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կենսաբանական գիտությունների թեկնածուի
գիտական աստիճանի հայցման ատենախոսության

ՍԵՂՄԱԳԻՐ

ԵՐԵՎԱՆ-2015

MINISTRY OF SCIENCE AND EDUCATION OF RA
YEREVAN STATE UNIVERISTY

SOGHOMONYAN DIANA RAZMIK

THE EFFECTS OF PHISICHAL AND OXIDOREDUCTION FACTORS ON GROWTH AND MEMBRANE ACTIVITY OF LACTIC ACID BACTERIA

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-2015

Ատենախոսության թեման հաստատվել է Երևանի պետական համալսարանում:

Գիտական ղեկավար՝	ՀՀ ԳԱԱ թղթակից անդամ, կենս. գիտ. դոկտոր, պրոֆեսոր Ա. Հ. Թռչունյան
Պաշտոնական ընդդիմախոսներ՝	ֆիզմաթ. գիտ. դոկտոր, պրոֆ. Վ.Բ. Առաքելյան, կենս. գիտ. դոկտոր Ա.Զ. Փեփոյան
Առաջատար կազմակերպություն՝	ՀՀ առողջապահության նախարարության ճառագայթային բժշկության և այրվածքների գիտական կենտրոն, Երևան

Ատենախոսության պաշտպանությունը տեղի կունենա 2015թ. հունիսի 16 –ին, ժամը 14:00-ին, Երևանի պետական համալսարանում գործող ՀՀ ԲՈՀ-ի 051 Կենսաֆիզիկայի մասնագիտական խորհրդի նիստում (0025, Երևան, Ալեք Մանուկյան փ. 1, ԵՊՀ, կենսաբանության ֆակուլտետ):

Ատենախոսությանը կարելի է ծանոթանալ Երևանի պետական համալսարանի գրադարանում:
Ատենախոսության սեղմագիրն առաքված է 2015թ. մայիսի 15–ին:

051 մասնագիտական խորհրդի գիտ. քարտուղար,
կենս. գիտ. թեկնածու, դոցենտ Մ.Ա. Փարսադանյան

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

Academic supervisor:	Corresponding Member of NAS RA, Dr. of Biological Sciences, Professor A. Trchounian
Official opponents:	Dr. of Physico-Mathematical Sciences, Professor V.B. Arakelyan Dr. of Biological Sciences A. Z. Pepoyan
Leading organization:	Research Center for Radiation Medicine and Burns, Ministry of Health RA, Yerevan

The defense of the dissertation will be held on 16th June, 2015, at 14:00, at the session of 051 Scientific Specialized Council on Biophysics of HAC of RA at Yerevan State University (0025, Yerevan, Alex Manoogian str. 1, YSU, Faculty of Biology).

The dissertation is available at the library of Yerevan State University.
The synopsis has been sent on 15th May , 2015.

Scientific Secretary of 051 Specialized Council,
PhD, Associate Professor M. A. Parsadanyan

INTRODUCTION

Topic's significance. Lactic acid bacteria (LAB) are heterogeneous group of gram-positive and catalase-negative microorganisms. They can synthesize lactic acid from lactose. Due to some metabolic properties, LAB were extensively contributed for different processes such as flavor development and ripening of fermented products (Soghomonyan and Trchounian, 2013). They produce a large number of antimicrobial compounds that are very important for food fermentation and preservation. Therefore, LAB are used as bio-preservative agents giving them great economic significance (Topisirovic et al., 2007). Moreover, they are important inhabitants of the gastrointestinal tract and some species are considered to have probiotic properties offering a number of benefits for health and well-being. One of the well-known species of this group is *Lactobacillus acidophilus*, which remains as the most widely recognized and commercially distributed probiotic culture, characterized as a homo-fermentative, gram-positive rod that grows optimally from 37 to 42° C (Altermann et al., 2005), which can overcome acid and bile barrier of stomach and intestine, respectively, and then beneficially affect host by improving its intestinal microbial balance (Kajfasz et al., 2011). There are many physicochemical and physical factors that can affect microorganism's growth, survival and membrane processes. The oxidation-reduction potential (ORP) is one of the most important parameter characterizing the state of bacterial growth medium and bacterial cells. LAB grown in anaerobic conditions possesses sugar (glucose) fermentation with the releasing of lactic acid and other organic acids. This process entails medium acidification and alternation of proton gradient, resulting the drop of medium pH, alteration of proton permeability, or activity of transport and enzymatic systems of the cell membrane (Vassilian and Trchounian, 2008; Soghomonyan et al., 2011). ORP and pH alteration studies in various conditions and the effects of oxidizers and reducers on the growth parameters of LAB and the processes linked with H^+ transfer are important for understanding of growth regulation mechanisms, evaluation of functional processes in the intestine of animals and humans, and for application of these bacteria for technological purposes(Vassilian and Trchounian, 2008). The bacterial effects of the extremely high frequency electromagnetic irradiation (EMI) with low (low-energy) intensity and with non-thermal action are interesting because of extremely high frequency EMI is widely applied in therapeutic practice, food and wine preservation (Geveke et al., 2009; Zhand et al., 2010). This EMI is also used by low-orbital systems of cosmic communication and different elements of mobile and, moreover, bacteria and the other cells can interact with each other through EMI (Trushin, 2003; Reguera, 2011). In the environment and different applications, small and very small doses of this EMI at the frequencies of 51.8 and 53 GHz have been determined to affect the growth and living properties of different bacteria, namely *Escherichia coli* (Tadevosyan et al., 2007; Torgomyan et al., 2011), *Enterococcus hirae* (Ohanyan et al., 2008; Torgomyan et al., 2012),

etc. Among cellular targets are water molecules, plasma membrane, probably the H^+-translocating F_0F_1-ATPase, the key enzyme in the membrane, and genome. These targets are considered as common ones for EMI effects on bacteria (Torgomyan and Trchounian, 2013), but exact primary targets and detailed mechanisms of the effects are not clear yet. Moreover, the specific mechanisms of induction of biological effects in LAB by these frequencies should be studied. The β-lactam natural antibiotics (cephalosporins, penicillins) are the largest group of antimicrobial drugs and have a leading role in the treatment of infectious diseases (Drawz and Bonomo, 2010), but antibiotics, as an external chemical factors, left their negative imprint on the life of organisms. Therefore, it is remarkable to investigate the effects of redox factors (oxidizers and reducers) as well as separate and combined effects of EMI and ceftazidime (cephalosporin, antibiotic of third generation) on LAB growth properties and membrane activity.

Research goals and tasks. The aim of this study was to investigate the separate and combined effects of physical (high frequency EMI) factor and antibiotic, and redox (oxidizers, reducers) factors on LAB growth survival and membrane activity.

Constituted tasks of the research were to:

1. Investigate the effects of oxidizers and reducers on *Lactobacillus acidophilus* and *Lactobacillus salivarius* growth rate and redox - oxidation/reduction potential (ORP) kinetics, as well as on bacterial membrane functional activity (H^+/K^+ exchange, membrane permeability).
2. Investigate the effects of coherent, extremely high frequency EMI, at the frequencies 51.8 and 53 GHz, on *L. acidophilus* growth parameters, such as growth rate, and survival (in minimal and rich, or solid and liquid media).
3. Observe the separate and combined effects of high frequency EMI and ceftazidime on antibiotic susceptibility and growth parameters.
4. Determine the separate and combined effects of EMI and ceftazidime on redox potential kinetics and changes of H^+ fluxes through bacterial membrane.
5. Reveal the separate and combined effects of EMI and ceftazidime on membrane vesicles ATPase activity dependence on pH of growth (pH 4.0 and pH 6.5) and assay (pH 4.0 and pH 6.5) media.
6. Explore the separate and combined effects of high frequency EMI and ceftazidime on survival and antibiotic susceptibility of *L. acidophilus* using *in vitro* model of human gastrointestinal tract.

Scientific novelty and applied value of the study. Within the scopes of this work it was established that different physical and redox factors affected the growth properties and membrane activity of LAB. Oxidizer ferrycianide and reducer DTT changed the dynamics of ORP during growth and affect the ion transport systems, moreover, they suppressed growth rate of *L. salivarius*, and this suppression depends on their concentration (Soghomonyan, et al., 2011). EMI at the frequencies 51.8 GHz and 53 GHz are suppressed *L.*

acidophilus growth and survival (Soghmonyan and Trchounian, 2013). This is evidenced by the differences between bacterial colony numbers, which were irradiated on solid or liquid media. It has been also shown that as a result of exposure the LAB sensitivity to ceftazidime was increased; ion fluxes and ATPase activity were changed. There was also an increase in sensitivity of irradiated bacteria not only to antibiotics, but also to other chemical substances (DCCD). The F_0F_1-ATPase complex, which is vital for LAB, could be considered as a target for EMI. It was shown the decreased number of irradiated *L. acidophilus* in *in vitro* model of gastrointestinal tract depending on growth medium pH and digest ferments and antibiotic (the antibiotic susceptibility was increasing). The investigation of oxidizers and reducers effects on LAB gives an opportunity to highlight cellular mechanisms of the impact and makes it possible to control processes of bacterial growth in dairy industry. Moreover, the study of different frequencies of EMI allows finding out the most effective frequencies in order to increase their application in food industry, agriculture and medicine. The increasing antibiotic susceptibility reduces the antibiotic resistance threshold and therefore reduces the gene transfer risk in gut medium. It should be noted that this frequencies of EMI have no thermal effects; therefore their application in food industry will not affect the organoleptic properties of food.

Main points to present at defense:

1. The effects of oxidizers and reducers on ORP dynamics during growth and on membrane activity (membrane permeability, H^+ and K^+ fluxes) of LAB.
2. The effects of low intensity EMI at the frequencies 51.8 GHz and 53 GHz on LAB growth parameters, membrane activity as well as antibiotic susceptibility.
3. The survival of irradiated LAB in *in vitro* model of the gastrointestinal tract, displaying medium pH dependence as well as digestive enzymes and antibiotic susceptibility.

Work approbation. The main results of the dissertation were discussed at Scientific seminars at Departments of Microbiology, Plants and Microbes Biotechnology and Department of Biophysics, Faculty of Biology, Yerevan State University; at scientific conferences: 14th European Bioenergetics conference (Moscow, Russia, 2006), 14th International Young Scientists Conference on Biology (Pushchino, Russia, 2010), 6th International Congress "Low and Super-low Fields and Radiations in Biology and Medicine" (Saint-Petersburg, Russia, 2012), ISTC International conference ''Radiation Safety Challenges in the 21st Century'' (Yerevan, Armenia, 2012), International Student, Postgraduate and Young Scientists Conference ''Lomonosov-2013'' (Moscow, Russia, 2012), 114th General Meeting of American Society for Microbiology, (Boston, USA, 2014), International Summer School for Young Scientists "Enhancing Health Promoting Compounds in Food" (Bazelati, Georgia, 2014), International Scientific Workshop "Trends in Microbiology and Microbial Biotechnology" (Yerevan, Armenia, 2014).

Publications. According to experimental data discussed in the dissertation, 13 works have been published, including 5 research articles, 3 of which in peer-reviewed journals, and 3 proceedings.

Volume and structure of dissertation. The dissertation contains following chapters: introduction, literature review (Chapter 1), experimental part (Chapter 2), results and discussion (Chapter 3), concluding remarks, conclusions and cited literature (total 176 papers and books). The document consists of 123 pages including 3 tables and 22 figures.

MATERIALS AND METHODS

Bacterial strains and their growth conditions. *L. acidophilus* VKMB-1660 wild type (Microbial Depositary Center Armenia, 11227), *L. salivarius* 1588, *L. salivarrius* 3823, *Lactococcus lactis* 3690 and *L. acidophilus* 101E (Armenian National Agrarian University, Yerevan) strains were used for experiments. These bacteria were grown in MRS broth and in adapted peptone medium (pH 6.5). The bacteria were grown at 37° C until stationary growth phase (20–24 h) under anaerobic conditions upon fermentation of glucose (20 g/l). Cells grown were concentrated by centrifugation (3,600 g) during 15 min, washed and diluted in bi-distilled water. Then, the bacterial suspension (at concentration of 10^7–10^8 colony-forming units (CFU)/ml) was transferred into the plastic plate (Petri dish) with suspension thickness of ~1 mm for subsequent irradiation or was assayed. Solid nutrient medium was prepared by the addition of agar (1.5 %) to MRS broth.

Electromagnetic irradiation of bacteria. The irradiation of bacterial suspension was performed by EMI generator; model G4-14, the coherent in time electromagnetic waves with the frequencies of 51.8 GHz and 53 GHz or millimeter waves of the 5.79 and 5.66 mm wavelengths in the option of amplitude modulation with frequency of 1 Hz (frequency stability was 0.05 %); the flux capacity was of 0.06 mW/cm^2 and this flux have no effect on the temperature of bacterial suspension during irradiation: the change in temperature of suspension was below 0.1° C during the exposure. The generator was supplied by Dr. V. Kalantaryan (Yerevan State University). After direct irradiation of bacterial suspension for 1 h, cells were immediately transferred into the fresh growth medium or assay mixture. In some experiments, when mentioned, the bacterial colonies on solid surface (nutrient medium in Petri plates) were irradiated for 1 h. Note, 1 h exposition has been shown to be effective for marked effects of low-intensity EMI on *E. coli* and the other bacteria (Tadevosyan and Trchounian, 2007; Ohanyan et al., 2008).

Bacterial growth and survival determination. Bacterial growth was monitored spectrophotometrically, via optical density (OD_{650}). The specific growth rate was determined by division of 0.693 (ln2) on OD doubling time and expressed as h^{-1}. For bacterial growth yield, colonies (CFU) number was calculated. Bacterial survival was evaluated by displacement of control and irradiated bacteria into minimal salt medium (46 mM K_2HPO_4; 23mM KH_2PO_4; 8 mM $(NH_4)_2SO_4$; 0.4 mM $FeSO_4$; and 6 mM $MgSO_4$; pH 6.5)

during 3 days. The survival was assessed by counting of CFU. Before inoculation of bacteria the 16-20 μM ceftazidime solution was added into the salt medium (Soghomonyan, Trchounian 2013).

Determination of antibiotic susceptibility. Antibiotic susceptibility for bacteria was studied by employing the method described by Bauer et al. (1966). This was a standardized antibiotic disk agar diffusion zone (halos) determination, which was giving a quantified parameter (zone linear sizes) of growth inhibition for antibiotics.

Determination of ORP kinetics of bacteria and H^+ transport assay. ORP was measured using pair of platinum (Pt) and titanium-silicate (Ti-Si) redox and/or combined ORP electrodes. The irradiated bacterial suspension (1 %) was inoculated into MRS broth. The antibiotic, or oxidizer and reducer added into the medium before inoculation. ORP changes during the growth were measured until stationary growth phase. The H^+ fluxes across bacterial membranes of whole cells were determined using appropriate selective electrode. The fluxes were expressed as mMol H^+ /min per mg dry weight of bacteria (DW). During the assays bacteria were incubated with ceftazidime (16 μM) and/or *N,N'*-dicyclohexylcarbodiimide DCCD (0.1 mM) for 7–10 min. DCCD-sensitive values were determined as differences between the values in the presence and the absence of DCCD in parallel assays (Soghomonyan et al., 2011; Soghomonyan, Trchounian, 2013).

Isolation of membrane vesicles. Right side out membrane vesicles were isolated by lysis of protoplasts with lyzosime using the Konings and Kabak method (Konings and Kaback, 1973):

ATPase activity. ATPase activity of membrane vesicles was measured by amount of liberated inorganic phosphate (P_i) after addition of 5 mM ATP, that was determined by the method of Taussky and Shorr (1953). Relative ATPase activity was expressed in nmol P_i per mg protein in 1 min. Membrane vesicles were incubated with ceftazidime (16-20 μM) and/or DCCD (0.1-0.2 mM) for 10 min.

Determination of membrane H^+ conductance. The membrane H^+ conductance (C_m^{H+}) was evaluated by registration of H^+ flux through the membrane up to achievement of electrochemical balance in the H^+ distribution on both sides of membrane by addition of small amounts of HCl (so-called "acid pulse" technique) (Akopyan and Trchounian, 2006). The H^+ flux was measured using a pH-meter with selective electrode. The membrane H^+ conductance was expressed in μM transferred H^+ per time (s) per unit of pH and DW.

Determination of survival of irradiated bacteria in *in vitro* model of gastrointestinal tract. The irradiated bacteria were passed through 6 tubes of MRS media with different pH values (7.5; 2.0; 3.0; 4.0; 5.0; 8.0 and 6.5 as a control), appropriate digest enzymes (lysozyme, pepsin, pancreatin) and bile salts. Ceftazidime was added in each tube with 20 μM final concentration. The viable counts were determined when irradiated bacteria incubated in 6 tubes for 0 min., 3 min., 20 min., 60 min. (Chen et al., 2009; Movsesyan et al., 2010), and

then the bacterial suspension was disseminated on MRS agar in Petri dishes, after appropriate dilutions.

Data processing. The average data are presented from 3 independent measurements; standard errors were not more 3 % if not indicated. The Student's validity criteria (p) were calculated to show the validity of difference between changed values and control.

RESULTS AND DISCUSSION

The redox potential changes during growth of LAB. The study of ORP dynamics during growth of *L. salivarius* shows, that the acidification by 1–1.5 units of initial pH (data not shown) and simultaneous decrease of ORP until low negative values (E_h, registered using platinum electrode) was observed in stationary growth phase, in growth medium (Soghomonyan et al., 2011). This decrease of E_h during growth, for instance *L. salivarius* 1588, occurred from positive (+50 ± 7 mV) in the beginning of log-phase until negative (–565 ± 23 mV) values (Fig. 1) with transition of culture to stationary phase (10–12h). The link between alterations of these parameters in bacterial media is polysemantic although the theory of oxidation-reduction processes suggests variability of pH and ORP alteration (Bagramyan et al., 2002). Dynamics of alterations of these values indicates the importance of reduction conditions for bacterial growth and supposes that decrease of ORP determines the growth of culture and its further transition to stationary phase. The value of E_h insufficiently increases (by 125 ± 12 mV) in the late stationary phase (over 22–24 h) that indicates acceleration of oxidative processes in culture. The use of a pair of oxidation-reduction electrodes platinum and titanium-silicate was remarkable. The decrease of ORP registered using Ti-Si-electrode in *L. salivarius* occurring with lower rate (Fig. 1); significant deviation in values of ORP during logarithmic growth phase has been observed. This difference was registered also in the presence of DTT (Fig.1). Oxidizer ferrycianide poorly permeating into the cell and supporting the positive values of ORP (+280 ±10 mV, Fig. 1) influenced on the growth of *L. salivarius* increasing almost by two times the duration of lag phase and decreasing of growth rate (Fig. 1). This influence depended on concentration of oxidizer within the range from 1 to 5 mM (Fig.2). Acidification of the medium increased and the drop of ORP until negative values (–490 ± 10 mV) was observed later during the logarithmic growth phase. DTT is known as the reducer of thiol groups of proteins (Kirakosyan et al., 2004; Poladyan et al., 2006). This reagent decreasing ORP until negative values (–160 mV at 1 mM DTT, Fig. 1) influenced bacterial growth by decreasing growth rate. The latter was depending on the concentration of DTT (from 1 to 5 mM, Fig. 2). This effect was observed at pH 6.5 and did not differ from DTT effect on the growth parameters of *E. coli* or other bacteria (Kirakosyan et al., 2004; Poladyan et al., 2006). Thus, ORP decreases until low negative values by stationary growth phase of LAB but the nature of this is not clear.

Influence of oxidizer and reducer on ions transport across bacterial membrane. LAB in experimental conditions in the medium contained glucose realizing H^+–K^+ exchange (Fig. 3). Ferrycianide decreased and DTT, in contrast, increased the H^+ and K^+ fluxes in *L. salivarius* (Fig. 3). The influence of these compounds can be explained as a result of direct simultaneous effect of the reagent onto one or both transport systems or indirectly by the effect on the state of thiol groups in these proteins by changing dithiol-disulfide channels

between them (Soghomonyan et al., 2011). There is probably an opportunity for conformational changes in proteins due to alteration of ORP value (Fig. 1) or other alterations resulting in shifts in C_mH^+ (Fig. 4) and $\Delta\mu H^+$ (data not shown).

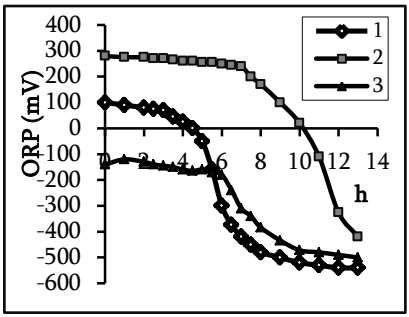

Fig.1. Decrease of ORP registered using platinum electrode (E_h) during anaerobic growth of *L. salivarius* 1588: 1-control without ferrycianide or DTT; 2-in presence of 1 mM ferrycianide; 3-in presence of 1 mM DTT. Ferrycianide or DTT added in growth medium

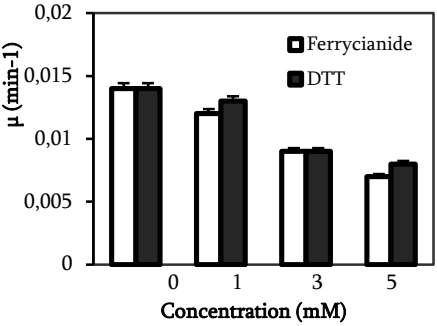

Fig. 2. Alteration of growth rate (μ) of *L. salivarius* 1588 in presence of ferrycianide or DTT in different concentrations: Bacteria were grown anaerobically, at pH 6.5, at fermentation of glucose.

Alteration of membrane permeability during growth of bacteria in different conditions. C_mH^+ of LAB can serve as indicator of membrane state. This parameter plays a role in the processes of energy transformation related with H^+ transport. It considerably changes during growth of *L. acidophilus* and *L. salivarius* (Fig. 4), and other LAB in media with different pHs. The values of C_mH^+ are different in various LAB and their strains: in *L. acidophilus* 101 is higher by 1.3–1.6 fold than in *L. salivarius* 1588 depending on pH (Soghomonyan et al., 2011).

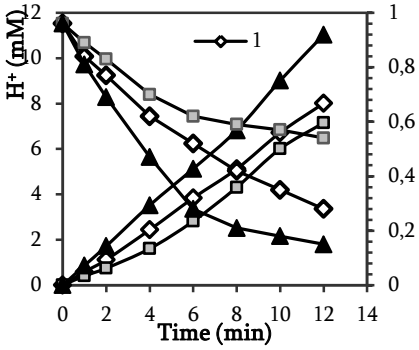

Fig. 3. Kinetics of H^+ release (a) and K+ uptake (b) by bacteria *L. salivarius* 1588 in the presence of 1 mM ferrycianide, 1 mM DTT. Glucose concentration-20 mM. Concentration of bacteria was 10^9 cells/ml.

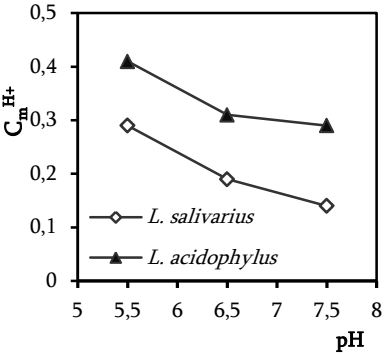

Fig. 4. C_mH^+ *L. acidophilus* 101 and *L. salivarius* 1588 grown in the media with different pHs. Y-axis is C_mH^+, μmol H^+/mg DW/unit pH. Other conditions are similar to Fig.3.

The value increased by ~1.4–2.1 fold (dependent on bacterial species) at pH decrease from slightly alkaline to acidic (Fig. 4) that was shown for other bacteria (Akopyan et al., 2002). Different values of C_m^{H+} can be related to alteration of membrane lipid and protein composition during growth of bacteria in different media (Yohannes et al., 2004). Alteration of C_m^{H+} correlated with ORP drop.

Comparable antibacterial effects of EMI and ceftazidime. The *L. acidophilus* colony number (Fig. 5a) and specific growth rate (μ) (Fig. 6) decreased after low-intensity EMI with the frequencies of 51.8 GHz and 53 GHz. In both irradiated suspension and irradiated bacteria on solid surface, the colony number changed markedly (see Fig. 5b). However, some differences between antibacterial effects for EMI were established (Fig. 5). This seems invteresting, since the organization of bacterial population, cell-to-cell interaction for colonies on solid surface and cells in suspension were different, so a further study is required. The results indicate EMI strong antibacterial effects on *L. acidophilus* for revealing EMI action on antibiotic susceptibility of *L. acidophilus* irradiated bacteria were exposed to influence of ceftazidime. The latter is known as inhibitor of cell wall synthesis (Ocana et al., 2006). This antibiotic in minimal inhibitory concentration (16 μM) affected *L. acidophilus* specific growth rate (Fig. 6). It was shown that 51.8 GHz and 53 GHz EMI effects were comparable with the ceftazidime inhibitory effects (Fig. 5b). Moreover, in cases of antibiotic susceptibility after EMI in irradiated suspension and irradiated dishes the diameter of halos increased for both frequencies ~1.7–2.5- folds (Fig.5b).

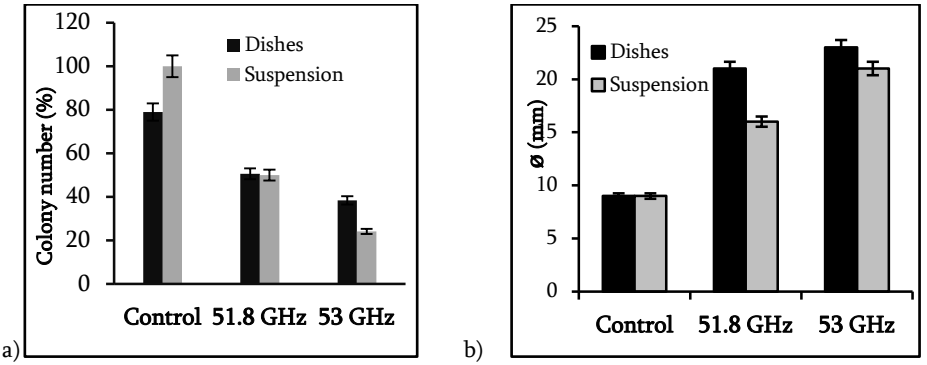

Fig. 5. The effects of 51.8 and 53 GHz frequency EMI on *L. acidophilus* number of colonies (a) and halo (**ø**) diameter for ceftazidime (b). Bacteria were irradiated in suspension (1) and on Petri dishes (2). The both controls were without EMI. The number of replicates was 3. The changes at the 51.8 GHz and 53 GHz were valid if compared with the appropriate controls ($p<0.001$).

This might indicate EMI-enhanced effect of ceftazidime on bacteria. The enhancing effects of EMI were observed with *L. acidophilus* survival in minimal salt medium after EMI and antibiotic action. For the 2nd and 3rd running days the differences in survival between the cells affected by 51.8 GHz and 53 GHz EMI and ceftazidime were seen clearly: EMI-

enhanced separate and combined effects of EMI and ceftazidime were established (Fig. 7). These results demonstrate decreasing colony number and cell growth properties, as well as bacterial survival after irradiation of bacteria by EMI at the frequencies 51.8 and 53 GHz. They indicate EMI strong antibacterial effects on *L. acidophilus*.

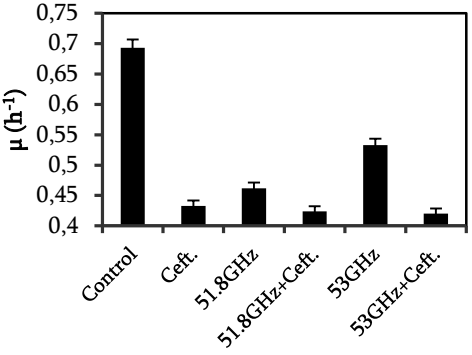

Fig. 6. *L. acidophilus* growth specific rate, in the control and after EMI of 51.8 and 53 GHz frequencies and ceftazidime. The antibiotic with glucose was added into thegrowth medium before inoculation. The changes at the 51.8 and 53 GHz and/or upon ceftazidime action were valid if compared with the control ($p<0.001$)

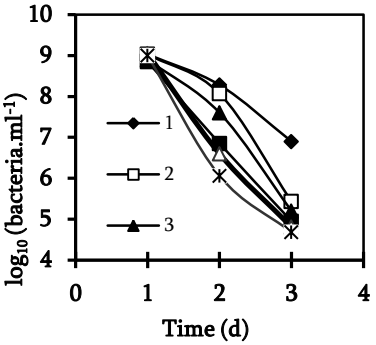

Fig. 7. The effects of high frequency EMI in combination with ceftazidime on *L. acidophilus* survival. Bacteria were held in the minimal salt medium during 3 days. 1-control, 2-ceft., 3-51.8 GHz, 4-51.8+ceft., 5-53 GHz, 6-53 GHz+ceft.

EMI and ceftazidime effects on ORP kinetics and H^+ fluxes across bacterial membrane. To reveal cellular targets and action mechanisms of EMI and ceftazidime effects on *L. acidophilus*, ORP kinetics and H^+ fluxes across the membrane were studied. ORP has been shown to be a determinant for *E. coli* and other bacterial growth under anaerobic conditions (Bagramyan, Trchounian, 1997; Soghomonyan et al., 2011). It had effects on H^+ fluxes across the membrane and membrane-associated enzymes activity. Indeed, ORP decreased up to -180±10 or -190±10 mV in 12 h and then increased to -160±5 mV in 24 h; the kinetics of ORP by *L. acidophilus* was changed by 51.8 GHz and 53 GHz EMI and ceftazidime (Fig. 8a). These changes were stronger by combined effects of EMI and ceftazidime, especially up to 12 h of growth (Fig. 8b). This might be indicating determinant effects of ORP in *L. acidophilus* when bacteria are entering the stationary growth phase. Moreover, 51.8 GHz and 53 GHz EMI did not change overall energy (glucose)-dependent H^+ effluxes across the *L. acidophilus* membrane but it increased DCCD-inhibited H^+ efflux (Fig. 9). In contrast, this EMI in combination with ceftazidime decreased DCCD-sensitive H^+ effluxes. These findings indicate that the F_0F_1 -ATPase, for which DCCD is specific inhibitor (Azzi et al., 1983; Trchounian, 2004) might be a target for EMI and ceftazidime. The finding is novel for LAB and similar to the effects on *E. coli* (Tadevosyan et al., 2008; Torgomyan et al., 2011) and the other bacteria (Ohanyan et al., 2008; Torgomyan et al., 2012). These results points out universal mechanisms of EMI effect for different bacteria. It

is interesting that EMI antibacterial action on *L. acidophilus* growth is comparable with ceftazidime inhibitory effect. This might be due to similar cellular mechanisms of EMI and

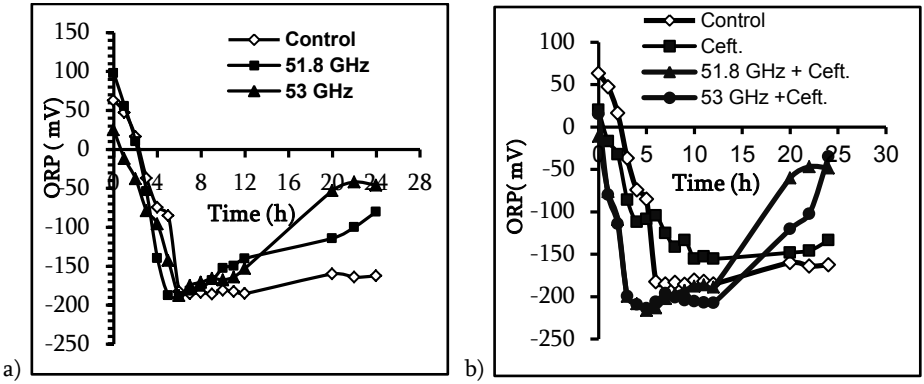

Fig. 8. The kinetics of *L. acidophilus* ORP after EMI of 51.8 and 53 GHz frequencies(a) and ceftazidime(b). Ceftazidime (16-20 μM) added into growth media, immediately before inoculation.

ceftazidime although different enhanced effects have been determined with *E. coli* and other bacteria and different antibiotics (Lee et al., 2009; Ohanyan, 2012). But, combination of EMI and ceftazidime enhanced the effects on *L. acidophilus* viability (Fig. 7).

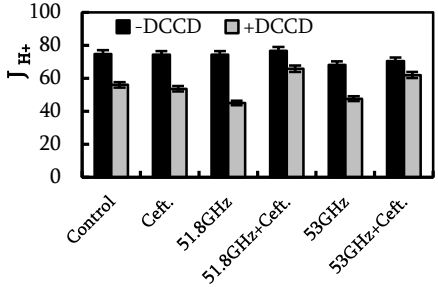

Fig. 9. The changes in energy-dependent total and DCCD-sensitive H^+ effluxes (J_{H+}) through the *L. acidophilus* membrane after EMI combined with ceftazidime. 1--DCCD, 2-+DCCD The changes in DCCD-sensitive fluxes were valid if compared with the appropriate controls ($p<0.01$).

These results might be explained by a proposal that survival requirements are different from growth mechanisms for *L. acidophilus* since different properties of LAB are discussed (Altermann et al., 2005; Jeanson et al., 2009). Among action targets and cellular mechanisms of extremely high frequency EMI and antibiotics effects on bacteria, changes of membrane properties of the cell are discussed in details (Torgomyan and Trchounian, 2013). The changed kinetics of ORP during bacterial growth (Fig. 8) and increased DCCD-inhibited H^+ efflux through the membrane by the F_0F_1-ATPase (Fig. 9) were revealed at the first time. They might confirm a target role of the F_0F_1-ATPase and a significance of membranous mechanisms in antibacterial effects of EMI and ceftazidime on *L. acidophilus*. This is similar to the proposal about enhanced effects of EMI and antibiotics on bacteria that include the F_0F_1-ATPase (Torgomyan and Trchounian, 2012; 2013).

The effects of EMI and ceftazidime on *L. acidophilus* membrane vesicles ATPase activity at pHs 6.5 and 4.0. 51.8 GHz and 53 GHz EMI didn't change overall energy (glucose)-dependent H^+ effluxes across the membrane, but it increased DCCD-inhibited H^+ efflux. In contrast to this, EMI in combination with ceftazidime decreased DCCD-sensitive H^+ effluxes. So it was suggested that the F_0F_1-ATPase might be a target for EMI and ceftazidime. Indeed, EMI at the frequencies 51.8 and 53 GHz suppressed the overall ATPase activity of *L. acidophilus* membrane vesicles ~1.3 fold and 2.6 fold, respectively (Fig. 10). The suppression was more considerable in the presence of 0.01 mM DCCD ~2.18 fold and 4.4 fold, respectively (Fig. 10). In case of combination EMI at both frequencies with ceftazidime a significant decrease in DCCD-inhibited ATPase activity of *L. acidophilus:* membrane vesicles after exposure of bacteria with EMI at the frequencies of 51.8 GHz or 53 GHz was determined in the absence and in the presence of ceftazidime. These data confirm a target role of F_0F_1 in bacterial action of EMI. Moreover, EMI at the frequency 53 GHz was stronger to suppress the DCCD- inhibited ATPase activity (Table 1). In case of combination EMI with 20μM ceftazidime, the inhibition of ATPase activity was much more expressed, especially in the presence of DCCD ~3.3 fold and 7.5 fold, respectively (Fig. 10). The results obtained in the presence of ceftazidime indicated that EMI enhanced the effect of ceftazidime. The changes in the F_0F_1-ATPase activity point out the membranous effects of low intensity extremely high frequency EMI on bacteria (Bulgakova et al., 1996).

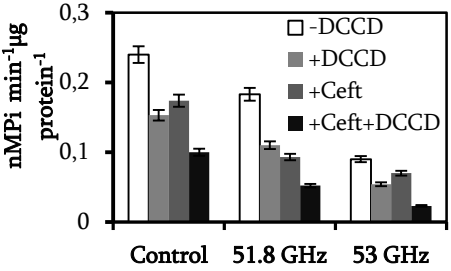

Fig.10. The separate and combined effects of EMI and ceftazidime (20μM) on overall ATPase activity of *L. acidophilus* membrane vesicles in presence and absence of 0.1mM DCCD.

They are similar to those with other bacteria as well as to the effects on lipid bilayers changing membrane permeability. Thus, in addition to inhibitory effects on *L. acidophilus* growth and survival and H^+ fluxes across the membrane (Soghomonyan et al, 2013), EMI at 51.8 GHz and 53 GHz frequencies enforced the influence of ceftazidime on DCCD-inhibited ATPase activity. It might be suggested that EMI combined with ceftazidime can cause conformational changes in F_0F_1 and lead to decrease in its activity. These effects are scientifically interesting and could be applied in biotechnology, when LAB are used in different ways. As shown before, the membrane-associated multi-subunit F_oF_1-ATPase, which links the production of ATP to the proton motive force (Paul and Colin, 2003; Skulachev et al., 2010), is an important element in response and tolerance to low pH in some bacteria through the controlling H^+ concentration between the cell cytoplasm and external medium (Martin-Galiano et al., 2005; Skulachev et al., 2010). In this work we

investigated ATPase activity of *L. acidophilus,* irradiated with EMI at frequencies 51.8 GHz and 53 GHz and in the presence of ceftazidime at low pH. The ATPase activity was assayed in two cases - when bacteria were grown at pH 4.0 or pH 6.5; but the assayed pH was

Ceftazidime (20μM)	ATPase activity (nM P_i/min/μg protein)		
	Control	51.8 GHz	53 GHz
-	43.5+2.2	36.5±2.0	18.0±1.1
+	37.0±2.0	20.5±1.3	22.5±1.4

Table 1. DCCD sensitive ATPase activity of *L. acidophilus* membrane vesicles under EMI with 51.8 and 53 GHz frequencies and in the presence of ceftazidime.

adjusted to 4.0. As shown in Fig. 11, EMI at both frequencies mentioned stimulated ATPase activity of membrane vesicles from *L. acidophilus* grown at pH 4.0 and 6.5 and assayed at pH 4.0. But when bacteria were grown at pH 4.0 ATPase activity was higher ~47 % than ATPase activity of bacteria which were grown at pH 6.5 (Fig. 11). When the assayed pH was adjusted to 6.5 EMI at both frequencies ATPase activity of membrane vesicles from *L. acidophilus* grown at pH 4.0 was suppressed ~1.2-1.8 folds (Soghomonyan et al., 2014) as it was shown before (Fig.10) for LAB grown at pH 6.5 (Soghomonyan, 2013), but at 53 GHz frequency it inhibited less than when grown at pH 4.0 (Fig. 11b).

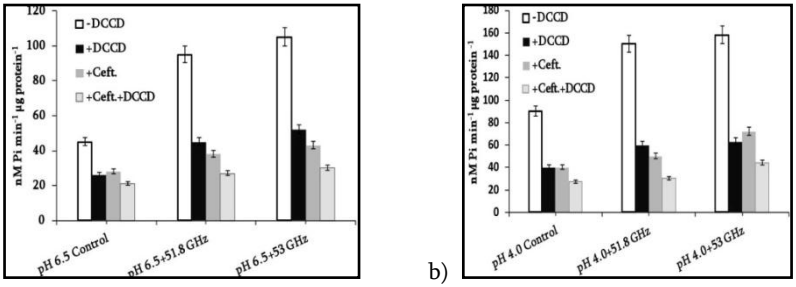

Fig. 11. The effects of 51.8 and 53 GHz frequencies EMI and DCCD on overall ATPase activity of *L. acidophilus* membrane vesicles in presence and absence of ceftazididme (20 μM). Control was without irradiation; 0.2 mM DCCD was added. pH of assay mixture is 4.0. pHs of growth media were 4.0 (b) and 6.5(a).

Either with pH 4.0 or pH 6.5 of growth media and the assays, 0.2 mM DCCD inhibited ATPase activity of membrane vesicles of non-irradiated (control) and irradiated cells and this inhibition was much more expressed in the presence of 20 μM ceftazidime. A significant decrease in DCCD-inhibited ATPase activity after exposure of bacteria with EMI in the frequencies of 51.8 GHz or 53 GHz was determined in the absence and in the presence of ceftazidime (Tabl. 2). The DCCD-inhibited ATPase activity was increased, when the assayed pH was adjusted to 4.0 and in the absence of ceftazidime (Tabl. 2).

However, the combination of EMI and ceftazidime suppressed the DCCD-sensitive ATPase activity. Thus, EMI with extremely high frequency stimulated the DCCD sensitive ATPase activity of membrane vesicles of *L. acidophilus* grown at acidic pH. F_0F_1-ATPase, a key enzyme of bacterial membrane, might be a target for EMI. These results are in accordance with the literature data (Chen et al., 2009; Kashket et al., 1987; Kobayashi et al., 1984).

Table 2. DCCD-sensitive ATPase activity of *L. acidophilus* membrane vesicles under 51.8 and 53 GHz frequencies EMI and in the presence of ceftazidime. pH of growth media 4.0 and 6.5 and assay pH 4.0 and 6.5.

pH of growth medium	Assay pH	ATPase activity (nM P_i/min/μg protein)	
		-ceftazidime	+ceftazidime
pH 4.0 control	pH 6.5	38.0±1.30	19.0±1.03
pH 4.0 + 51.8 GHz		50.0±1.60	23.0±1.10
pH 4.0 + 53 GHz		35.0±1.04	25.0±1.12
pH 4.0 control	pH 4.0	40.0±1.21	13.0±0.60
pH 4.0+51.8 GHz		90.0±3.24	20.0±1.02
pH 4.0+53 GHz		95.0±3.31	28.0±1.10
pH 6.5 control		19.0±1.05	7.7± 0.40
pH 6.5+51.8 GHz		50.0±1.60	11.0±0.53
pH 6.5+53 GHz		53.0±1.60	13.0±0.70

In summary, in addition to inhibitory effects on *L. acidophilus* growth and survival and H^+ fluxes across the membrane at pH 6.5 (Soghomonyan and Trchounian, 2013) EMI at 51.8 GHz and 53 GHz frequencies enforced the influence of ceftazidime on DCCD-inhibited ATPase activity at different pHs. But it is of interest that at low pH EMI stimulated the DCCD sensitive ATPase activity but its combination with ceftazidime and DCCD decreased ATPase activity. These changes serve as a basis to suggest that F_0F_1-ATPase is a target for EMI even in low pH.

The effects of EMI and ceftazidime on survival of *L. acidophilus* in *in vitro* model of gastrointestinal tract. *L. acidophilus* is a probiotic strain and the acid tolerance is one of the main characteristics of probiotics, thus, due to this feature, the *L. acidophilus* is able to overcome the acidic barrier of digestive tract and have a beneficial effect on human health (Kajfasz et al., 2011). Moreover, these bacteria regulate their internal pH close to neutral via F_0F_1-ATPase complex. The bacteria in the digestive tract exposed with different pH values (pH 7.5; 2.0; 3.0; 4.0; 5.0; 8.0) as well as digestive enzymes (pepsin, pancreatin and etc.) and bile salts. Nowadays, to the adverse conditions of human gastrointestinal tract, added up two more conditions, which are high frequency EMI and antibiotics. Therefore it is interesting to investigate the separate and combined effects of EMI at frequencies 51.8 and 53 GHz and ceftazidime on survival of *L. acidophilus* in *in vitro* model of human gastrointestinal tract. It was shown, that the cell number sharply reduced at pH 2.0 without

irradiation and antibiotic, but when after 20 min the bacteria were transferred into medium with higher pH, then they survive, but the repressive effects of EMI and ceftazidime are displayed in all segments (in all appropriate pHs) of digestive tract especially in stomach pH media. It should be noted that decrease in the number of bacteria at pHs 2.0 and 3.0 is also due to the highly acidic pH. Especially at pHs 2.0 and 3.0 the viability of irradiated by 51.8 and 53 GHz frequencies bacteria reduced 75 and 83 %, 71.4 and 77% respectively. Although at pHs 4.0 and 5.0 the cell number of irradiated at both frequencies bacteria declined 43.3 and 45.7 %, 42.9 and 40.8% respectively, consequently it depends on pH for this section of digestive tract (in presence of 0.3 % pepsin), but compared to the control were also noticeable the reducing effects of both frequencies.

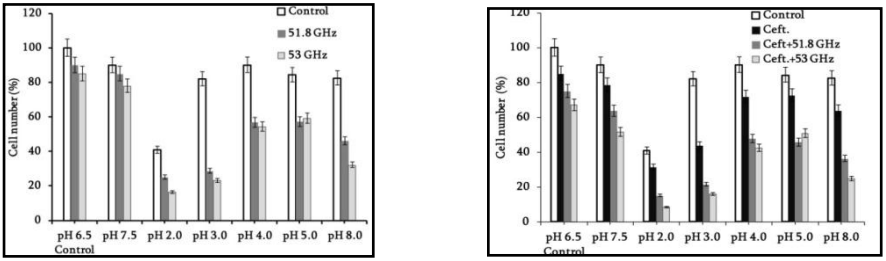

Fig.12. The separate and combined effects of EMI and ceftazidime on viability of *L.acidophilus* in *in vitro* model of gastrointestianl tract. Ceftazidime was (20μM) added to medium before inoculation of bacteria. Control without digest enzymes, antibiotic and irradiation.

It is evident that at pH 8.0, in the presence of bile salts (0.45%) and pancreatine (0.1%) the cell number of irradiated at both frequencies bacteria decreased by 54 and 68 %, respectively. It is also interesting, that there was an increase of antibiotic susceptibility in all sections of this *in vitro* model, especially at pH 8.0 the cell number drops by 75.3%. Thus the EMI at 51.8 GHz and 53 GHz frequencies and ceftazidime have repressive effects on viability of *L.acidophilus* in gastrointestinal tract. Moreover the irradiation enhanced the effect of ceftazidime.

CONCLUSIONS

The following conclusions were made based on experimentally obtained data:

1. The oxidizer ferrycianide and reducer DTT changed the ORP dynamics of LAB during growth under anaerobic conditions; moreover they suppress the growth rate of *L. salivarius*, depending on concentrations of these compounds.
2. Ferrycianide decrease and DTT, in contrast, increased the H^+ and K^+ fluxes across the membrane of *L. salivarius*, which can be explained as a result of changes in transport systems due to alteration of ORP value or other alterations resulting in shifts in C_m^{H+}.
3. The exposure of anaerobically grown *L. acidophilus* suspension with low intensity, coherent EMI at frequencies 51.8 GHz and 53 GHz led to decreasing the specific growth rate, cell number and viability; more expressed effects had observed at the 53 GHz

frequency. Moreover, EMI with indicated frequencies was causing increase in antibiotic susceptibility of *L. acidophilus*.

4. The exposure of anaerobically grown *L. acidophilus* suspension with above mentioned EMI led to changes in the ion transport systems' activity by the changes of H^+ fluxes and DCCD-inhibited ATPase activity.
5. The irradiation of *L. acidophilus* suspension changed the ORP kinetics, especially, in stationary phase of growth, due to membrane associated alterations.
6. In the case of *L.acidophilus* suspension irradiation, the target is the proton translocating F_0F_1-ATPase complex which is situated in bacterial membranes. This enzyme is target in optimal pH as well as in acidic pH.
7. The low intensity EMI at frequencies 51.8 GHz and 53 GHz and antibiotic ceftazidime supresses the viability of *L. acidophilus* in *in vitro* model of human gastrointestinal tract.
8. In the case of effects of oxidizers and reducers on LAB and due to their irradiation, occured alternations in plasma membrane proteins activity, especially in F_0F_1-ATPase activity.

LIST OF PUBLICATIONS AS A PART OF DISSERTATION TOPIC

1. **Soghomonyan D.R**., Kalantaryan V.P., Trchounian A.H. Changes in the F_0F_1-ATPase activity of irradiated *Lactobacillus acidophilus* in the presence of ceftazidime at low pH. *Biological Journal of Armenia* 2014, v. 66, pp 39-43.
2. **Soghomonyan D**., Trchounian A. Changes in the F_0F_1-ATPase Activity of Irradiated *Lactobacillus acidophilus* in the Presence of Ceftazidime at Low pH. In *Trends in Microbiology and Microbial Biotechnology, International Scientific Workshop,* Yerevan, Armenia, 2014, p. 91.
3. **Soghomonyan D**. The Survival of Irradiated Lactic Acid Bacteria in the Simulated Gastrointestinal Conditions With Antibiotic Ceftazidime. In *Enhancing Health Promoting Compounds in Food, International Summer School for Young Scientists*, Bazelati, Georgia, 2014, pp 24-25.
4. **Soghomonyan D**., Trchounian A., Proton-Translocating F_0F_1 ATPase as a Target for Combined Effects of Low-Intensity Electromagnetic Irradiation and Antibiotics on *Lactobacillus acidophilus*. In *ASM-2014 114th General Meeting*, Boston, USA, 2014, K-754.
5. **Soghomonyan D.**, Trchounian A., Comparable Effects of Low-intensity Electromagnetic Irradiation at the Frequency of 51.8 and 53 GHz and Antibiotic Ceftazidime on *Lactobacillus acidophilus* Growth and Survival. *Cell Biochemistry and Biophysics*. 2013, v. 67, pp 829–835
6. **Soghomonyan D**., Extremely High Frequency Electromagnetic Irradiation Effect On *Lactobacillus acidophilus* Sensitivity Toward Antibiotic Ceftazidime. In *International*

Student, Postgraduate and Young Scientists Conference "Lomonosov-2013", Moscow, Russia, 2013, p. 181.

7. **Soghomonyan D.R.**, The Effects of Low-Intensity Electromagnetic Irradiation at the Frequencies of 51.8 and 53 GHz and Antibiotic Ceftazidime on *Lactobacillus acidophilus* F_oF_1 -ATP-ase Activity, *Biological Journal of Armenia,* 2013, v. 65, pp 89-92.
8. Torgomyan H., Ohanyan V., **Soghomonyan D.**, KalantaryanV.,Trchounian A., The Low Intensity Extremely High Frequency Electromagnetic Irradiation Universal Effects on Anaerobic Bacteria. In *International conference " Radiation Safety Challenges in the 21st Century"* Yerevan, Armenia, Abstract book, 2012, pp 119-120.
9. Trchounian A., Torgomyan H., Ohanyan V., **Soghomonyan D.**, Kalantaryan V. Extremely high frequency electromagnetic Irradiation with low-Intensity enhanses antibiotics antibacterial effects on different bacteria. In *"Low and superlow fields and radiations in biology and medicine" Scientific Proceedings of the 6th International Congress*, Saint-Petersburg, Russia, 2012, p. 192.
10. **Soghomonyan D.**, Akopyan K., Trchounian A., pH and Oxidation-Reduction Potential Change of Environment during a Growth of Lactic Acid Bacteria: Effects of Oxidizers and Reducers. *Applied Biochemistry and Microbiology*, 2011, V.47, pp 27-31.
11. **Soghomonyan D**., Trchounian A., The role of redox potential of growth medium during growth of lactic acid bacteria: effects of oxidisers and reducers. In *14th International Conference of Young Scientists, Pushchino*, Russia, Abstract book, 2010, V. 2, pp 262-263 (in Russian).
12. Kirakosyan G., **Hakobyan D. (Soghomonyan since 2007)**, Poladyan A.,Vassilian A., Trchounian A., Proton ATPase in Redox Sensing by Bacteria, In *14th European Bioenergetics Conference,* Moscow*,* Russia, *BBA-Bioenergetics* Short reports 2006, v.14, pp. 311-312.
13. Kirakosyan G., **Hakobyan D. (Soghomonyan since 2007),** Trchounian A. Redox Sensing of Anaerobic Bacteria Connected with Proton: Phenomenon, Mechanisms and Role in Physiology of Fermenting Bacteria. In *Proceedings of the International Conference "Advanced Biotechnology: Perspectives of Development in Armenia"*, Tsakhkadzor, Armenia, 2006, p. 218 (in Russian).

ՖԻԶԻԿԱԿԱՆ ԵՎ ՕՔՍԻԴԱՎԵՐԱԿԱՆԳՆՈՂԱԿԱՆ ԳՈՐԾՈՆՆԵՐԻ ԱԶԴԵՑՈՒԹՅՈՒՆԸ ԿԱԹՆԱԹԹՎԱՅԻՆ ԲԱԿՏԵՐԻԱՆԵՐԻ ԱՃԻ ԵՎ ԹԱՂԱՆԹՆԵՐԻ ԱԿՏԻՎՈՒԹՅԱՆ ՎՐԱ

Ամփոփագիր

Հանգուցային բառեր. *Lactobacillus acidophilus*, *Lactobacillus salivarius*, ԱԵՖազային ակտիվություն, գաղութների թիվ, էլեկտրամագնիսական ճառագայթում, իոնների հոսք, կենսունակություն, հակաբիոտիկ, օքսիդավերականգնողական պոտենցիալ:

Կաթնաթթվային բակտերիաները (ԿԹԲ) Գրամ-դրական և կատալազ-բացասական մանրէների հետերոգեն խումբ են, որոնք կարող են կաթնաթթու սինթեզել լակտոզից: ԿԹԲ-ն ի շնորհիվ իրենց նյութափոխանակային առանձնահատկությունների ներառվում են սննդի արտադրության տարբեր փուլային գործընթացներում. սննդամթերքի որոշ տեսակների հոտային որակների բարելավման և հասունացման համար (Soghomonyan and Trchounian, 2013): Բացի այդ, ԿԹԲ նաև մարսողական ուղու «բնակիչներ» են և այս խմբի ամենահայտնի տեսակներից է հոմոֆերմետային *Lactobacillus acidophilus*-ը, որը սննդի արդյունաբերության մեջ լայնորեն կիրառվող պրոբիոտիկ կուլտուրա է (Altermann et al., 2005): Այն կարող է հաղթահարել մարսողական ուղու «անբարենպաստ» պայմանները (թթվային pH, լեղի, մարսողական ֆերմենտներ) և բարերար ազդեցություն ունենալ տիրոջ առողջության վրա՝ վերականգնելով աղիների մանրէների քանակական հավասարակշռությունը (Chen et al., 2009; Kajfasz et al., 2011): Հարկ է նշել, որ մանրէներն ենթարկվում են նաև միջավայրի ֆիզիկակաքիմիական և ֆիզիկական գործոնների ազդեցությանը, որոնք ազդում են բակտերիաների աճը բնութագրող չափորոշիչների և թաղանթակապ գործընթացների վրա: Օքսիդավերականգնողական պոտենցիալը (ՕՎՊ) բակտերիալ բջիջների և նրանց աճման միջավայրի վիճակը բնութագրող կարևորագույն չափորոշիչներից է: Բակտերիաների անաերոբ աճի՝ գլյուկոզի խմորման պայմաններում տեղի է ունենում միջավայրի pH-ի անկում, թաղանթային թափանցելիության և բջջաթաղանթի ֆերմենտային և տեղափոխիչ համակարգերի ակտիվության փոփոխություն (Vassilian and Trchounian, 2008; Soghomonyan et al., 2011): Աճի ընթացքում ՕՎՊ-ի և pH-ի փոփոխության, ինչպես նաև բակտերիաների աճը բնութագրող չափորոշիչների և թաղանթով H^+ իոնների տեղափոխման վրա օքսիդիչ և վերականգնիչ նյութերի ազդեցության ուսումնասիրությունները կարևոր են մանրէների աճը կարգավորող մեխանիզմների, ինչպես նաև մարդու և կենդանիների աղիքային միջավայրում ընթացող գործընթացների պատկերացման և այս բակտերիաների տեխնոլոգիական կիրառությունների համար: Միևնույն ժամանակ ցածր ինտենսիվությամբ, ծայրահեղ բարձր հաճախականությամբ (ԾԲՀ) էլեկտրամագնիսական ճառագայթման (ԷՄՃ)՝ մանրէների վրա ունեցած ոչ ջերմային ազդեցությունները հետաքրքիր են, քանի որ ԷՄՃ-ն արդեն լայնորեն կիրառվում է թերապևտիկ պրակտիկայում, սննդամթերքի և գինու պահպանման գործընթացներում (Geveke et al., 2009; Zand et al., 2010): Կենդանի օրգանիզմները, այդ թվում և մանրէները, մմ-յին ալիքների միջոցով են հաղորդակցվում և ղեկավարում իրենց հիմնական ֆիզիոլոգիական գործառույթները (Nikolaev, 2000; Trushin, 2003; Reguera 2011): Ցույց է տրվել, որ 51.8 և 53 ԳՀց հաճախությունները ազդում են *Escherichia coli* (Tadevosyan et al.,2007; Torgomyan et al., 2011), *Enterococcus hirae* (Ohanyan et al., 2012; Torgomyan et al., 2012) և այլ մանրէների աճի և կենսագործունության վրա: β-լակտամային հակաբիոտիկները (ցեֆալոսպորիններ,

պենիցիլիններ) հակամանրէային դեղամիջոցների ամենամեծ խումբն են և առաջատար դեր են կատարում վարակիչ հիվանդությունների բուժման գործընթացում (Drawz and Bonomo, 2010), սակայն, որպես քիմիական գործոններ, իրենց բացասական հետքն են թողնում կենդանի օրգանիզմների, այդ թվում և ԿԹԲ-ի վրա: Հիմք ընդունելով վերը շարադրվածը՝ առավել արդիական և իրատեսական է ուսումնասիրել օքսիդիչ ֆերիցիանիդի և վերականգնիչ դիթիոտրեյտոլի (ԴԹՏ, 1-5 մՄ վերջնական կոնցենտրացիաներով) ազդեցությունը ԿԹԲ աճը բնութագրող չափորոշիչների, այդ թվում և՝ ՕՎՊ-ի փոփոխության վրա, իոնների հոսքերի վրա: Ինչպես նաև՝ 51.8 և 53 ԳՀց հաճախականությամբ ԷՄՃ-ի (ճառագայթման տևողությունը 1ժ, հոսքի ուժգնությունը 0.06 մՎտ/սմ^2) և երրորդ սերնդի β-լակտամային հակաբիոտիկ ցեֆտազիդիմի (16-20 մկՄ նվազագույն ազդող կոնցենտրացիայով) առանձին և զուգակցված ազդեցությունները ԿԹԲ-ի աճը բնութագրող չափորոշիչների, այդ թվում և ՕՎՊ-ի փոփոխության վրա, հակաբիոտիկի նկատմամբ զգայունության և թաղանթակապ գործընթացները բնութագրող իոնների հոսքերի, թաղանթային թափանցելիության, ԱԵՖազային ակտիվության (աճի համար նպաստավոր և թթվային pH-ներում) վրա: Ուսումնասիրվել է նաև ԷՄՃ և ցեֆտազիդիմի առանձին և զուգակցված ազդեցությունները *L. acidophilus* VKMB-1660 շտամի՝ աղեստամոքսային տրակտի *in vitro* մոդելում կենսունակության և հակաբիոտիկի նկատմամբ զգայունության վրա:

Կատարված ուսումնասիրությունների արդյունքում ցույց է տրվել, որ ֆերիցիանիդի և ԴԹՏ-ի ազդեցությամբ ճնշվում է *L. salivarius-ի* աճի տեսակարար արագությունը և տեղի է ունենում իոնների հոսքերի փոփոխություն, փոխվում է նաև աճման միջավայրի ՕՎՊ-ի կինետիկան: Ցույց է տրվել նաև, որ 51.8 և 53 ԳՀց հաճախություններով ԷՄՃ-ը կարող է արձագանքային համարվել *L. acidophilus* բակտերիաների համար, քանի որ այս հաճախությունները ճնշում են աճի տեսակարար արագությունը, նվազում է գաղութների թիվը: Կախված ճառագայթվող բակտերիաների միջավայրից՝ պինդ (ՄՌՇ ագար), կամ հեղուկ (կախույթ), ԷՄՃ-ը ազդում է նաև միջբջջային փոխազդեցության վրա (Soghomonyan and Trchounian, 2013): Տեղի են ունենում H^+ իոնների հոսքերի և ԱԵՖազային ակտիվության նվազում, նաև աճի ստացիոնար փուլում ՕՎՊ-ի վերընթաց փոփոխություն (Soghomonyan and Trchounian 2013): ԷՄՃ-ի և հակաբիտիկ ցեֆտազիդիմի զուգակցման դեպքում տեղի է ունենում վերը թվարկված աճի բնութագրիչների, ինչպես նաև իոնների հոսքերի և ԱԵՖազային ակտիվության վրա հակաբիոտիկի ճնշիչ ազդեցության ուժեղացում (Soghomonyan, 2013; Soghomonyan, Trchounian, 2013): Նորույթ կարելի է համարել, օքսիդիչ և վերականգնիչ նյութերի և ԷՄՃ միջոցով ԿԹԲ-ի աճի վերահսկման հնարավորությունը, նաև այն դիտարկումը, որ ճառագայթման և ցեֆտազիդիմի զուգակցված ազդեցության դեպքում մարսողական ուղու *in vitro* մոդելում տեղի է ունենում *L. acidophilus*-ի կենսունակության նվազում (անկախ թթվային pH-ի, մարսողական ֆերմենտների և լեղու աղերի ճնշիչ ազդեցությունից) և հակաբիոտիկի նկատմամբ զգայունության մեծացում (Soghomonyan, 2014): Հետաքրքիր է որ F_0F_1-ԱԵՖազային համալիրը ճառագայթման թիրախ է հանդիսանում նույնիսկ թթվային 4.0 pH-ում (Soghomonyan et al., 2014): Այսպիսով՝ օքսիդիչ և վերականգնիչ նյութերի, ինչպես նաև ԷՄՃ-ի ուղղակի, կամ միջնորդավորված (ջրի մոլեկուլներ) ազդեցությամբ ԿԹԲ- թաղանթակապ սպիտակուցային համակարգերում տեղի են ունենում կառուցվածքագործառութային փոփոխություններ (Soghomonyan, 2013, Soghomonyan and Trchounian, 2013, Soghomonyan et al., 2014): Ստացված արդյունքները ավելի հստակ պատկերացում են տալիս օքսիդիչ և վերականգնիչ նյութերի և ՇԲՀ ԷՄՃ-ի ազդեցության մեխանիզմների և սննդի արդյունաբերության, բժշկության և գյուղատնտեսության մեջ նշված գործոնների կիրառական հնարավորությունների վերաբերյալ:

СОГОМОНЯН ДИАНА РАЗМИКОВНА

ВЛИЯНИЕ ФИЗИЧЕСКИХ И ОКИСЛИТЕЛЬНО- ВОССТАНОВИТЕЛЬНЫХ ФАКТОРОВ НА РОСТ И АКТИВНОСТЬ МЕМБРАН МОЛОЧНОКИСЛЫХ БАКТЕРИЙ

РЕЗЮМЕ

Ключевые слова: *Lactobacillus acidophilus*, *Lactobacillus salivarius*, антибиотик, АТФазная активность, выживаемость, окислительно-восстановительный потенциал, поток ионов, число колоний, электромагнитное излучение.

Молочнокислые бактерии (МБ) – гетерогенная группа Грам-положительных, каталаза-отрицательных микроорганизмов, которые синтезируют молочную кислоту из лактозы. Многие штаммы благодаря своим метаболическим особенностям используются в различных процессах производства пищевых продуктов для созревания некоторых пищевых продуктов и улучшения их запаха. Кроме того, МБ являются обитателями пищеварительного тракта. Один из известных видов – широко используемая в пищевой промышленности в качестве пробиотической культуры гомоферментативная бактерия *Lactobacillus acidophilus* (Altermann et al.,2005), которая способна преодолеть “неблагоприятные” условия пищеварительного тракта (кислотный барьер, желчь, пищеварительные ферменты) и благотворно влиять на здоровье человека посредством восстановления микробного баланса (Chen et al., 2009; Kajfasz et al., 2011). Следует отметить, что бактерии подвергаются воздействию физико-химических и физических факторов окружающей среды, которые влияют на параметры, характеризующие рост бактерий и мембранносвязанные процессы. Окислительно-восстановительный потенциал (ОВП) – один из наиболее важных параметров, характеризующих состояние бактериальной клетки и среды роста. Во время анаэробного роста бактерий, при сбраживании глюкозы, наблюдается падение pH среды, а также меняется мембранная проницаемость и активность ферментных транспортных систем (Vassilian and Trchounian, 2008; Soghomonyan et al., 2011). Изучение изменения ОВП среды, а так же влияния окисляющих и восстанавливающих соединений на параметры роста и транспорт ионов, важно для выявления механизмов регулирования бактериального роста, процессов происходящих в кишечной среде, а также для технологического применения МБ. В то же время интерес к нетепловым эффектам низкоинтенсивного, крайневысокочастотного (КВЧ) электромагнитного излучения (ЭМИ) на бактерии объясняется широким применением этого излучения в терапевтической практике, в процессах хранения вин и пищевых продуктов (Geveke et al., 2009; Zand et al., 2010). Кроме того, микроорганизмы взаимодействуют друг с другом и регулируют свои основные физиологические процессы с помощью миллиметровых волн (Nikolaev, 2000; Trushin, 2003; Reguera 2011). Было показано, что 51.8 и 53 ГГц частоты влияют на рост и жизнедеятельность *Escherichia coli* (Tadevosyan et al., 2007; Torgomyan et al., 2011), *Enterococcus hirae* (Ohanyan et al., 2012; Torgomyan et al., 2012) и других бактерий. β-лактамные антибиотики являются самой большой группой антимикробных препаратов и выполняют ведущую роль в лечении инфекционных заболеваний (Drawz and Bonomo, 2010), но в качестве химических

факторов они оставили свой отрицательный отпечаток на живые организмы, в том числе и на МБ. Исходя из вышеизложенного, можно сказать, что актуальным является изучение влияния 1-5 мМ окислителя феррицианида и восстановителя дитиотреитола (ДТТ) на параметры роста, в том числе изменение ОВП, и мембранносвязанные процессы (поток ионов, проницаемость). Также было изучено влияние отдельных и комбинированных эффектов β-лактамного антибиотика третьего поколения цефтазидима с минимальной действующей концентрацией16-20 мкМ и ЭМИ с частотами 51.8 и 53 ГГц (продолжительность облучения 1 ч, сила потока 0.06 мВт см^2) на параметры роста, в том числе изменение ОВП, чувствительность к антибиотику, поток ионов и АТФазную активность (при оптимальном и кислом pH). Кроме того было изучено влияние отдельных и комбинированных эффектов ЭМИ и цефтазидима на выживаемость и чувствительность *L. acidophilus* VKMB-1660 к антибиотику на *in vitro* модели пищеварительного тракта человека.

В результате исследований было показано, что феррицианид и ДТТ подавляют удельную скорость роста *L. salivarius,* а также меняется поток ионов и кинетика ОВП (Soghomonyan et al., 2011). ЭМИ с частотами 51.8 и 53 ГГц могут быть резонансными для бактерий *L. acidophilus,* поскольку подавляется удельная скорость роста и уменьшается число колоний. В зависимости от облучаемой среды: твердой – в виде МРШ агаризованной среды, или жидкой – бактериальной суспензии облучаемых бактерий, ЭМИ КВЧ влияют на межклеточное взаимодействие (Soghomonyan and Trchounian, 2013). Кроме того, происходит падение АТФазной активности, замедление ионных потоков и изменение ОВП в стационарной фазе роста. При комбинированном воздействии антибиотика и ЭМИ КВЧ данных частот наблюдается усиление воздействия антибиотика на вышеперечисленные параметры роста, а также на АТФазную активность и ионные потоки (Soghomonyan, 2013; Soghomonyan and Trchounian, 2013). Новизна данной работы – в выявлении возможности регулирования роста бактерий с помощью окисляющих и восстанавливающих соединений и ЭМИ КВЧ. На *in vitro* модели пищеварительного тракта также наблюдается снижение выживаемости и повышение чувствительности к цефтазидиму, несмотря на условия с низким pH, желчными солями и пищеварительными ферментами (Soghomonyan, 2014). Надо отметить, что F_0F_1-АТФазный комплекс является мишенью для ЭМИ КВЧ данных частот даже при кислом pH (4.0) (Soghomonyan et al., 2014).

Таким образом, при влиянии окисляющих и восстанавливающих соединений, а также при прямом или опосредованном (молекулы воды) действии ЭМИ КВЧ и антибиотика, в бактериальной мембране, точнее в мембранносвязанных белковых молекулах происходят структурно-функциональные изменения (Soghomonyan, 2013; Soghomonyan and Trchounian, 2013; Soghomonyan et al., 2014). Полученные результаты позволят лучше понять механизмы воздействия как окисляющих и восстанавливающих соединений, так и ЭМИ КВЧ, а также представить возможности их практического применения в пищевой промышленности, медицине и в сельском хозяйстве.