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կենսաբանական գիտությունների թեկնածուի
գիտական աստիճանի հայցման ատենախոսության

Ս Ե Ղ Մ Ա Գ Ի Ր

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MINISTRY OF SCIENCE AND EDUCATION OF RA
YEREVAN STATE UNIVERISTY

OHANYAN VAHE ASHOT

THE GROWTH AND SURVIVAL OF BACTERIA UNDER SOME PHYSICAL FACTORS AND ANTIBIOTICS ACTION

S Y N O P S I S

of dissertation for conferring of scientific degree of
Candidate of Biological Sciences
in the specialty of 03.00.02 – Biophysics

Yerevan 2012

Ատենախոսության թեման հաստատվել է Երևանի պետական համալսարանում:

Գիտական ղեկավար՝ ՀՀ ԳԱԱ թղթակից անդամ, կենսաբանական գիտությունների դոկտոր, պրոֆեսոր Ա. Հ. Թռչունյան

Պաշտոնական ընդդիմախոսներ՝ կենս. գիտ. դոկտոր, պրոֆ. Հ. Ռ. Վարդապետյան, կենս. գիտ. դոկտոր Ա. Զ. Փեփոյան

Առաջատար կազմակերպություն՝ ՀՀ առողջապահության նախարարության ճառագայթային բժշկության և այրվածքների գիտական կենտրոն, Երևան

Ատենախոսության պաշտպանությունը տեղի կունենա 2012թ. հունիսի 8-ին, ժամը 14:00-ին Երևանի պետական համալսարանում գործող ՀՀ ԲՈՀ-ի 051 «Կենսաֆիզիկա» մասնագիտական խորհրդի նիստում (0025 Երևան, Ալեք Մանուկյան 1, ԵՊՀ, կենսաբանության ֆակուլտետ):

Ատենախոսությանը կարելի է ծանոթանալ Երևանի պետական համալսարանի գրադարանում:

Ատենախոսության սեղմագիրն առաքված է 2012թ մայիսի 7-ին:

051 մասնագիտական խորհրդի գիտական քարտուղար,
կենսաբանական գիտությունների դոկտոր, պրոֆեսոր՝ Լ. Հ. Նավասարդյան

The theme of the dissertation has been approved at Yerevan State University.

Academic advisor: Corresponding Member of NAS RA, Doctor of Biological Sciences, Professor A. Trchounian

Official opponents: Dr. of Biological Sciences, Professor H. Vardapetyan
Dr. of Biological Sciences A. Pepoyan

Leading organization: Research Center for Radiation Medicine and Burns, Ministry of Health of RA, Yerevan

The defense of the dissertation will be held on June 8th, 2012, at 14:00 at the session of 051 Specialized Council «Biophysics» of RA SCC at Yerevan State University (0025 Yerevan, Alex Manoogian 1, YSU, Faculty of Biology).

The dissertation is available at the library of Yerevan State University.
The synopsis has been sent on May 7th, 2012.

Scientific Secretary of the 051 Specialized Council,
Doctor of Biological Sciences, Professor L. Navasardyan

INTRODUCTION

Topic significance. The living organisms and the environment itself are always disturbed by various physical factors and chemical affecting agents. The typical examples are light, electromagnetic and gravitational fields, temperature and heliophysical fluctuations, partial pressure of oxygen, osmotic pressure, etc.

The electromagnetic fields (EF) in extremely high frequencies (EHF) with low intensity are among the ones that interest the scientists the most, because biological objects, including bacteria, show high sensitivity toward those fields (Deviatkov et al. 2000; Petin, 2006; Belyaev, 2005; Trchounian, 2010). The investigation of these fields is necessary and verified based on several aspects and points of view. The industrial and medical applications of these waves were established based on biological effects of electromagnetic irradiation (EMI) in EHF. But, almost in all cases the selection of used waves and the methods of application were based on practical observations. There is neither a simple explanation that is able to reveal the affecting mechanisms of the waves, nor fundamental knowledge about the biophysical and other alternations that occur after the irradiation (Berkinblit, Glagoleva, 1998; Deviatkov et al. 2000; Pakhomov, Murphy, 2002; Belyaev, 2005; Alexandrov et al., 2006; Petin, 2006; Ruediger, 2009). These kinds of observations are important in terms of ubiquitous presence of artificial sources of EMI in environments of living organisms and particularly human beings. The most obvious sources are telecommunication technologies and remote control systems. Therefore, in order to eliminate or decrease the possibility of potential adverse effects of biological objects and to improve human safety standards, the research of the affecting mechanisms of electromagnetic waves becomes significant.

There are several hypothesis concerning biological effects of EMI. They are based on resonant interactions of EMI and biological systems (Deviatkov et al. 2000; Sinitsyn, Petrosyan, 2000). But they are unable to explain the non-thermal effects of EMI in EHF in details. The physicochemical mechanism of the effects still stains unrevealed. Particularly, was shown, that probably the primary target of EMI in living cells is the cell membrane (Trchounian, 2010; Torgomyan et al., 2012). The membrane is able to convert the fluctuations of affecting electromagnetic waves into acoustic-electric variations (Deviatkov et al., 1991), while water seems to appear as mediator (Fesenko et al., 1995). It should be mentioned that the effects of EMI in EHF on bacteria is also important, because the cell-to-cell interactions through these waves is possible (Trushin, 2003), since bacteria itself are able to irradiate in ultrasonic range (Matsuhashi et al., 1998).

Using *Escherichia coli* bacteria was shown that EMI in extremely high, including 51.8 and 53 GHz, frequencies express different biological effects, including bactericide one, and depend on type and specifications of bacteria, growth phase and parameters, growth medium composition and pH, affecting agents, particularly the frequency, intensity and duration of used waves of irradiation and many other similar parameters (Tadevosyan, Trchounian 2009; Trchounian et al., 2001; Isakhanyan, Trchounian, 2005; Tadevosyan et al., 2006, 2007, 2008; Torgomyan et al., 2011, 2012). But similar effects on other bacteria are partially or not researched. From these perspective Enterococcus hirae anaerobic bacteria appears to be interesting, as their membrane properties and metabolism are quite different comparing to *Escherichia coli* bacteria (Trchounian, Kobayashi, 1998; Kakinuma, 1999; Akopyan, Trchounian, 2005; Poladyan et al., 2006). It is important to evaluate the effects of EMI in EHF with low intensity based on differences in their growth and survival parameters. It is also necessary to reveal the membrane mechanism of these effects.

Under explosure of different physical affecting agents the *Pseudomonas putida* and *Erwinia herbicola* – biotechnologically important aerobic bacteria - are also interesting to study (Hass, Defago, 2005; Charkowski, 2007; Ma et al, 2007). Particularly, their survival evaluation can be conducted through assessment of different property alternations, including oxygen absorption that is a characteristic feature of these bacteria.

Research goals and tasks. Within the scopes of conducted work, the main goal was to investigate the changes in growth and survival parameters of some anaerobic and aerobic bacteria, under EMI in EHF and effects of other physicochemical various factors.

Constituted tasks of the research were to:

1. reveal the effects of low intensity EMI at EHF (51.8 and 53.0 GHz) on growth and survival characteristics of *E. hirae* anaerobic bacteria;
2. investigate the consequences of EMI on bacteria based on duration of irradiation and medium properties;
3. establish the effective frequencies of EMI that alter H^+/K^+ exchange through the membrane and membrane-associated ATP-ase activity of bacteria;
4. study the EMI and widely used antibiotics' combined effects on bacterial growth and survival;
5. reveal a link between the survival and oxygen absorption velocity of *P. putida* and *E. herbicola* in different growth phases;
6. investigate the effects of ultrasonic irradiation, temperature and osmotic shocks on oxygen absorption velocity of these bacteria.

Scientific novelty and applied value of the topic. Within the scopes of the topic was revealed, that 51.8 GHz and 53.0 GHz frequencies with low intensity electromagnetic coherent waves affect *E. hirae* anaerobic bacteria's growth and survival rates and appear to have bactericide effect. This is associated with the changes that occur in proton (ion) fluxes through the membrane and alternations in membrane-associated enzymatic activity. In the mean time, the combined effects with widely used antibiotics and EMI are more severe and have more strongly expressed bactericide appearance. Based on the results, it is apparent, that irradiated bacteria alter their sensitivity towards antibiotics. This can serve as a good basis to solve applied problems in food protection, medical and industrial aspects using combined approaches.

The link between oxygen absorption and survival in aerobic bacteria *P. putida* and *E. herbicola* makes probable to use this method in biotechnology in order to evaluate the viability of bacteria.

Main points to present at defense.

1. The low-intensity 51.8 GHz and 53.0 GHz frequency EMI effects on *E. hirae* growth and survival rate, as well as alternations in H^+/K^+ exchange and ATPase activity.
2. The enhanced combined effects of EMI in EHF and antibiotic on *E. hirae* bacteria.
3. The link between survival rate and oxygen absorption and effects on oxygen absorption velocity under physicochemical factors (ultrasonic irradiation, temperature and osmotic shocks) in aerobic bacteria *P. putida* and *E. herbicola*.

Work approbation. Main results of the dissertation were discussed at seminars in Department of Biophysics, Biology Faculty of Yerevan State University and at scientific conferences: 8^{th} International Congress of the European BioElectromagnetics Association (Bordeaux, France, 2007), International conference and DAAD Alumni seminar "Biotechnology and Health – 4" (Yerevan, Armenia, 2010), 17^{th} International Biophysics Congress (IUPAB) and Chinese Biophysics Congress (Beijing, China, 2011).

Publications. Based on experimentally obtained data 7 works, including 4 papers in peer reviewed journals were published.

Volume and structure of dissertation. Dissertation contains: introduction, literature review (Chapter 1), experimental part (Chapter 2), results and discussion (Chapter 3), conclusions and cited references (total 150 papers and books). The document consists of 104 pages, contains 2 tables and 27 graphs and figures.

MATERIALS AND METHODS

Bacteria: All experiments were conducted on *Enterococcus hirae* ATCC 9790, *Pseudomonas putida* 772, *Erwinia herbicola* 5 wild type stains.

Bacterial cultivation and preparation for experiments. *E. hirae* were cultivated at 37^oC for 18-22 h in anaerobic conditions by direct transfer from nutrient agar surface in Petri dish into liquid tryptone growth medium containing tryptone 1%, yeast extract 0.5%, KH_2PO_4 1%, pH = 8,0. *P. putida* and *E. herbicola* were cultivated in aerobic conditions in complex growth medium containing fish paste 20 g, yeast extract 5 g, ammonium fumarate 1 g, pH = 7.0. All values are calculated for 1 l volume. The growth of bacteria was monitored based on bacterial suspension optical density (OD) value. The bacterial suspension was washed and concentrated by centrifugation at 3600 g speed for 15 min and transferred into distilled water or assay solution for further assays.

Bacterial irradiation. Bacteria were irradiated in distilled water by a G4-141 type electromagnetic generator (irradiating coherent electromagnetic waves at the range from 5.6 up to 8.3 mm, flow flux intensity is 0.06 mW/cm^2) which was supplied by Dr. V. Kalantaryan (Yerevan State University).

Determination of bacterial growth characteristics. Specific growth rate of bacteria was determined as 0.693/time doubling of OD of bacterial suspension (when logarithm of OD increases linearly during the time). The growth lag phase duration was determined graphically (OD correlation curve based on growth duration) (Trchounian et al. 2012).

Determination of bacterial survival. Survival rate of bacteria was determined by transferring them into distilled water or saline medium (46 mM $KHPO_4$, 23 mM KH_2PO_4, 8 mM $(NH_4)_2SO_4$, 0.4 $FeSO_4$, 6 µM $MgSO_4$) as described (Markaryan et al., 2002). In some experiments different antibiotics were added into the medium. The quantity of bacteria in unit volume was determined in the same time during 4 days using spectrophotometer at 600 nm wave length and by counting colony forming units (CFU).

Obtaining bacterial membrane vesicles. Right side out membrane vesicles were obtained by lysis of protoplasts with lyzosime using the methods offered by Konings and Kabak (1973).

Transport of protons and potassium ions through bacterial membrane. The transport of ions through membrane was investigated by analyzing the ion activity in external medium that was determined by potentiometric method using ion-selective electrodes (Belustin et a., 1992; Trchounian et al., 2012). The assay mixture was Tris-phosphate buffer, pH 8.0, containing 0.4 mM $MgSO_4$, 1 mM KCl and NaCl.

ATPase activity assay of bacteria. ATPase activity in membrane vesicles was determined by released inorganic phosphorus, after the reaction of bacterial suspension with ATP (3 mM) (Hutchins et al., 2001). The assay mixture was Tris-HCl buffer, pH 7.5, containing 0.4 mM $MgSO_4$, 100 mM KCl (Trchounian and Vassillian, 1994). Protein was determined by the Lowry method (Lowry et al., 1951).

Determination of respiratory activity of *P.putida* and *E. herbicola.* The respiratory activity of bacterial cells was determined based on diluted oxygen absorption rate (Chance, Williams, 1955; Ohanyan et al., 2009).

RESULTS AND DISCUSSION

The effect of electromagnetic irradiation on *E. hirae* growth and survival characteristics

There are several points of view about effects and mechanisms of affection on living organisms about EFs (Bingi, 2002, 2011). There is an accepted hypothesis that the electromagnetic waves affect water, altering its physicochemical properties and hydration activity. These alternations lead to resonant effects in biological systems. The main system that water corresponds with is cell membrane; therefore there is a proposal that membranes are affected directly or indirectly as well, altering its physicochemical properties (Petin, 2006). Therefore, the membranes with different lipid structures and protein activity have different resonant effects.

This is the possible mechanism that explains that different microorganisms have different responses to EFs affection. The structure and functional properties, as well as the medium of microorganism have great role in this process.

The most suitable biological objects for this kind of investigation are bacteria, as if the consist of singular cell and directly are affected by external affecting factors. It is assumed that EMI has two main targets in bacteria. The targets are cell membrane and genome (nucleotides) (Torgomyan et al., 2012). Cell membranes are various based on their structure, chemical composition and sensitivity towards external affecting factors, transport system and many other properties (Trchounian et al., 2001). In some cases there are no other organisms, which have the membranes with similar properties (Trchounian et al., 2001; Lobishev et al., 2003). For example, the membranes of many oligotrophs hold leucine containing fats, which regulate sustainability of membranes toward irradiation (Batrakov, Nikitin, 1996).

The most interest is sharing the effects of coherent EMI in EHF, particularly 51.8 GHz and 53.0 GHz resonant frequencies. Based on literature and data of our experiments, we assume that the resonant is caused by membranotropic effect. Proteins and macromolecules also participate during this process.

All mentioned effects depend on bacterial species and type, growth medium and conditions. The investigation of the effects caused by EMI in EHF on bacteria, grown in anaerobic conditions, is among the most interesting ones, because these are the optimum conditions for growth of most pathogenic bacteria. Similar conditions are favorable also for non pathogenic bacteria, particularly *E. hirae* ATCC 9790. Therefore, in scopes of this work, many investigations were carried on these bacteria, grown in anaerobic conditions.

It was shown, that *E. hirae* being exposed to irradiation in low intensity coherent EMI in EHF range (49-53GHz) or millimeter waves, during 0.5 and 1 h, significantly elongates the growth lag phase, in the mean time, decreasing the specific growth rate (Fig. 1). The suppressing effect is noticeable the most at the range from 49 GHz up to 53 GHz. But it depends on the irradiation duration. It was revealed that depending on the duration of irradiation the effect is enhancing, although, this effect is noticeable only up to 1 h of irradiation. After 1 h, more irradiation, particularly for 2 h, does not enhance the effect (Fig. 2). It was also shown that, in contrast to *E. coli* (Tadevosyan et al., 2008), this effect is not correlated with bacterial growth medium pH (pH 6.0 or pH 8.0).

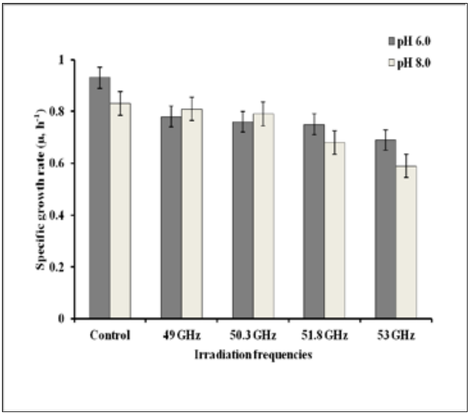


Fig. 1. Variation in growth parameters of *E. hirae* ATCC 9790 after their exposure to coherent EMI in EHF at various pHs of medium. The specific growth rate of the bacteria (μ) was determined as 0.693/ doubling time of optical density of the suspension (when the optical density logarithm increased linearly in time); exposure time of 1 h; control – unexposed bacteria. Mean values of at least 3 independent experiments with standard errors are presented.

It was assumed that bacteria develop a defense or reconstructing mechanism which compensates the effects of irradiation (Ohanyan et al, 2008). Experimentally was shown, that *E. hirae* significantly decreases the specific growth rate after the exposure to 49 GHz, 51.8 GHz and 53.0 GHz EMI for 0.5 and 1 h.

The lag phase duration was elongated by 1.6 and 2.0 times after *E. hirae* was irradiated by 51.8 GHz and 53.0 GHz waves, correspondingly (Fig. 1). In the mean time, EMI in EHF is enhanced in a correlation with increasing frequencies, starting from 49 GHz up to 53 GHz. The most bolded results are noticeable at 53.0 GHz frequency. This assumption is in good agreement with the concept on resonance frequencies of 51.8 GHz and 70.6 GHz for *E. coli*, determined earlier (Belyaev et al., 1996).

An interesting fact was revealed that the effect of electromagnetic waves on the *E. hirae* growth characteristics does not depend on medium pH. The decrease of specific growth rate after the exposure to irradiation in different frequencies was almost identical for pH 6.0 and pH 8.0. The finding is explained based on the statement that these bacteria grow pretty well in the mentioned pH range.

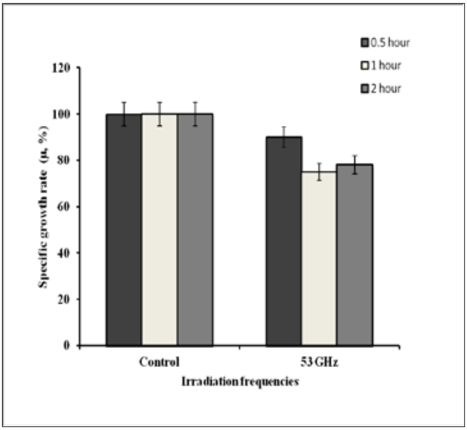


Fig. 2. Dependences of the coherent EMI in EHF effect on specific growth rate of *E. hirae* ATCC 9790 versus duration of their exposure, pH 8.0. On the y axis, specific growth rate of bacteria (μ, %). The control value of μ (unexposed) is 100%. Other conditions are designations are the same as in Fig. 1.

The experimentally obtained data state that the effect of low-intensity coherent EMI in EHF ranging from 49 GHz up to 53 GHz on *E. hirae* leads to alternations of growth characteristics; the results can serve as a good basis for further study for revealing the mechanisms of irradiation to explain possible effects. Consequently, the fundamental knowledge will find practical applications in biotechnology and medicine.

The effects of electromagnetic irradiation in combination with antibiotics on ion transport through membrane and ATP-ase activity of *E. hirae*

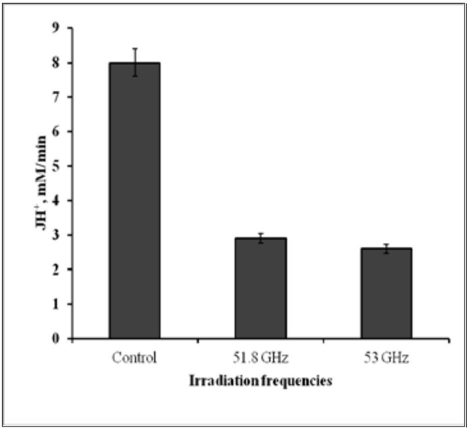
In the basis of alternations of surface, bioenergetic and transport properties of membrane is the EMI in EHF (Bulgakova et al., 1996; Trchounian et al., 2001; Tadevosyan et al., 2008; Tadevosyan, Trchounian, 2009). This statement serves to indicate the membrane-tropic mechanism of the effect that can cause significant alternations in bacterial cell cycle by decreasing survival rate (Tadevosyan et al, 2007): proton translocating F_oF_1- ATPase, which is a main bioenergetic mechanism in the membrane, most probably, is the main target of the irradiation.

Here was shown, that the effects of some antibiotics (based on membrane-tropic action) and membrane protein activity suppressors, are enhanced due to EMI in EHF. The combined effects are more effective rather than singular treatments that were shown for *E. coli* and other bacteria, such as *Staphilococcus* species (Bulgakova et al., 1996; Tadevosyan et al., 2008; Tadevosyan, Trchounian 2009, Torgomyan et al., 2011).

The investigation of affecting mechanisms of EMI in EHF on *E. hirae* may reveal novel properties, because these bacteria are quite different comparing to *E. coli* or other bacteria mentioned. For example, the difference from *E. coli* either structurally or by membrane process, by metabolic specifications and cell cycle (Kakinuma, 1999). Therefore, investigations of the effects of EMI in EHF on *E. hirae*, particularly at 51.8 GHz and 53.0 GHz frequencies on ion transfer, as well as the effects in combination with antibiotics – ceftriaxone and kanamycin, are interesting and verified from scientific point of view.

E. hirae acidify the growth medium by fermenting glucose, releasing H^+ from the cell, either through H^+ transferring F_oF_1 ATPase, or in form of organic acid (Poladyan, Trchounian, 1999). Simultaneously K^+ is absorbed through K^+ absorbing constitutive TrkA system (Trchounian, 2009). This kind of H^+/K^+ exchange plays a important role in bacterial physiology and its modifications can affect bacterial cell cycle and activity.

The irradiation of *E. hirae* with 51.8 GHz and 53.0 GHz frequency electromagnetic waves results the significant depression of energy-dependent H^+ efflux (J_H^+) by 2.8 and 3.2 fold, correspondingly. Its effect on K^+ flux (J_K^+) is more important: J_K^+ is depressed almost 2.8 and 6 folds, correspondingly (Figs.3, 4).



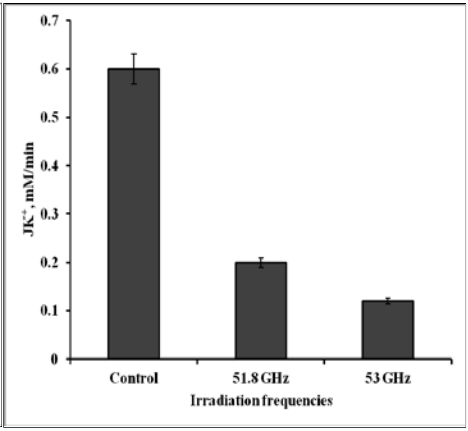


Fig. 3. Suppression of energy-dependent H^+ flux through *E. hirae* ATCC9790 membrane after irradiation with EMI at frequencies 51.8 GHz and 53.0 GHz. J_i is 10^{12} cells on average based on 3 independent experiments with standard error. Bacteria in control probe were not irradiated.
Fig. 4. Suppression of K^+ flux through *E. hirae* ATCC9790 membrane after irradiation with EMI at the frequencies 51.8 GHz and 53.0 GHz. J_i is for 10^{12} cells on average based on 3 independent experiments with standard errors. Bacteria in the control were not irradiated

The dramatic decrease of J_H^+ as a result of irradiation may also be final stage fermentations' that depresses the production of organic acids, as well as F_oF_1 ATPase's effects consequence. But the drastic decrease of J_K^+ may give evidence about direct affection of K^+ uptaking constitutive TrkA-like system and F_oF_1 ATPase, as was shown (Trchounian, Kobayashi, 1998; Trchounian, 2009) that these two systems interact with each other forming protein-protein super-complex. These complexes act in membranes as one, joined mechanism. Moreover, the results indicate that the membrane-tropic effects on the growth characteristics of EMI are in good agreement with previously conducted experiments data with same frequencies and object - *E. hirae* (Ohanyan et al., 2008).

The EMI at the 51.8 GHz and 53.0 GHz frequencies causes dramatic decrease in either overall or DCCD-sensitive ATPase activity of *E. hirae* 1.5 and 2 fold, correspondingly (Fig. 5). The results indicate about direct effect of EMI on F_oF_1-ATPase. For this bacterium DCCD acts as the F_oF_1-ATPase specific inhibitor (Kakinuma, 1999; Pedersen, 2008 Poladyan, Trchounian, 2009).

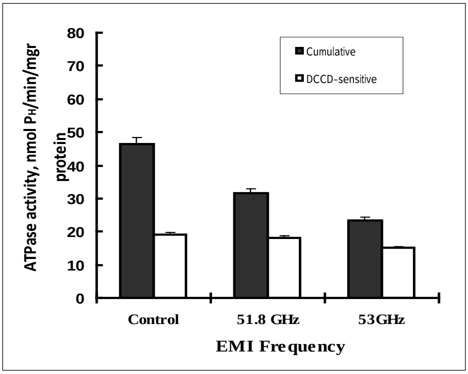


Fig. 5. Change in ATPase activity in membrane vesicles of *E. hirae* ATCC9790 either overall or DCCD-sensitive, after irradiation with EMI at the frequencies 51.8 GHz and 53.0 GHz. DCCD was applied in 0.1 mM concentration, vesicles were incubated for 10 min and ATP was added with 3 mM concentration. The control represents non-irradiated bacteria.

The investigation of combined effects of EMI in EHF and antibiotics on *E. hirae* may create new opportunities for applications. The used antibiotics were selected from different groups of antibiotics. Kanamycin and ceftriaxone are aminoglycozides and cefalosporines – third generation semi-synthetics. These antibiotics, most possibly, have different affecting mechanisms (Alekhin, 2000; Torgomyan, Trchounian, 2012). Therefore, the enhanced effects are due to combined effects that alter *E. hirae* growth characteristics – lag phase duration (τ) and specific growth rate (μ) (Figs. 6,7), as well as changing ion transport through the membrane (Figs. 8,9).

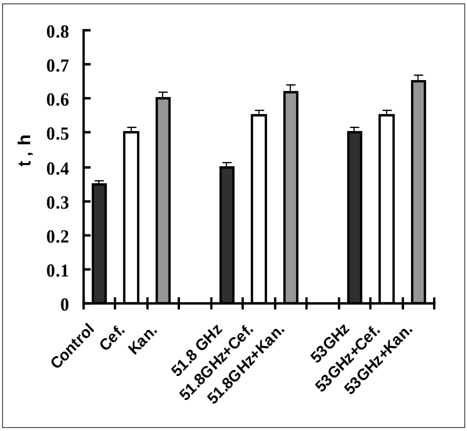


Fig. 6. Change of duration of growth lag-phase of *E. hirae* ATCC9790 after irradiation with coherent EMI EHF at the frequencies 51.8 GHz and 53.0 GHz in combination antibiotics – ceftriaxone (100 μM) and kanamycin (200 μM), which were added into the growth medium, in parallel with glucose.

The duration of growth lag phase increases, almost by the same rate (Fig. 6). But, the decrease of specific growth rate is more noticeable, when the bacterial suspension was irradiated with extremely high frequency electromagnetic waves in combination with ceftriaxone. The combination with kanamycin is less expressed (Fig. 7). The specific growth rate was decreased by 1.9 and 2.3 folds as a result of ceftriaxone and 51.8 GHz and 53.0 GHz frequencies, correspondingly. While the samples, where bacterial suspension was treated with ceftriaxone only, the decrease was 1.3 fold comparing to the control. In the samples, which were treated with the 51.8 GHz and 53 GHz frequency electromagnetic waves, the specific growth rate decrease was 1.1 and 1.2 fold, correspondingly, compared to the control. The effects are important and are explained with the increase of sensitivity towards ceftriaxone and kanamycin after irradiation. The increased sensitivity can be the consequence of structural and conformational changes of membranes due to irradiation. Particularly, the membrane proteins, that are linked with mediated effects of antibiotics (Alekhin, 2000; Tadevosyan et al., 2008; Torgomyan et al., 2011).

Energy dependent J_H^+ and J_K^+ were investigated in order to reveal their possible role and to understand the enhanced effects on bacterial growth after combined exposure to antibiotics and EMI in EHF.

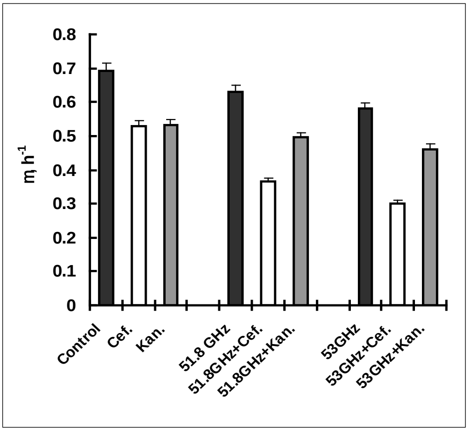
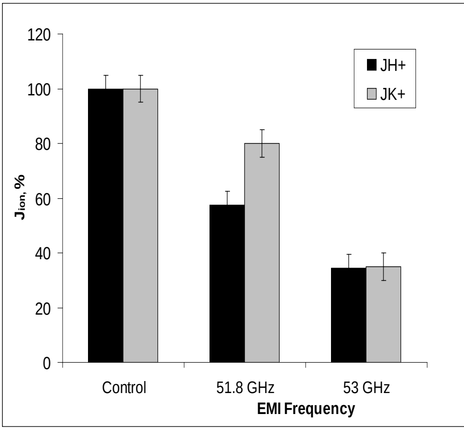


Fig. 7. Change of specific growth rate of *E. hirae* ATCC9790 after irradiation with coherent EMI EHF at the frequencies 51.8 GHz and 53.0 GHz in combination with antibiotics – ceftriaxone and kanamycin. For the others see legends to Fig. 6.

Antibiotic ceftriaxone, suppresses J_H^+ and J_K^+ 1.6 and 3 fold, correspondingly, while kanamycin – 1.3 and 2.3 folds. Most possibly, the F_oF_1-ATPase and its activity are depressed as a result of direct effect of antibiotic that affects the both fluxes (Hong, Pedersen, 2008). The effect enhancement as a result of antibiotics and EMI combined exposure that was also recorded, depresses both fluxes (Figs. 8,9). Moreover, the combined effect of 53 GHz wave and ceftriaxone has the most enhanced effect on both fluxes, while in combination with kanamycin, J_K^+ was dramatically decreased (Fig. 9).

It should also be mentioned that the combinations of both mentioned frequencies with kanamycin more effectively depress J_K^+. Moreover, the decrease of J_K^+ was about 1.6 and 2.5 folds more comparing to the samples that were only irradiated (comp. Figs. 3, 4 and Figs. 8,9).

The results indicate that combined effects of EMI and antibiotics enhance the effects on J_H^+ and J_H^+ through bacterial membrane of *E. hirae*. The obtained data is consistent with the results about the effects on growth parameters of the same bacteria (Figs. 7, 8). This issue verifies the membrane-tropic effect of both factors, either irradiation or antibiotics on bacteria. Additionally, the effects of used antibiotics on J_K^+ also indicate about the direct influence on TrkA system. The same should be said, concerning F_oF_1-ATPase with this system. Taking into consideration that these links could occur through dithiol-disulfide transitions, there is a possibility of destruction of intra- but not intermolecular



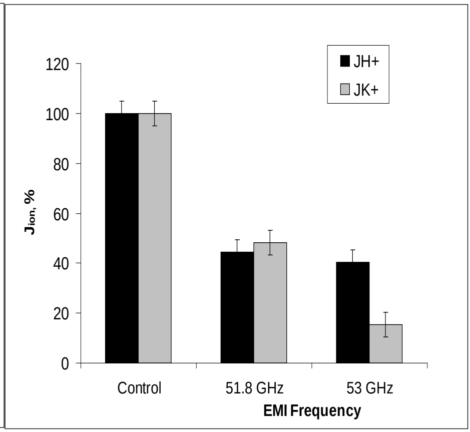


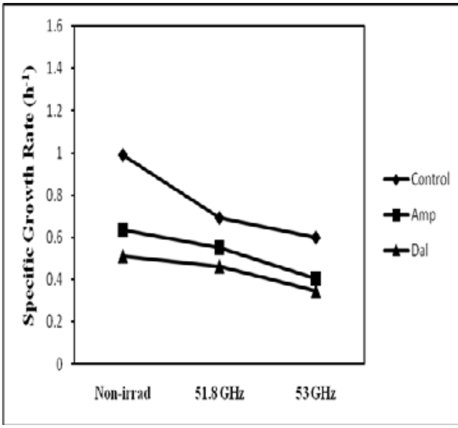
Fig. 8. EMI effect at the frequencies 51.8 GHz and 53.0 GHz in combination with ceftriaxone on H^+ and K^+ fluxes by *E. hirae* ATCC9790. The control was treated with antibiotics only and the flux is accepted as 100%. Bacteria were incubated for 10 min in the presence of antibiotics.
Fig. 9. EMI effect at the frequencies 51.8 GHz and 53.0 GHz in combination with kanamycin on H^+ and K^+ fluxes by *E. hirae* ATCC9790. For the others see legends to Fig. 8.

connections may affect TrkA-like system. There is also a possibility that the different effects of antibioitics are due to different pathways of affection.

Obviously under extremely high frequency EMI specific growth rate radically decreased (Tadevosyan et al., 2007; 2008). In order to reveal all possible correlations of combined effects several combinations of irradiation and/or antibiotics were considered. Several groups of bacteria were created to observe the effects. First group was the control group, which was neither radiated nor treated with antibiotics. Second group was the radiated group; bacterial suspension was irradiated with 51.8 GHz EMI. Bacterial suspension in third group was treated with 53 GHz frequency EMI. Bacterial suspension in forth group was treated with ampicillin only, while the fifth group was treated with dalacin only. Sixth group was either radiated with 51.8 GHz or treated with ampicillin and seventh group was irradiated with 53 GHz and treated with ampicillin. Eighth group was irradiated with 51.8 GHz and treated with dalacin and ninth group was irradiated with 53 GHz and treated with dalacin. Thus, particularly when the bacterial suspension was irradiated with 51.8 GHz waves, μ had decreased approximately by 30%, while with 53 GHz, growth specific rate was decreased by 40% comparing to control group (Fig. 10).

The data and calculated results demonstrate that ampicillin decreases μ also by 30% affecting separately, while dalacin decreases μ by about 50% (Fig. 10). Moreover, antibiotics in combination with EMI affect even more severely. The combinations of 51.8 GHz and ampicillin; 53 GHz and ampicilin; 51.8 GHz and dalacin; 53 GHz and dalacin affect more effectively and decrease μ of bacteria by 45%, 60%, 44% and 65%, correspondingly (Fig. 10). Growth lag phase duration data was also obtained as a secondary indicator of electromagnetic suppression effect. In comparison with the control group, the lag phase was prolonged about

20% and 45%, when bacterial were irradiated with 51.8 GHz and 53 GHz, respectively (Fig. 11).



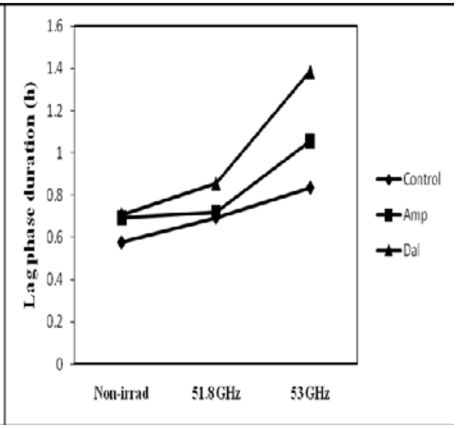


Fig. 10. Changes in specific growth rate - μ for *E. hirae* ATCC9790 affected by EMI of 51.8 GHz and 53 GHz and ampicillin and dalacin. Standard errors were within the designations.
Fig. 11. Changes in growth lag phase duration for *E. hirae* ATCC9790 affected by EMI of 51.8 GHz and 53 GHz and ampicillin and dalacin. For the others, see legends to Fig. 10.

The combination of 51.8 GHz and 53 GHz frequencies with ampicillin prolonged the lag phase by 25% and 83% respectively, while ampicillin only prolonged the duration of lag phase by 20%. The combination of 51.8 GHz and 53 GHz frequencies with dalacin prolonged the lag phase duration by 48% and 2.4 fold, respectively, while the lag phase duration in the group treated only with dalacin was prolonged by 23%.

The survival rate during 4 days also was observed on *E. hirae;* the treatments were the same (i.e. irradiation and/or antibiotics). Based on the results it is apparent that bacterial suspension radiated either with 51.8 GHz and 53 GHz waves eventually contains ~10% less viable bacteria than the control (Fig. 12). The number of CFU decreased gradually depending on antibiotic. While ampicillin had no significant effect on survival, dalacin had noticeable bactericide effect. Dalacin decreased the number of CFU by 7% in 4 days of survival. The combination of 51.8 GHz and dalacin decreases CFU ~2.5 fold, while the combination of 53 GHz and dalacin was less effective (Fig. 13).

It is apparent that combined treatment is more effective than singular influence and particularly 53 GHz was more effective rather than 51.8 GHz irradiation regarding to specific growth suppression (Fig. 10). Nevertheless, for survival with antibiotics and 51.8 GHz was more effective than in case with 53 GHz (Fig. 11). This might suggest different structures and pathways that are essential for bacterial growth and survival, a further study is required.

Apparently 51.8 GHz and 53 GHz EMI affect membranes of the cells (Trchounian 2001; Tadevosyan et al., 2007; 2008). Previously was shown that the growth of *E. coli* was also depressed due to similar waves (Ohanyan et al., 2008).

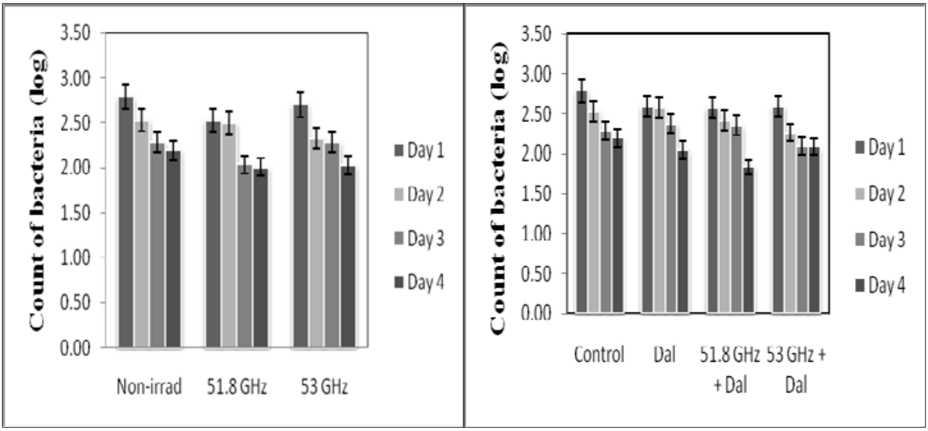


Fig. 12. *E. hirae* ATCC9790 survival non-irradiated versus irradiated with EMI of 51.8 GHz and 53 GHz.
Fig. 13. *E. hirae* ATCC9790 survival non-irradiated versus irradiated with EMI of 51.8 GHz and 53 GHz in combination with dalacin.

The observed data are comparable with *E. coli* results (Trchounian 2001; Tadevosyan et al., 2007; 2008). Consequently, the analogous effect of EMI using different bacteria is evident, despite of the differences of *E. coli* and *E. hirae*. It is interesting, because these bacteria have different membrane structures and composition due to their types.

More in depth conclusions about the affecting mechanisms of EMI are considerable. The targets have resonant interaction with EMI. The cluster structure of water alters; DNA gains conformational transformations and the permeability of membrane changes as the samples are treated with EMI. So, these alternations increase accessibility/transparency of the membrane. That is why combined effects of antibiotic and EMI dramatically enhance the bactericide effect.

It is also clear that the effect is significant not only during the bacterial growth but also during the survival. The survival duration decreases when the bacterial suspension is treated with combined physical and chemical agents.

The effects of physical factors on survival rate of bacteria. Determination of bacterial survivability based on respiratory activity.

Nowadays, there are several approaches to maintain bacterial survivability. These approaches depend on bacterial type and species and they may be specific. The accepted and most convenient method is the periodic re-cultivation of bacteria on solid nutrient growth medium and calculation of CFU, to describe the survivability (Arkadiev et al., 1989). It was shown that for aerobic bacteria determination of dissolved oxygen in the medium and its absorption velocity show a certain link with number of viable bacteria (Shtevneva, Sudakova, 1986; Stitt et al., 1997). This allows evaluating bacterial survivability. It is obvious, that in this case, there is a possibility that determination of respiratory activity of bacteria, based on O_2

absorption rate, can become an excellent, convenient and fast parameter to determine the survivability of aerobic bacteria.

The used *P. putida* 772 and *E. herbicola* 5 were in different growth phases. The O_2 absorption rate was determined during different growth phases. The experimental data show, that the concentration of O_2 had dropped dramatically up to its absence. When O_2 was provided again, the specific absorption rate was recovered. The absorption stabilizes as bacteria moves into stationary growth phases and drastically decreases as soon as bacteria move into death phase (Fig.14).

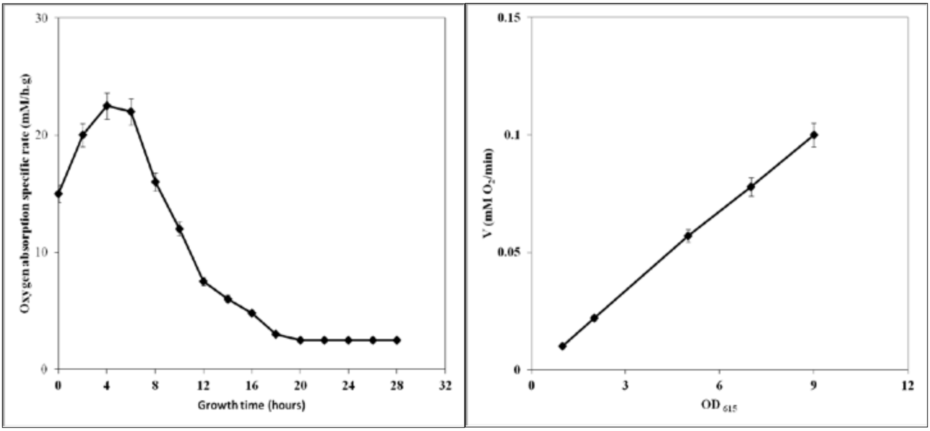


Fig. 14. Dependence of O_2 absorption rate in *P. putida* 772 bacterial growth phase.
Fig. 15. Correlation between number of viable bacterial cells and value of O_2 absorption rate in case with *E. herbicola* 5.

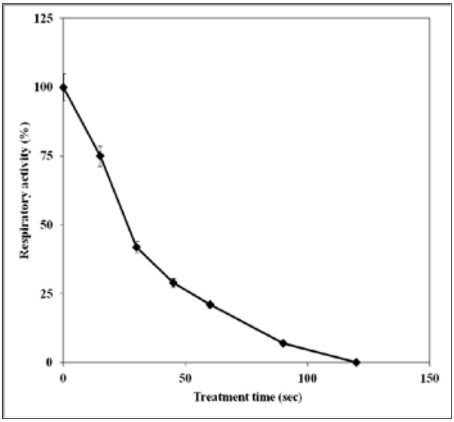
The experiments revealed that there is a linear correlation between the number of viable bacterial cells and the value of *E. herbicola* 5 absorption rate (Fig. 15). These data are consistent with data of other authors (Shtevneva, Sudakova, 1986) and verifies the possibility of bacterial cell quantity determination, based on O_2 absorption rate.

The respiratory activity is considered as the functional descriptor of aerobic bacteria and it depends on bacterial growth phase and other factors, so it is assumed that this parameter shows survivability of the bacterial cells and it can be the descriptor of survivability.

The effects of different physical and chemical factors on bacterial O_2 absorption rate were also investigated. The factors were ultrasonic irradiation, temperature and osmotic shocks. These effects were researched, as if they should be determined to decrease the bias and to find the more precise correlation between the viable cells and O_2 absorption rate (Elpiner, 1963; Morozov et al., 1986; Ribkin, Ravin, 1986).

It was shown that after 30 sec treatment of bacterial suspension with ultrasonic irradiation the O_2 absorption rate by *P. putida* decreased dramatically. In 120 sec the rate was totally depressed (Fig. 16).

The temperature shocks showed that they also affected O_2 absorption rate of *E. herbicola* 5 and at 40°C after 2 h it started to decrease and became about 40% after 8 h. In case with 60°C the decrease was more noticeable (Fig. 17).



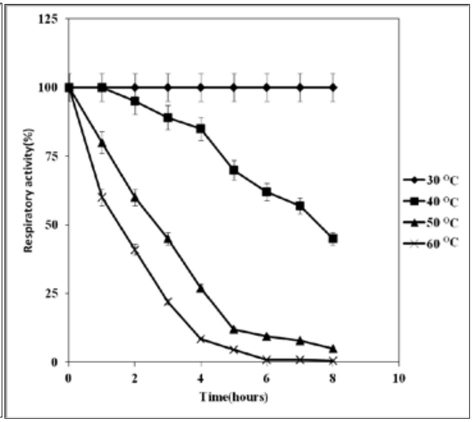


Fig. 16. Relative rate of O_2 absorption dependence on *P. putida* 772 treatment duration.
Fig.17. Temperature effects on *E. herbicola* 5 respiratory activity.

The osmotic effects which were created by transferring bacteria into distilled water or physiological solution influence on the respiratory activity. The O_2 absorption rate dramatically decreased in *E. herbicola* 5 suspension, when they were transferred into water. Almost 15 h later, the residual rate was about 10% in contrast to starting O_2 absorption rate. In case with physiological solution, the O_2 absorption rate started to decrease after 9 h. These results indicate that bacterial respiratory activity is sensitive towards osmotic effects.

Summarizing the results it can be stated that, if the bacterial survivability is linked with sustainability of cellular structures and the respiratory activity is undoubtedly linked with it, so O_2 absorption rate can be used as a parameter that precisely describes the survivability of bacteria.

CONCLUSIONS

Several conclusions were made based on experimentally obtained data:

1. The irradiation of *E. hirae* ATCC 9790, grown in anaerobic conditions over glucose fermentation, with low-intensity extremely high frequency coherent electromagnetic waves affects their growth characteristics: increasing lag growth phase duration and decreasing the specific growth rate as well as the survivability of bacterial cells.

2. Duration and frequency of low-intensity electromagnetic waves are significantly important factors during irradiation of *E. hirae*. The most bactericide effect is determined when the

bacterial suspension is irradiated for 1 h by 51.8 GHz and 53.0 GHz frequencies and is not correlated with medium pH.

3. The irradiation of *E. hirae* with low-intensity 51.8 GHz and 53.0 GHz frequency electromagnetic waves during 1 h in combination with different antibiotics in minimal affecting concentrations (ampicillin, dalacin, kanamycin, ceftriaxone), enhances the bactericide effect, resulting the most dramatic changes in bacterial growth characteristics and significantly decreasing survivability of bacterial cells.

4. The irradiation of *E. hirae* with low-intensity 51.8 GHz and 53.0 GHz frequency electromagnetic waves during 1 h results alternations in proton and potassium ion (this ion transfer is associated with proton movement) transfer through the membrane and changes ATPase activity. These changes can be considered as evidence about membrane-tropic effects of EMI.

5. The survivability of *P. putida 772* and *E. herbicola* 5 is linked with O_2 absorption rate by the bacterial cells. The rate is different during bacterial growth and death phases and is linearly correlated with the number of bacterial cells.

6. The O_2 absorption velocity in *P. putida and E. herbicola* significantly changes either when the bacteria are exposed to temperature and osmotic shocks or treated with ultrasonic irradiation.

7. The *P. putida* and *E. herbicola* survivability can be evaluated by O_2 absorption activity.

LIST OF PUBLICATIONS AS A PART OF DISSERTATION TOPIC

1. Torgomyan H., Ohanyan V., Blbulyan S., Kalantaryan V., Trchounian A. (2012) Electromagnetic irradiation of *Enterococcus hirae* at low intensity 51.8 and 53.0 GHz frequencies: changes in bacterial cell membrane properties and enhanced antibiotics effects. *FEMS Microbiol. Lett.*, **329**, 131–137.

2. Ohanyan V. (2012) Combined effects of extremely high frequency electromagnetic field and antibiotics on *Enterococcus hirae* growth and survival. *National Academy of Sciences of Armenia Reports*, **112**, 87–94

3. Torgomyan H., Ohanyan V., Blbulyan S., Trchounian A. (2011) *Enterococcus hirae* membrane properties and antibiotics effects after low intensity electromagnetic irradiation, in 17th International Biophysics Congress (IUPAB) and 12th Chinese Biophysics congress, Beijing, China, p. 430.

4. Torgomyan H., Ohanyan V., Tadevosyan H., Trchounian A. (2010) Electromagnetic radiation and antibiotics effects on *Enterococcus hirae*: changes in ion fluxes through the membrane, International conference and DAAD Alumni seminar “Biotechnology and Health -4”, Yerevan, 106-107.

5. Ohanyan V., Gukasyan G., Trchounian A. (2009) Relationship of respiratory activity of *Pseudomonas putida* and *Erwinia herbicola* bacteria with their survival. *Technolog. jivih syst.*, **6**(3), 19-25. (Оганян В., Гукасян Г., Трчунян А. (2009) Связь дыхательной активности бактерий *Pseudomonas putida* и *Erwinia herbicola* с их выживаемостью. *Технологии живых систем*, **6**(3), 19- 25)

6. Ohanyan V., Sargsyan H., Tadevosyan H. Trchounian A (2008). The action of low-intensity extremely high frequency electromagnetic radiation on growth parameters for bacteria *Enterococcus hirae. Biophysics* (Moscow), **53**, 406–408.

7. Tadevosyan H., Sargsyan H., Ohanyan V., Trchounian A. (2007) Bacterial effects of the extremely high frequency electromagnetic radiation with low intensity: inhibition of the growth, 8th International congress of the European BioElectromagnetics Association, Bordeaux, 31-32.

ՕՀԱՆՅԱՆ ՎԱՀԵ ԱՇՈՏԻ

ԲԱԿՏԵՐԻԱՆԵՐԻ ԱՃԸ ԵՎ ԿԵՆՍՈՒՆԱԿՈՒԹՅՈՒՆԸ ՈՐՈՇ ՖԻԶԻԿԱԿԱՆ ԳՈՐԾՈՆՆԵՐԻ ԵՎ ՀԱԿԱԲԻՈՏԻԿՆԵՐԻ ԱԶԴԵՑՈՒԹՅԱՆ ՆԵՐՔՈ

Ամփոփագիր

Հանգուցային բառեր՝ *Enterococcus hirae, Pseudomonas putida, Erwinia herbicola*, էլեկտրամագնիսական ճառագայթում, կենսունակություն, հակաբիոտիկներ, շնչառական ակտիվություն:

Այս աշխատանքի շրջանակներում ուսումնասիրվել են տարբեր ֆիզիկական և քիմիական գործոնների, մասնավորապես ծայրահեղ բարձր հաճախություններում էլեկտրամագնիսական ճառագայթման և հակաբիոտիկների, ինչպես նաև ուլտրաձայնի, ջերմային և օսմոսային շոկերի ազդեցությունները անաերոբ՝ *Enterococcus hirae* բակտերիաների աճի և կենսունակության, իոնային հոսքերի և ԱԵՖ-ազային ակտիվության, ինչպես նաև աերոբ՝ *Pseudomonas putida, Erwinia herbicola* բակտերիաների կենսունակության և շնչառական ակտիվության վրա:

Բացահայտվել է, որ գլյուկոզի խմորման ընթացքում անաերոբ պայմաններում աճեցված *E. hirae* բակտերիաների 51.8 ԳՀց և 53.0 ԳՀց ցածր ուժգնության էլեկտրամա·նիսական կոհերենտ ալիքներով ճառագայթումը առաջացնում է բակտերիասպան ազդեցություն, որը կախված է ճառագայթման տևողությունից: Սակայն ցույց է տրվել, որ մեկ ժամ տևողությունից երկար ճառագայթումները էական տարբերություն չեն ցուցաբերում: Բացահայտվել է նաև, որ այս բակտերիասպան ազդեցությունը ուժգնանում է, երբ անաերոբ բակտերիաների վրա, ներկայումս լայն կիրառություն ունեցող, առաջին և երկրորդ, ինչպես նաև երրորդ և չորրորդ սերնդի հակաբիոտիկները, մասնավորապես ցեֆտրիաքսոնը, կանամիցինը, ամպիցիլինը ու դալացինը և վերը նշված էլեկտրամագնիսական ալիքները ներգործում են համակցված կերպով: Այդ դեպքում դիտարկվում է աճման տարբեր ցուցանիշների՝ աճման տեսակարար արագության, աճման գաղտնի լագ փուլի, իոնային հոսքերի և ԱԵՖազային ակտիվության ավելի կտրուկ փոփոխում, որը և պատճառ է բակտերիասպան ազդեցության ի հայտ գալուն:

Աճման լագ փուլի գնահատումը ցույց տվեց, որ նույն հաճախություններում ճառա·այթահարումը և/կամ հակաբիոտիկների հետ համակցված ազդեցությունը հանգեցնում է փուլի տևողության երկարաձգմանը 20 %-ից մինչև 83 %-ը՝ կախված համադրությունից: Այս պայմաններում փոփոխվում է նաև աճման տեսակարար արագությունը, տարբեր պայմաններում նվազելով մինչև 65 % -ով: Փորձերի արդյունքում բացահայտվեց, որ բակտերիաների աճման տարբեր հատկանիշների վրա ամենահզոր ազդեցությունը թողնում է 53.0 ԳՀց ցածր ուժգնության էլեկտրամագնիսական կոհերենտ ալիքների և դալացին ու ցեֆտրիաքսոն հակաբիոտիկների համակցված ազդեցությունները:

51.8 ԳՀց և 53.0 ԳՀց ցածր ուժգնության էլեկտրամագնիսական կոհերենտ ալիքներով ճառագայթահարման արդյունքում փոփոխվում էին նաև թաղանթով իոնային հոսքերը, հանգեցնելով բջջից դեպի դուրս H^+ իոնների (JH^+) հոսքի

նշանակալից ճնշմանը, համապատասխանաբար, 2.8 և 3.2 անգամ, իսկ դեպի բջիջ K^+ իոնների (JK^+) ներհոսքը ճնշվում էր, համապատասխանաբար, 2.8 և 6 անգամ: Հակաբիոտիկների հետ կատարված փորձերից ստացված արդյունքները վկայում են, որ հակաբիոտիկների և ծայրահեղ բարձր հաճախություններում էլեկտրամագնիսական ճառագայթման համակցությունները հզորացնում են *E. hirae* բակտերիաների թաղանթով JH^+ և JK^+-ի վրա ազդեցությունը: Օգտագործված հակաբիոտիկների ազդեցությունը JK^+-ի վրա վկայում է այն մասին, որ տեղի է ունենում TrkA համակարգի վրա ուղղակի ներգործություն, կամ նրանք ազդում են F_OF_1- ԱԵՖազի և այս համակարգի կապի վրա: ԱԵՖազային ակտիվությունը գնահատվել է, թե գումարային ակտիվության ձևով և թե արգելակչի՝ դիցիկլոհեքսիլկարբոդիիմիդի ներկայությամբ: Ցույց է տրվել, որ ԱԵՖազային ակտիվությունը նվազում է համապատասխանաբար 1.2 և 3.5 անգամ:

Հետազոտությունների ընթացքում գնահատվել է նաև *E. hirae* բակտերիաների կենսունակությունը չորս հաջորդական օրերի ընթացքում վերը նշված նմանատիպ ֆիզիկական և քիմիական առանձին ու համակցված գործոնների ազդեցության ներքո: Ակնհայտ է նաև, որ այդ ազդեցությունները նկատելի են ոչ միայն բակտերիաների աճման, այլ նաև կենսունակության ընթացքում: Կենսունակության ժամանակահատվածը նվազում է, երբ բակտերիալ կախույթը մշակվում է նշված գործոններով:

Նոր բացահայտումներ են կատարվել նաև աերոբ բակտերիաներ՝ *Pseudomonas putida*-ի և *Erwinia herbicola*-ի հետ կապված: Մասնավորապես ցույց է տրվել, որ նրանց շնչառական ակտիվությունը կամ լուծված թթվածնի կլանման արագությունը, որը գնահատվել է նրանց աճման տարբեր փուլերում և տարատեսակ ֆիզիկական ներգործությունների, մասնավորապես՝ ուլտրաձայնային մշակման, ջերմային ու օսմոսային շոկերի առաջացման պայմաններում, ուղղակիորեն կապված է միջավայրում առկա կենսունակ բակտերիալ բջիջների թվաքանակի հետ: Կենսունակության և թթվածնի կլանման միջև ուղղակի կապը ցույց տալու նպատակով, ինչպես արդեն նշվեց, բակտերիաների կենսունակությունը գնահատվել է տարբեր ֆիզիկական գործոնների ազդեցության ներքո: Մասնավորապես, թթվածնի կլանման արագությունը ուլտրաձայնով մշակելիս արդեն իսկ 10 վրկ հետո նվազել է 20 %-ով, իսկ 60 վրկ հետո թթվածնի կլանումը գրեթե բացակայել է: Ջերմաստիճանային ու օսմոսային շոկերը նույնպես հանգեցնում էին թթվածնի կլանման արագության կտրուկ նվազմանը:

Ուստի առաջարկվել է այս բակտերիաների կենսունակության որոշման արագ ու արդյունավետ մեթոդ՝ հիմնված թթվածնի կլանման որոշման վրա:

Ամփոփելով ամբողջ աշխատանքը, կարելի է նշել, որ կատարված հետազոտությունների արդյունքում ցույց է տրվել բակտերիաների վրա մի շարք ֆիզիկական և քիմիական գործոնների առանձին կամ համակցված ազդեցությունը, որի հիման վրա, բակտերիաների աճման և կենսունակության գնահատման համար, առաջարկվել են հստակ մոտեցումներ և մեթոդները: Ստացված արդյունքները հակաբակտերիալ պաշտպանության արդյունավետ միջոցների շարքում կարող են գտնել կիրառություն և արդյունաբերության մեջ ու կենսատեխնոլոգիայում և բժշկության մեջ:

ОГАНЯН ВАГЕ АШОТОВИЧ

РОСТ И ВЫЖИВАЕМОСТЬ БАКТЕРИЙ ПОД ВЛИЯНИЕМ НЕКОТОРЫХ ФИЗИЧЕСКИХ ФАКТОРОВ И АНТИБИОТИКОВ

РЕЗЮМЕ

Ключевые слова: *Enterococcus hirae, Pseudomonas putida, Erwinia herbicola,* электромагнитное излучение, выживаемость, антибиотики, дыхательная активность.

В рамках этой работы были исследованы действие разных физических и химических факторов: электромагнитного излучения (ЭМИ) крайне высоких частот, тепловых и осмотичесых шоков, ультразвука, и влияние антибиотиков на рост и жизнеспособность, ионный транспорт и АТФ-азную активность анаэробных бактерий *Enterococcus hirae*, а также выживаемость и дыхательную активность аэробных бактерий *Pseudomonas putida, Erwinia herbicola*.

Обнаружено, что ЭМИ в 51.8 ГГц и 53.0 ГГц малой интенсивности оказывает бактерицидное действие на бактерии *E. hirae*, выращенных в анаэробных условиях при сбраживании глюкозы. Это действие зависит от длительности облучения. Однако доказано, что ЭМИ длительностью более 1 часа не оказывает существенной разницы. Также было выявлено, что бактерицидное действие ЭМИ усиливается, когда на эти бактерии совместно действуют ЭМИ и антибиотики первого, второго, а также третьего и четвертого поколения, в частности, цефтриаксон, далацин, ампицилин, канамицин. В этом случае наблюдается более резкое изменение разных показателей: удельная скорость роста, длительность лаг фазы роста, ионный транспорт и АТФазная активность, что и является причиной уничтожения бактерий.

Оценка продолжительности лаг фазы роста показала, что ЭМИ в тех же частотах и/или совмещенное влияние антибиотиков приводит к более длительной фазе - от 20 % до 83 % в зависимости от совместного действия. В этих условиях изменялась также удельная скорость роста, уменьшаясь в разных условиях на 65 %. В результате опытов было выявлено, что на разные характеристки роста бактерий самое мощное влияние оказывает совместное действие ЭМИ с частотой в 53.0 ГГц низкой интенсивности и антибиотиков далацина и цефтриаксона.

В результате ЭМИ когерентными волнами низкой интенсивности при частотах 51.8 ГГц и 53.0 ГГц, изменялись также ионные потоки в мембранах, способствуя значительному усилению выхода H^+ ионов (JH^+) из клетки, соответственно в 2.8 и 3.2 раз, и уменьшению а потоков K^+ (JK^+) в клетку соответственно в 2.8 и 6 раз. По результатам опытов, проведенных с антибиотиками, становится ясно, что совмещение ЭМИ крайне высоких частот и антибиотиков усиливает влияние на JH^+ и JK^+ у *E. hirae*. Влияние использованных антибиотиков на JK^+ свидетельствует о том, что существует прямое влияние на TrkA систему или же они действуют на связь F_OF_1- АТФазы с этой системой. АТФазная активность E. hirae была оценена как в виде суммарной активности, так и в присутствии ДЦКД. Было доказано, что АТФазная активность мембранных везикул снижается соответственно в 1.2 и 3.5 раз.

В результате исследований также была оценена жизнеспособность *E. hirae* в течение 4 дней в идентичных условиях. Очевидно, что действие выше упомянутых

факторов выявляются не только в процессе роста бактерий, но и их выживаемости. Выживаемость сокращается, когда бактериальная суспензия обрабатывается вышеназванными факторами.

Были получены новые результаты, связанные с аэробными бактериями *Pseudomonas putida* и *Erwinia herbicola*. В частности, было показано, что их дыхательная активность или скорость поглощения растворенного кислорода, которая была оценена в разных фазах их роста, и в условиях разных физических и химических факторов - тепловых и осмосных шоков, ультразвука, электромагнитного излучения напрямую связаны с числом жизнеспособных бактериальных клеток, существующих в среде. Для выявления прямой связи между выживаемостью и поглощением кислорода, как было отмечено, было изучено действие разных физических факторов. В частности, после воздействия ультразвука, скорость поглощения кислорода после 10 с уменьшилась на 20 %, а через 60 с - поглощение почти исчезло. Тепловой и осмотический шоки также приводили к резкому сокращению скорости поглощения кислорода.

Итак, был предложен быстрый и эффективный метод определения выживаемости этих бактерий, основанный на определении поглощения кислорода бактериями.

Обобщая всю работу, можно отметить, что в результате проведенных исследований на бактериях были показаны результаты отдельного и совмещенного влияния физических и химических факторов на бактерии, на основе чего, для оценки роста и жизнедеятельности бактерий, были предложены четкие подходы и методы, которые максимально описывают влияние того или иного фактора.

Полученные результаты могут найти применение как в промышленности и биотехнологии, так и в медицине.