action of EMF is offered also on structure and properties of water (Gapochka L.D. et al. *Moscow Univ. N. Physics, Astrophysics* 1994. V. 35, P. 71-76; Fesenko E.E. et al. *FEBS Lett.* 1995. V. 366. P. 49-52; Gapeyev A.B. et al. *Bioelectrochem. Bioenerg.* 1997. V. 43. P. 217-220; Potselueva M.M. et al. *Reports Biochem. Biophys.* 1998. V. 359. P. 415-418; Bechkii O.V. et al. *Crit Rev. Biomed. Eng.* 2000. V. 28. P. 247-268; Tadevosyan H. et al., *Biophysics*, 2007, V. 52, P. 893-898; Torgomyan H. et al., *Cell Biochem. Biophys.* 60 (2011) 275-281) leading
 - for instance, to change the water molecules electronic structure and intermolecular structure; to increase in chemical activity of water or hydration of proteins and other cellular structures. These could also relate to modifications of structure, properties and function of membrane, especially membrane proteins.

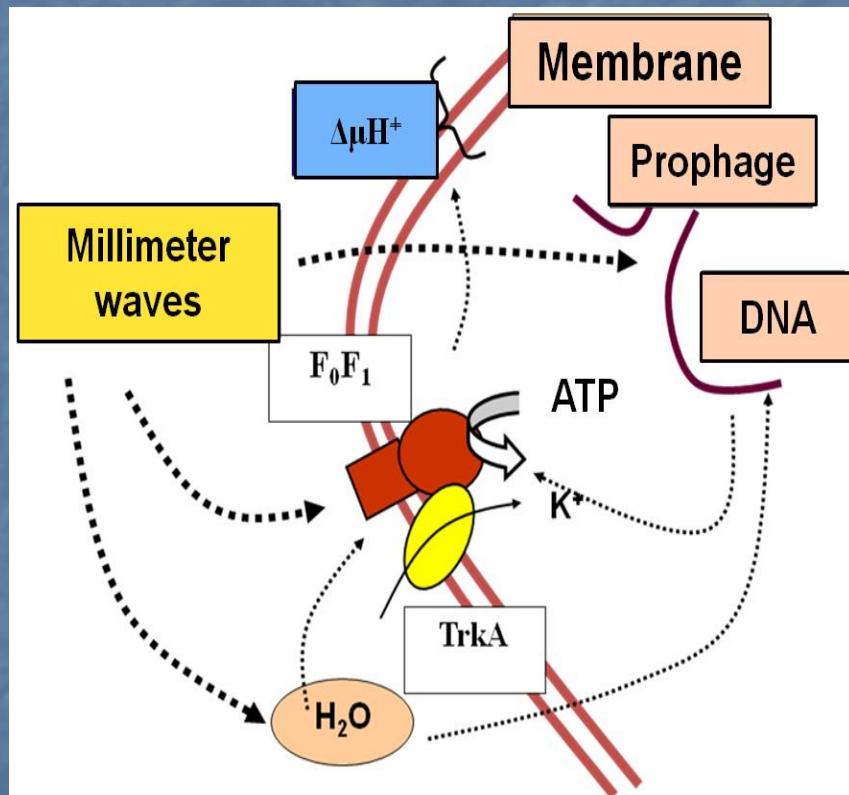
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- Conformational changes in bacterial genome or DNA are also suggested (Belyaev I.Ya. et al. *Z. Naturforsch.* 1992. 47:621-627). Bactericidal effect might be determined by transition of bacterial pro-phages from lysogenic to lytic state (Lukashevsky K.V., Belyaev I.Ya. *Med. Sci. Res.* 1990. 18:955-957; Belyaev I.Ya. et al. *Bioelectromagnetics*. 1996. 17:312-321).
- Effects of EMF can be a result of the resonant interaction, when in case with *E coli* it is shown that, regardless of intensities of EMF of extremely high frequency, resonance is at the frequencies of 41.5, 51.8 or 70.6 GHz (Lukashevsky K.V., Belyaev I.Ya. *Med. Sci. Res.* 1990. 18:955-957; Belyaev I.Ya. et al. *Bioelectromagnetics*. 1996. 17:312-321).

Consequence:

EMF effects on bacteria and cells in generally, especially primary cellular mechanisms of these effects, require the further study.

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Probable ways and three main primary cellular targets for extremely high frequency EMF in bacteria:

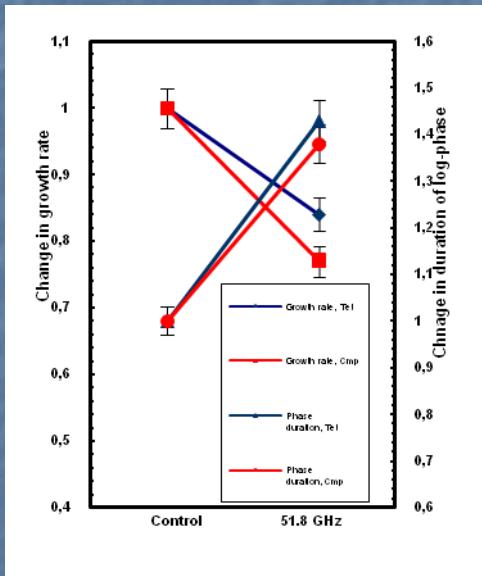
- **membrane proteins** (the proton F_0F_1 -ATPase (F_0F_1), K^+ transporter (TrkA)),
- **water molecules (H_2O)**
- **genome.**

F_0F_1 generates proton electrochemical gradients across the membrane ($\Delta\mu H^+$).

(Torgomyan H., Trchounian A.
Crit. Rev. Microbiol. 2012, Epub
Jun 6)

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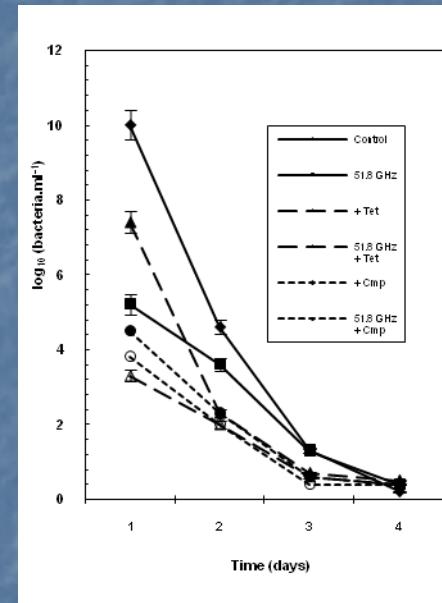
Enhancing sensitivity to antibiotics



The effects of EMI with the frequency of 51.8 GHz on the *E. coli* K12 growth properties in the presence of 4 mM tetracycline (Tet) or 4 mM chloamphenicol (Cmp).

The lag phase duration and specific growth rate without EMI was 1.

(Tadevosyan et al.
Cell Biochem.
Biophys. 51 (2008)
97-103)

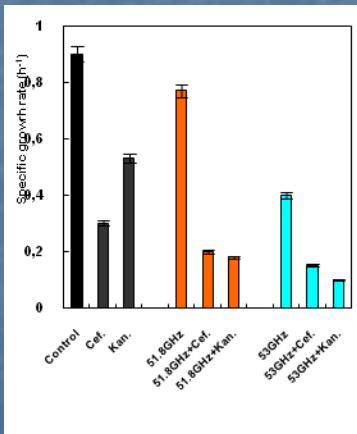
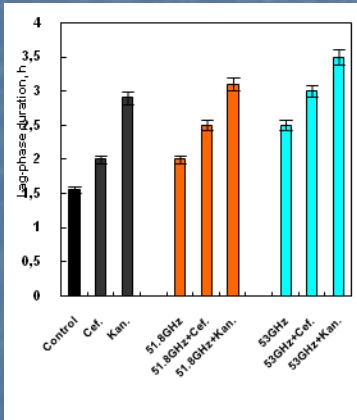


E. coli survival during 4 days in the minimal salt medium after EMI of the frequency of 51.8 and 53 GHz and in the presence of tetracycline and chloramphenicol.

Bacteria grown till the stationary phase were radiated for 1 h, and 4 μM Tet or 4 μM Cmp was added.

(Tadevosyan H. et al. *Cell Biochem. Biophys.* 2008, 51, 97-103)

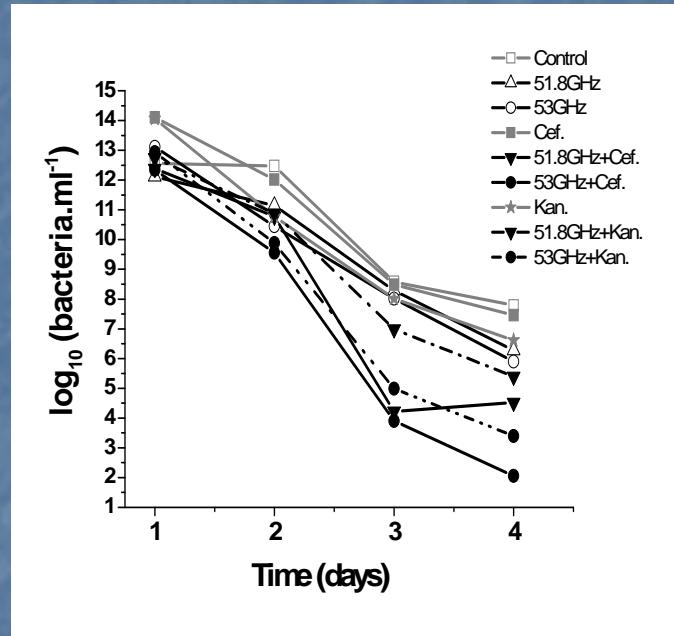
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Enhancing sensitivity to antibiotics

E. coli growth properties after EMF of the frequency of 51.8 and 53 GHz and in the presence of ceftriaxone and kanamycin.

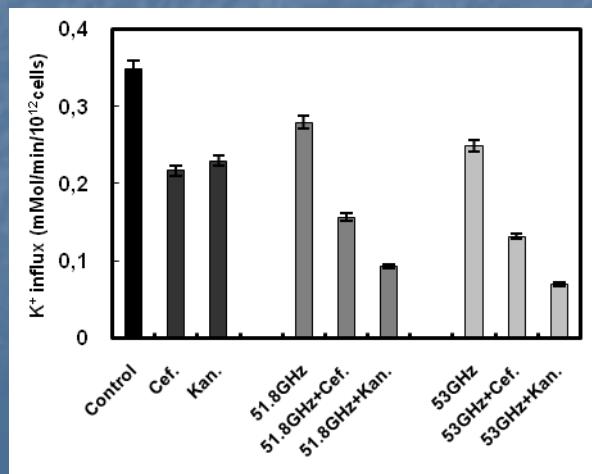
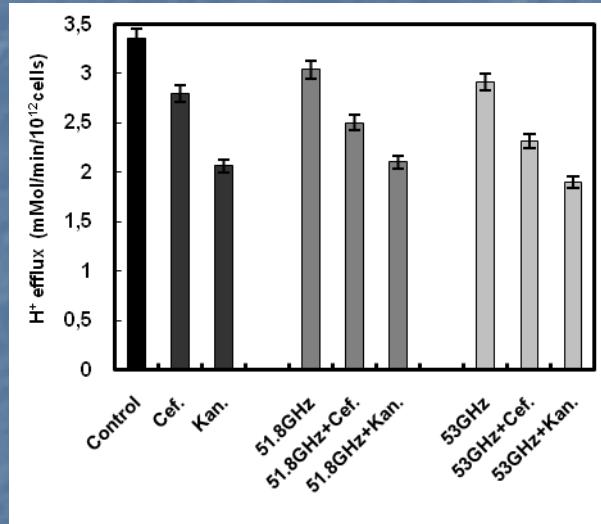
Control was without EMI or antibiotics. Ceftriaxone (Cef) and kanamycin (Kan) were added to the growth medium immediately before inoculation in the concentrations of 0.4 µM and 15 µM, respectively.



E. coli survival during 4 days in the minimal salt medium after EMI of the frequency of 51.8 and 53 GHz and in the presence of ceftriaxone and kanamycin.

(Torgomyan H. et al., *Curr. Microbiol.*, 2011, 62, 962-967)

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Enhancing sensitivity to antibiotics

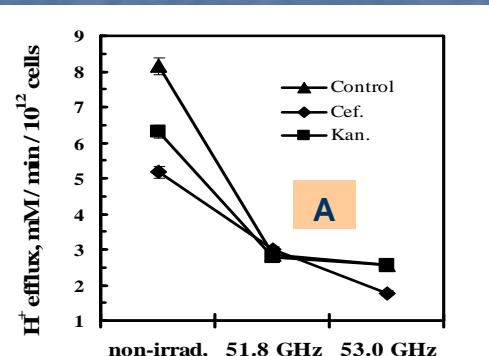
The changes in energy-dependent proton secretion and potassium ions uptake by *E. coli* K12 after EMI of the frequencies of 51.8 and 53 GHz and in the presence of antibiotics.

➤ EMF depressed energy (glucose)-dependent ion transport and it was considered well in the case of 53 GHz (~2-fold).

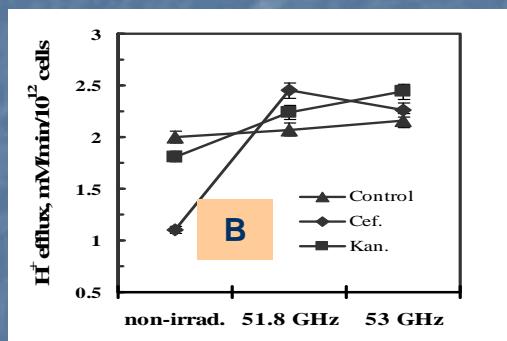
➤ Moreover, used antibiotics, such as kanamycin and ceftriaxone enforced the inhibitory effects of MMW on H⁺ efflux in ~1.2-fold and K⁺ influx in ~2.1 or ~2.7-fold, so it was stronger with kanamycin.

These might point out membranous aspect: modifications of structure, stability, interaction with each other, enzymatic activity and function of membrane proteins (Bulgakova V. et al. *Biophysics* 1996, 41:1289-1293; Tadevosyan H. et al. *Cell Biochem. Biophys.* 2008, 51:97-103; Tadevosyan H., Trchounian A. *Biophysics*, 2009, 54:1055-1059), especially a role of F₀F₁-ATPase, in radiation and antibiotics effects on *E. coli* (Tadevosyan H., Trchounian A. *Biophysics*, 2009, 54:1055-1059).

Bactericidal effects of low-intensity extremely high frequency electromagnetic field

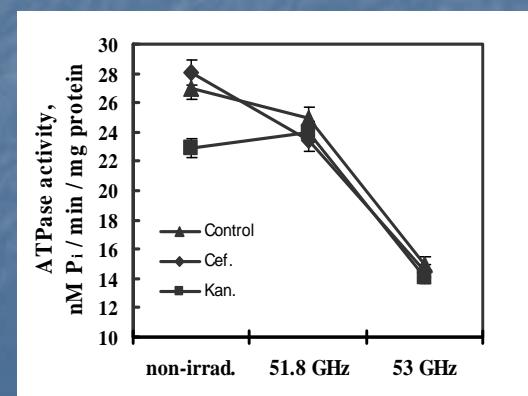
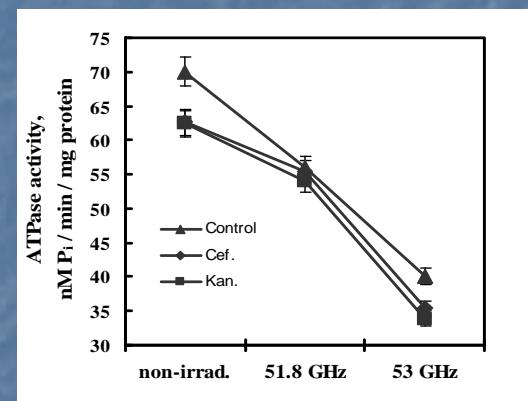
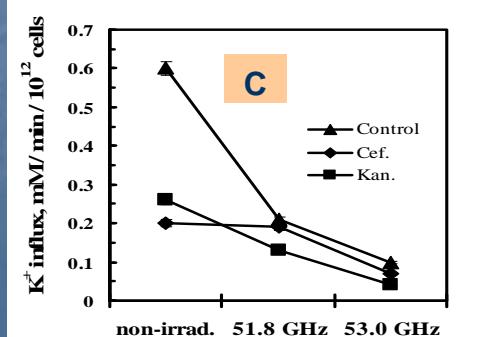


The changes in energy-dependent total (A) and DCCD-sensitive (B) H^+ and total K^+ (C) fluxes through the *E. hirae* ATCC9790 membrane after 51.8 and 53.0 GHz frequencies EMI and in combination of antibiotics.



The changes in either overall (A) or DCCD-sensitive (B) ATPase activity in *E. hirae* ATCC9790 membrane vesicles, after 51.8 and 53.0 GHz frequencies EMI and in combination of ceftriaxone and kanamycin. DCCD (0.1 mM) and ATP (3 mM) were used.

(Torgomyan et al.
FEMS Microbiol. Lett.
2012. 329:131-137)



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Concluding remarks (for anaerobic fermentative conditions):

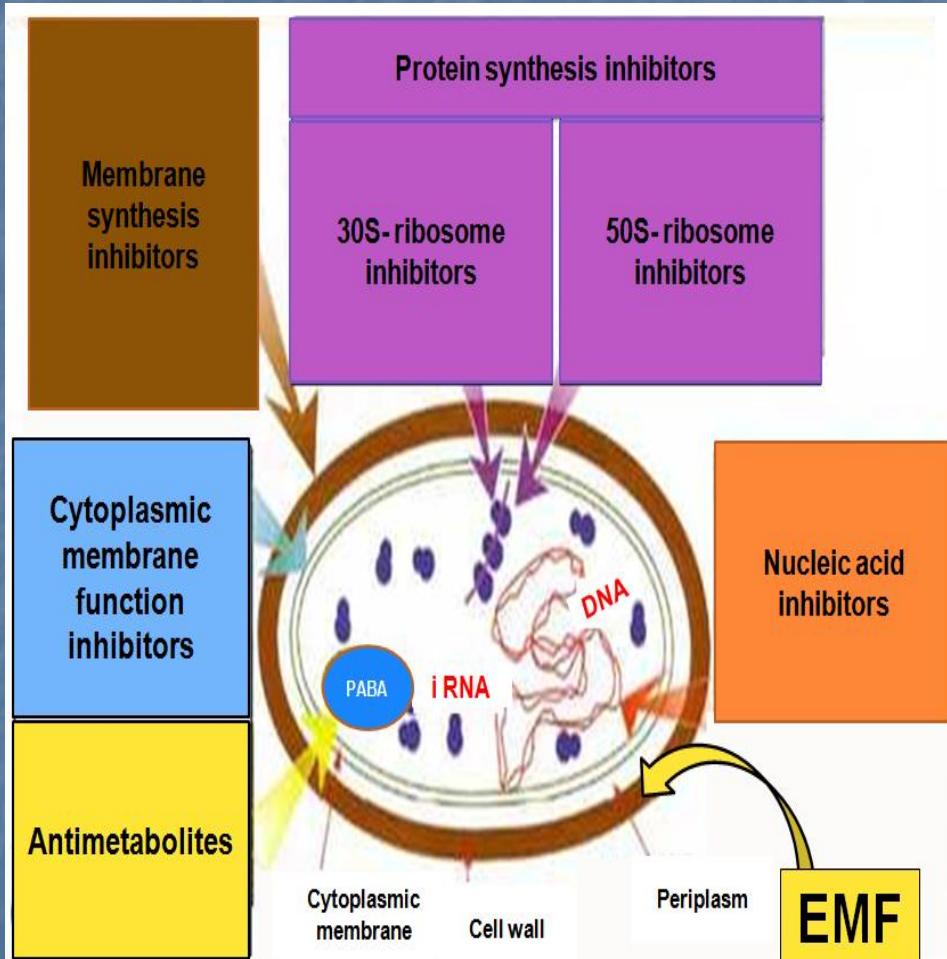
Enhanced effects are observed by both EMF and antibiotics, such as tetracycline, chloramphenicol, kanamycin and ceftriaxone, action. Those are maximally achieved at the frequency of 53 GHz.

Proposal:

EMF can change the sensitivity to antibiotics, or have combinatory effects, or synergetics with antibiotics.

This seems to be in accordance with results reported by Bulgakova et al. (*Biophysics*. 1996. V. 41. P. 1289-1293) that EMF with non-thermal intensity could change the sensitivity of *Staphylococcus* to various antibiotics having membranotropic properties.

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Combined action of extremely high frequency EMF and antibiotics on bacteria. EMF signals are primary transformed to change water cluster structuring, membrane proteins properties, DNA or prophage conformations and other cellular structures. H₂O by its turn can change membrane-associated metabolic processes by altering membrane protein conformation, their hydration degree, electrochemical gradients across the membrane and other properties; a metabolic energy is required. These all create conditions, when the action of antibiotics is facilitated to bring enhanced cell damage and subsequently cell death.

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Main conclusions:

- ✓ Membranous effects might be accounted in biological action of EMF on bacteria;
- ✓ Water has a role in the effects of EMF on bacteria.

Significance:

Bacterial effects of “noise” and coherent MMW with low intensity shown by different groups are of significance to understand distinguishing role of bacteria in biosphere nowadays leading to changed metabolic pathways and, for instance, to antibiotics resistance

(for review, see *Bacterial Stress Responses*. Storz G., Hengge-Aronis R. (Eds.). ASM Press. Washington DC. 2000. 502 p.).

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Further concepts:

A progress in understanding of interaction mechanisms and cellular and subcellular (molecular) targets of EMF in bacteria can be stated so as this knowledge will provide a basis for extrapolation of the findings to future microbial and other technologies.

The perspectives for EMF application in

- **ecology** are to change distribution of bacteria in biosphere;
- **food industry** - to increase safety and nutritional attributes;
- **biotechnology** - to obtain of biomass and end-products;
- **medicine** - to treat the broad range of bacterial diseases.

(Torgomyan H., Trchounian A. *Crit. Rev. Microbiol.* 2012. Epub Jun 6)

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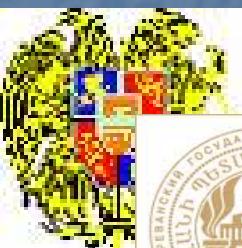
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