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THE EFFECTS OF LOW-INTENSITY ELECTROMAGNETIC IRRADIATION AT THE FREQUENCIES OF 51.8 AND 53 GHZ AND ANTIBIOTIC CEFTAZIDIME ON *LACTOBACILLUS ACIDOPHILUS* F_0F_1 ATP-ASE ACTIVITY

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The effects of low intensity electromagnetic irradiation (EMI) at the frequencies 51.8 and 53 GHz and antibiotic ceftazidime on N,N'-dicyclohexilcarbodiimide (DCCD), inhibited ATP-ase activity of *Lactobacillus acidophilus* membrane vesicles were investigated. It was shown that both frequencies decreased the ATP-ase activity, moreover, ceftazidime increase the sensitivity of cells to DCCD, inhibitor of the F_0F_1-ATP-ase. EMI combined with ceftazidime and DCCD markedly decreased the ATP-ase activity. The F_0F_1-ATP-ase is suggested can be a target for the effects observed.

L. acidophilus – electromagnetic irradiation – ATPase activity

Ուսումնասիրվել է ցածր ուժգնությամբ, 51,8 և 53 ԳՀց հաճախականությամբ էլեկտրամագնիսական ալիքների և հակաբիոտիկ ցեֆտազիդիմի ազդեցությունը *Lactobacillus acidophilus* կաթնաթթվային բակտերիաների թաղանթային բշտիկների N,N'-դիցիկլոհեքսիլկարբոդիիմիդի (ԴՑԿԴ) նկատմամբ զգայուն ԱԵՖ-ազային ակտիվության վրա: Յույց է տրվել, որ երկու հաճախություններն էլ ճնշում են ԱԵՖ-ազային ակտիվությունը, ավելին՝ ցեֆտազիդիմը մեծացնում է ԴՑԿԴ-ի՝ F_0F_1-ԱԵՖ-ազի արգելակչի նկատմամբ բջիջների զգայնությունը, իսկ էլեկտրամագնիսական ալիքների, ցեֆտազիդիմի և ԴՑԿԴ-ի զուգակցումը զգալիորեն ճնշում է ԱԵՖ-ազային ակտիվությունը: Ենթադրվում է, որ F_0F_1-ԱԵՖ-ազը դիտարկված ազդեցությունների թիրախ է:

L. acidophilus – էլեկտրամագնիսական ճառագայթում – ԱԵՖազային ակտիվություն

Было изучено влияние низкоинтенсивных электромагнитных волн с частотами 51,8 и 53 ГГц и антибиотика цефтазидима на N,N'-дициклогексилкарбодиимид (ДЦКД) чувствительную АТФ-азную активность мембранных везикул молочнокислых бактерий *Lactobacillus acidophilus*. Было показано, что обе частоты снижают АТФ-азную активность, более того, цефтазидим повышает чувствительность клеток к ДЦКД, ингибитору F_0F_1-АТФ-азы, а комбинированное действие цефтазидима, электромагнитных волн и ДЦКД значительно снижает АТФ-азную активность. Предполагается, что F_0F_1-АТФ-аза является мишенью наблюдаемых эффектов.

L. acidophilus – электромагнитное излучение – АТФ-азная активность

The lactic acid bacteria (LAB) are arguably the second only to yeast in importance in their service to human. They have been used worldwide in the generation of safe, storable and organoleptically pleasing foodstuffs [1]. One of the well-known LAB species is a probiotic culture *Lactobacillus acidophilus*.

LAB are confronted with several challenges such as electromagnetic irradiation (EMI) effects and influence of chemicals including different antibiotics. The bacterial effects of extremely high frequency electromagnetic irradiation (EMI) with low (low-energetic) intensity and non-thermal action are interesting due to that extremely high

frequency EMI is widely applied in therapeutic practice, food and wine preservation [2]. Recently it has been shown that there are two frequencies of EMI – 51.8 and 53 GHz which can affect these bacteria [3]. Different cellular targets are suggested to explain the effects of EMI–water molecules, plasma membrane and genome [4, 5, 6]. Among membrane components the *N,N′*-dicyclohexylcarbodiimide (DCCD) sensitive H^+-translocating F_0F_1-ATP-ase, the key enzyme of bioenergetics means, might be a target for EMI and some antibiotics [7]. The direct effects on this ATP-ase can disturb proton motive force generation and pH homeostasis maintaining to enhance cellular effects of those factors. However these effects should be further studied.

In our work we have investigated the effects of EMI and ceftazidime on DCCD inhibited ATPase activity of *Lactobacillus acidophilus* membrane vesicles.

Materials and methods. VKMB-1660 wild type strain was used for all experiments. These bacteria were grown in MRS broth (pH 6.5) [3,8]. The bacteria were grown at 37^0C until stationary growth phase (20-24 h) under anaerobic conditions upon fermentation of glucose (20 g/ l) [3, 9]. Cells grown were concentrated by centrifugation (3,600×g) during 15 min, washed and diluted in bi-distilled water. Then, the bacterial suspension (at the concentration of 107-108 colony-forming units (CFU)/ml) was transferred into the plastic plate (Petri dish) with suspension thickness of 1 mm for subsequent irradiation during 1 h as described elsewhere [5, 7, 9]. The latter was performed by EMI generator; model G4-141 (State Scientific-Production Enterprise "Istok", Fryazino, Moscow Region, Russia) radiating (with conical antenna) the coherent in time electromagnetic waves with the frequencies of 51.8 GHz and 53 GHz 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 [3, 7]. The generator was assembled at the Institute of Radiophysics and Electronics, the National Academy of Sciences of Armenia, and supplied by Kalantaryan. Membrane vesicles were isolated from cells as described [7]. ATPase activity was determined by amount of liberated inorganic phosphate (Pi) after adding 5 mM ATP by Taussky and Shorr method [13]. The corrections were made for blanks without ATP or membrane vesicles. Relative ATP-ase activity was expressed in nmol Pi per mg protein in 1 min. The assay mixture contains 50 mM Tris– HCl (pH 6.5), 0.4 mM $MgSO_4$ and 100 mM KCl When it was necessary, membrane vesicles were preincubated with ceftazidime (20 □M) or DCCD (0.01 mM) for 10 min. Average data from triple measurements were represented with standard errors determined as before [3, 7, 9, 10].

Results and Discussion. As shown before [3], 51.8 and 53 GHz EMI has strong anti-bacterial effects on *L. acidophilus* and enhanced the effect of ceftazidime on their growth and survival. Moreover, EMI has effects on H^+ fluxes across the membrane [3, 7]. EMI at the frequencies both did not 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 [3]. So it was suggested that H^+-translocating F_0F_1-ATP-ase, for which DCCD is specific inhibitor [7, 10], might be a target for EMI and ceftazidime. Indeed, EMI at the frequencies 51.8 and 53 GHz suppressed the overall ATP-ase activity of *L. acidophilus* membrane vesicles 1.3 fold and 2.6 fold, respectively. The suppression was more considerable in the presence of DCCD (0.01 mM) – 2.18 fold and 4.4 fold, respectively (fig.1). In case of combination EMI at both frequencies with ceftazidime (20 μM) the inhibition of ATP-ase activity was much more expressed, especially in the presence of DCCD – 3.3 fold and 7.5 fold, respectively (fig. 2).

We had determined the DCCD sensitive ATPase activity had been calculated from parallel measurements (tabl.1): a significant decrease in DCCD-inhibited ATP-ase activity of *L. acidophilus* membrane vesicles after exposure of bacteria with EMI at the frequency of 51.8 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 ATP-ase activity (tabl. 1). The results obtained in the presence of ceftazidime indicated that EMI enhanced the effect of cefta zidime.

The changes in the F_0F_1-ATPase activity point out the membranous effects of low intensity extremely high frequency EMI on bacteria. They are likely to those with the other bacteria [14] as well as to the effects on lipid bilayers changing membrane permeability [13].

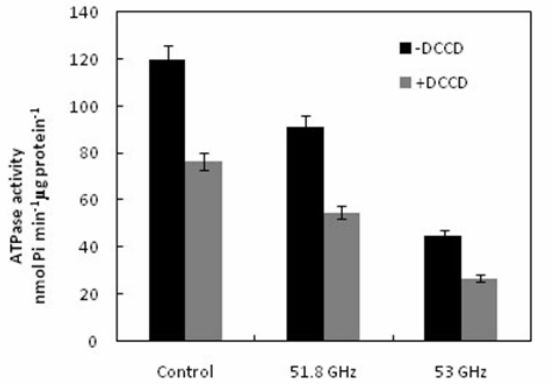

Fig. 1. The effects of 51.8 and 53 GHz frequencies EMI and DCCD on overall ATPase activity of *L. acidophilus* membrane vesicles. Control was without irradiation; 0.01 mM DCCD was added when mentioned. For the others, see "*Materials and Methods*"' section

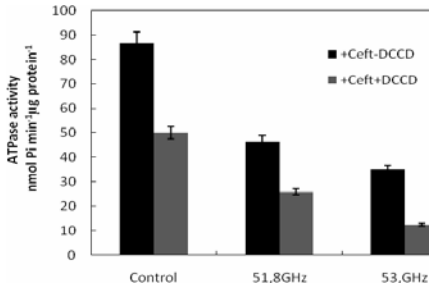

Fig. 2. The effects of 51.8 and 53 GHz frequencies EMI and DCCD on overall ATPase activity of *L. acidophilus* membrane vesicles in the presence of ceftazidime (20 µM). For the others, see legends to fig. 1.

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

Antibiotic Ceftazidime (20µM)	ATPase activity (nmol 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

Thus, in addition to inhibitory effects on *L. acidophilus* growth and survival and H^+ fluxes across the membrane [3], EMI at 51.8 and 53 GHz frequencies enforced the influence of antibiotic 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 of interest and could be applied in biotechnology, when LAB are used in different paths.

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