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CHANGES IN THE F_0F_1-ATPASE ACTIVITY OF IRRADIATED *LACTOBACILLUS ACIDOPHILUS* IN THE PRESENCE OF CEFTAZIDIME AT LOW pH

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The aim of this study was the investigation of the effects of low intensity electromagnetic irradiation (EMI) at the frequencies of 51.8 and 53 GHz and of antibiotic ceftazidime on the N,N'-dicyclohexilcarbodiimide (DCCD) inhibited ATPase activity of membrane vesicles of lactic acid bacteria Lactobacillus acidophilus grown at low pH (pH 4.0 or 6.5) and assayed at the same pH. It was shown that both frequencies EMI stimulated ATPase activity of L. acidophilus grown at pH 4.0, but EMI combined with ceftazidime and DCCD decreased ATPase activity at pH 4.0 and pH 6.5. It was suggested that the F_0F_1-ATPase might be a target for EMI even at low pH.

L. acidophilus – low pH – ATPase activity

Աշխատանքի նպատակն է ուսումնասիրել ցածր ուժգնությամբ, 51.8 և 53 ԳՀց հաճախականությամբ էլեկտրամագնիսական ալիքների (ԷՄԱ) և հակաբիոտիկ ցեֆտազիդիմի ազդեցությունը ցածր pH-ում (pH 4.0 և pH 6.5) աճեցրած *Lactobacillus acidophilus* կաթնաթթվային բակտերիաների թաղանթային բշտիկների N,N'-դիցիկլոհեքսիլկարբոդիիմիդի (ԴՑԿԴ) նկատմամբ զգայուն ԱԵՖազային ակտիվությունը նույն pH-ներով փորձարարական լուծույթներում: Ցույց է տրվել, որ ԷՄԱ երկու հաճախություններն էլ խթանում են pH 4.0-ում աճեցրած *Lactobacillus acidophilus* թաղանթային բշտիկների ԱԵՖազային ակտիվությունը, բայց ԷՄԱ-ի, ցեֆտազիդիմի և ԴՑԿԴ-ի զուգակցումը զգալիորեն ճնշում է ԱԵՖ-ազային ակտիվությունը ցածր pH-ում: Ենթադրվում է, որ F_0F_1-ԱԵՖազը կարող է ԷՄԱ թիրախ հանդիսանալ նույնիսկ ցածր pH-ում:

L. acidophilus – ցածր pH – ԱԵՖազային ակտիվություն

Целью данной работы явилось изучение влияния низкоинтенсивных электромагнитных волн (ЭМВ) с частотами 51.8 и 53 ГГц и антибиотика цефтазидима на N,N'-дициклогексилкарбодиимид (ДЦКД) чувствительную АТФазную активность мембранных везикул молочнокислых бактерий *Lactobacillus acidophilus*, выращенных при низком pH (pH 4.0 и 6.5) и в экспериментальных растворах с тем же pH. Было показано, что обе частоты ЭМВ стимулируют АТФазную активность мембранных везикул молочнокислых бактерий *L. acidophilus*, выращенных при pH 4.0, но комбинированное действие цефтазидима, ЭМВ и ДЦКД значительно снижает АТФазную активность при обоих pH. Предполагается, что F_0F_1-АТФаза является мишенью для ЭМВ даже при низком pH.

L. acidophilus – низкий pH – АТФазная активность

Lactic acid bacteria, including lactobacilli, are known to be present in different foods and have an excellent record for safety. They are used as starter cultures in food fermentation and as probiotics – health - promoting microbes. Intestinal lactobacilli have several interactions with host organisms and have been linked with numerous health benefits. As probiotic bacteria, lactic acid bacteria are confronted with several challenges such as acidic

pH and high bile salt concentration. *Lactobacillus acidophilus* is one of the widespread probiotic bacteria, which can overcome acid and bile barrier of stomach and intestine, respectively, and then beneficially affect host by improving its intestinal microbial balance.

As shown before, the cell membrane multi-subunit F_oF_1-ATPase, which links the production of ATP to the proton motive force [1], is an important element in response and tolerance to low pH in some bacteria trough the controlling H^+ concentration between the cell cytoplasm and external medium [2]. Nowadays in addition to these intestinal unfavorable conditions there are many physical factors and chemicals which can affect bacteria and lead to disturbance of their functional activity.

One of the physical factors affecting bacteria is the high frequency electromagnetic irradiation (EMI), which is widely used in telecommunication as well as in therapeutic practice, food and wine preservation; it has different application. 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 growth, survival and living properties of different bacteria, namely *Escherichia coli* [3-5], *Enterococcus hirae* [6], and *Lactobacillus acidophilus* [7]. Among cellular targets for EMI are water molecules, plasma membrane, the N,N'-dicyclohexylcarbodiimide (DCCD) sensitive H^+-translocating F0F1-ATPase, the main membrane-associated enzyme of bioenergetics relevance, and bacterial genome. These targets are considered as common ones for EMI effects on bacteria [8-10] but exact primary targets and detailed mechanisms of the effects are not clear yet. Previously, it has been shown that EMI at both frequencies affected ATPase activity of *L. acidophilus* grown at pH 6.5 [10] but effects on bacteria grown at low pH are unknown.

In this work we investigated ATPase activity of *L. acidophilus*, irradiated with EMI at frequencies 51.8 and 53 GHz and in the presence of antibiotic ceftazidime at low pH. The ATPase activity of the membrane vesicules of *Lactobacillus acidophilus* was assured in two cases: when bacteria were grown at pH 4.0 or pH 6.5 but the assayed pH was adjusted to 4.0.

Materials and methods. For all experiments *L. acidophilus* VKMB-1660 wild type strain (laboratory stock) was used. The bacterial growth, isolation of membrane vesicles and preparation to assays as well as irradiation of bacteria with EMI were detailed in previous paper [10].

ATPase activity was determined by amount of liberated inorganic phosphate (Pi) after adding 5 mM ATP by Taussky and Shorr method [12]. The corrections were made for blanks without ATP or membrane vesicles. Relative ATPase activity was expressed in nmol Pi per mg protein in 1 min. The assay mixture contains 50 mM Tris-HCl (pH 6.5 and 4.0), 0.4 mM $MgSO_4$ and 100 mM KCl. When it was necessary, membrane vesicles were pre-incubated with ceftazidime (20 μM) or DCCD (0.2 mM) for 10 min.

Average data from triple measurements were represented with standard errors determined as before [4, 7, 11, 13].

Results and Discussion. It was shown previously, that the low intensity, extremely high frequency EMI suppressed the membrane vesicles overall ATPase activity of *L. acidophilus* grown at pH 6.5 1.3-fold and 2.6-fold, respectively [10]. The suppression was more considerable in the presence of DCCD, inhibitor for the F0F1-ATPase (0.1 mM), – 2.18-fold and 4.4-fold, respectively, and when EMI was combined 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 [10]. Moreover, 51.8 and 53 GHz EMI had strong antibacterial effects on *L. acidophilus* and enhanced the effect of ceftazidime on their growth, survival and H^+ fluxes across the membrane, but it increased DCCD-inhibited H^+ efflux. In contrast to this, EMI in combination with ceftazidime decreased DCCD-sensitive H^+ effluxes [7].

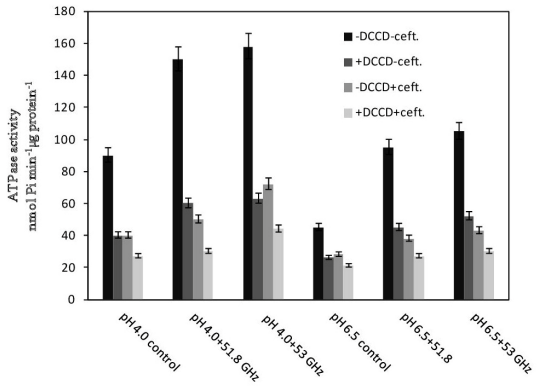

Figure 1. The effects of 51.8 and 53 GHz frequencies EMI and DCCD on overall ATPase activity of *L. acidophilus* membrane vesicles in presence and absence of ceftazididme (20 μM). Control was without irradiation; 0.2 mM DCCD was added. pH assay mixture 4.0. pHs of growth media 4.0 and 6.5. For the others, see "Materials and Methods".

As shown in fig. 1, EMI at both frequencies mentioned (the flux intensity of 0.06 mW/cm^2) 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 on ~47 % than ATPase activity of bacteria which were grown at pH 6.5 (see fig. 1). When the assayed pH was adjusted to 6.5 (by 0.1 M HCl), EMI at both frequencies was suppressed ATPase activity of membrane vesicles of L. acidophilus grown at pH 4.0 in 1.2-1.8 folds (fig. 2) as it was shown before for LAB grown at pH 6.5 [10], but at 53 GHz frequency it inhibited less than when grown at pH 4.0 (see fig. 2). As at pH 4.0 or pH 6.5 of growth media and upon the assays, DCCD (0.2 mM) inhibited ATPase activity of membrane vesicles of non-irradiated (control) and irradiated cells and this inhibition was much more expressed in the presence of antibiotic ceftazidime (20 μM).

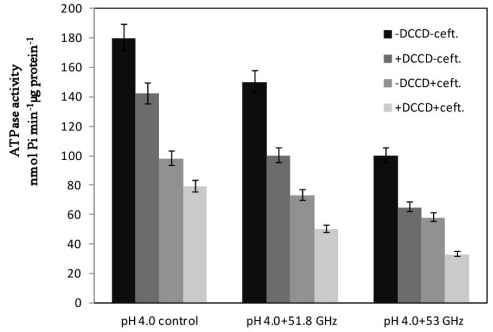

Figure. 2. The effects of 51.8 and 53 GHz frequencies EMI and DCCD on overall ATPase activity of *L. acidophilus* membrane vesicles in presence and absence of ceftazididme (20 μM). pH of assay mixture 6.5, pH of growth medium 4.0. For the others, see legends to Figure 1.

The DCCD-sensitive ATPase activity had been calculated from parallel measurements (tab. 1): 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 or 53 GHz was determined in the absence and in the presence of ceftazidime. The DCCD-inhibited ATPase activity was increased, when the assayed pH was adjusted to 4.0 and in the absence of ceftazidime (tab. 1). However, the combination of EMI and ceftazidime suppressed the DCCD-sensitive ATPase activity.

Table 1. 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	-ceftazidime	+ceftazidime
pH 4.0 control	pH 6.5	38.0±0.60	19.0±0.81
pH 4.0 + 51.8 GHz		50.0±1.00	23.0±0.73
pH 4.0 + 53 GHz		35.0±0.50	25.0±0.90
pH 4.0 control	pH 4,0	40.0±0.70	13.0±0.71
pH 4.0 + 51.8 GHz		90.0±0.75	20.0±0.64
pH 4.0 + 53 GHz		95.0±0.90	28.0±1.00
pH 6.5 control		19.0±0.50	7.7± 0.88
pH 6.5 + 51.8 GHz		50.0±0.80	11.0±0.73
pH 6.5 + 53 GHz		53.0±0.92	13.0±0.90

Thus, EMI with extremely high frequency stimulated the DCCD sensitive ATPase activity of membrane vesicles of L. acidophilus grown at acidic pH. The H^+ translocating F_0F_1-ATPase, a key enzyme of bacterial membrane, might be a target for EMI. Interestingly, it was shown for Lactobacillus casei, that the expression of the H^+-ATPase coded gene expression increased along with the increased acidity of incubation: expressions was increased 3.3-times and 2.8-times at pH 4.0 and pH 5.0, respectively, than at pH 6.5; so there may be some relationship between H^+-ATPase and acid tolerance in *L. casei* [14].

It should be noted that at a low external pH (< 3.5), *L. acidophilus* can maintain cytoplasmic pH at values close to neutral [15]. In 1984, Kobayashi et al. reported that H^+-ATPase content of *S. faecalis* increased when cells are grown at a pH of less than 8.0 in the presence of protonophores. To maintain the cytoplasmic pH at 7.6–7.8, cells expel protons at pH of less than 7.6, and then extrusion diminishes after the cytoplasmic pH reaches 7.6. It was suggested that this is the reason for the increase in H^+-ATPase content during growth at a low cytoplasmic pH [16].

In summary, in addition to inhibitory effects on *L. acidophilus* growth and survival and H^+ fluxes across the membrane at pH 6.5 [7], EMI at 51.8 and 53 GHz frequencies enforced the influence of antibiotic ceftazidime on DCCD-inhibited ATP ase activity at different pHs. But it was of interest that at low pH EMI stimulated the DCCD sensitive ATPase activity but it's combination with ceftazidime and DCCD decreased ATPase activity. These changes allow to suggest that H^+ translocating F_0F_1 ATPase is a target for EMI even in low pH.

L. acidophilus is a probiotic strain and the acid tolerance is one of the main characteristics of probiotics, thus, these results could be applied in biotechnology and food industry.

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