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Electromagnetic and acoustic technologies in antibacterial preparation development

Subject and Purpose. The present paper is concerned with the use of wave technologies in the development of antibiotics-alternative approaches for pathogenic microflora suppression. Lactobacilli strains picked in different ecological niches and their activity against pathogenic strains are studied with a focus on a targeted modification of adhesive and antagonistic properties of lactobacilli by exposing them to low-intensity electromagnetic (EM) fields and the ultrasound.

Methods and Methodology. Lactobacilli picked in different ecological niches are experimentally studied, including (1) standard strains from probiotic preparations and (2) circulating strains picked in humans and bees. For the ultrasonic and electromagnetic radiation sources, G3-109 and G3-F and G4-141 and G4-142 generators are taken, respectively. The adhesive properties of *Lactobacillus* spp. strains and their antagonistic activity are estimated against *C. diphtheriae*, *S. aureus*, and yeast-like fungi of *Candida* genus in aerobic and microaerophilic culture conditions. Statistical technology is employed in the data processing and analysis.

Results. It has been established that *L. plantarum* strains picked in the gut of healthy bees are most antagonistic towards pathogens. It has been demonstrated that the priority culture conditions for lactobacilli are microaerophilic conditions simulating their stay in vivo. It has been shown that it is possible to modify properties of microorganisms by their exposure to ultrasound and low-intensity electromagnetic fields in narrow bands of the EHF range. The effect efficiency versus frequency has a dispersion character. Individual features of various pathogenic strains have been recognized.

Conclusion. The obtained results open up prospects for electromagnetic and acoustic technologies in the development of safe alternative means to antagonize persisting pathogens and increase human body resilience. Fig. 6. Tabl. 6. Ref.: 24 items.

Key words: electromagnetic field, ultrasound, lactobacilli, diphtheria, golden staphylococcus, adhesive and antagonistic activity, modification.

Nowadays, environmentally friendly technologies have received extensive attention in medicine, vaccinology, pharmacology, microbiology. A strong need exists in new methods and means based on physical impacts on biological objects of different classes, including microorganisms, to purposefully modify their functional properties [1, 2]. Ultrasound and low-intensity electromagnetic (EM) fields in the extremely high frequency (EHF) range are considered such impact factors, with environmental requirements and maximum allowable levels and doses of radiation taken into account [1, 3, 4].

Antibiotics that are widely used in today's medicine can quickly and effectively suppress various infections, including particularly dangerous ones. Yet a positive effect from antibiotic therapy is accompanied by such functional disorders affecting the human body as slower hematopoiesis, inhibited metabolic processes, maldigestion, etc. Under the action of antibiotics, polyresistant strains of the agents increase their diversity, thus causing atypical forms of common infectious diseases, such as pneumonia, tuberculosis, meningitis, typhoid, etc. Therefore, in non-life threatening conditions, pro-, pre- and synbiotic preparations must be taken to

keep the flora of the macro-organism in the normal state [5, 6].

A search for new sources of pro-, pre- and synbiotic preparations against pathogenic bacteria is very important. Synbiotic preparations based on representatives of normal flora and having high colonization capacity and high antagonistic activity towards pathogenic and opportunistic microorganisms [6–9] hold promise. Probiotics, which include lactobacilli living on the body mucous membranes, are most commonly used to cope with dysbiotic conditions. Their species composition is very diverse [8, 10–14]. At present, certain lactobacilli species that persevere in the human body are only studied for their general biological properties. Consequently, it is necessary to find and select candidates for production strains among representatives of alternative ecosystems.

The differentiation and identification of probiotic bacteria is a critical point in the main microbiological study. Potential strain candidates should not only be certified according to their basic characteristics, but “probiotic” properties as well, among which are, first of all, adhesive and antagonistic activities. The adhesion property determines the bacteria ability to gain a foothold in an ecosystem and provides colonization resistance to the mucosa. The antagonistic properties in biocenosis ensure production of antimicrobial compounds of protein origin (bacteriocins), which exerts a positive effect on the human body [15].

We seek to investigate abilities of lactobacilli strains isolated from different ecosystems in an effort to antagonize pathogenic strains of diphtheria, golden staphylococcus, and yeast-like fungi of the genus *Candida* under different culture conditions (aerobic and microaerophilic). Of interest is also a targeted modification of adhesive and antagonistic properties of lactobacilli by exposing them to a low-intensity EM field and the ultrasound.

1. Equipment and measurement technique.

The irradiation of microorganism strains and their exometabolites was carried out on a special experimental bench.

To implement the EM field effect in a narrow frequency band of the EHF range, generators G4-141 ($f = 37.5...53.57$ GHz) and G4-142 ($f = 53.57...78.33$ GHz) were used for $P \leq 5$ mW power signals. During the irradiation, the generator waveguide outputs were loaded with horn an-

tennas of 6.0×5.0 cm² and 8.5×6.5 cm² apertures. The examined objects were placed 5...7 cm away from the horn mouth, i.e. in the antenna near zone. The power flux density (PFD) was 0.1 mW/cm² with an irradiation non-uniformity of no more than 3 dB in the object location. The non-uniformity is connected with the near zone specifics, finite sizes of the aperture and irradiated objects and with a low impedance of the load.

To implement the ultrasound effect, generators G3-109 ($f = 60$ kHz, $P = 5$ W) and G3-F ($f = 18$ kHz, $P = 16$ W) under the load of circular piezoceramic converters-radiators of PZT type (radiators based on synthetic Plumbum Zirconate Titanate ceramics) were used. When irradiated with G3-109 generator, the excitation amplitude of the signal was $U = 15$ V at a load of $R = 50 \Omega$ ($P = 5$ W). The coefficient of the electric into acoustic power transformation was $\eta \approx 5\%$, i.e. the average power of acoustic vibrations in the biological object location reached (0.25...0.50) W. The bacterial suspension tubes were located in the near zone of the radiator. The irradiation took place in an aqueous medium.

The conditions for microaerophilic bacteria culture were created in microanastates using Generator GENbox microaer gas generating packs (*bio-Merieux, France*) or an industrially manufactured gas mixture consisting of O₂ (5%), CO₂ (10%), and N₂ (85%). The examination was given to strains of *Lactobacillus spp.* picked in different ecological niches, including: 1) probiotic preparations: strain *L. acidophilus* from preparation “Symbilact” produced by *Vivo, Ukraine*, strain *L. rhamnosus* from preparation “PREMA[®]” produced by *Delta Medical Promotions, Switzerland*, 2) *L. plantarum* strain picked in the gut of bees (19 bee families) with the participation of NPC “IEKVM” NAAN, and 3) strain from the mucosa of the upper respiratory tract and the contents of the intestine of people aged 17 to 23.

For pathogenic bacteria, we use circulating and museum strains *Corynebacterium diphtheriae* and *S. aureus* obtained by the *City Student Hospital of Kharkov*. The reference strain is *S. aureus 209 P (ATCC 6538-R)* from the laboratory of medical microbiology in cooperation with the *Museum of Microorganisms of SA “IMI NAMN”*.

The electromagnetic exposure was carried out in discrete 42.2 and 61.0 GHz bands for 3 hours. The ultrasonic exposure was carried out in the

18.0 and 60.0 kHz bands for 1 hour. Standard probiotic strains of lactobacilli: *L. rhamnosus*, *L. acidophilus* and strains of *L. plantarum* picked in the gut of bees, were exposed to the EM field and the ultrasound.

The evaluation was given to: 1) adhesive activity of lactobacilli using the mean adhesion index (MAI), adhesion coefficient (AC) and the micro-organism adhesion index (MOAI) [16, 17] and 2) antagonistic activity of lactobacilli and their exometabolites towards pathogenic strains of *C. diphtheria*, of *S. aureus* and yeast-like fungi of the genus *Candida*. The method of delayed antagonism on the spectrum of action was used, i.e. it was considered the ability to suppress the vital activity of different number of test cultures with the indication of growth retardation zones and determination of their biofilm formation during inter-microbial interaction [18, 19].

The results were processed according to the rules of variation statistics [20] using standard programs.

2. Discussion of the exposure results. Preliminarily potential strain-candidates of lactobacilli were selected from alternative ecoiniches, specifically from practically healthy people aged 17 to 23 and among representatives of normocenosis of bees. Nineteen bee families were examined to pick $10^5 \dots 10^6$ CFU/g of lactobacilli in the gut of healthy bees.

The cultivation of bacteria was held under aerobic and microaerophilic conditions. The main reason is that in the biological niches of the human body *in vivo*, the culture conditions of the bacteria differ significantly when extracted *in vitro*. One of important parameters of the existence and development of microorganisms is the gas composition

of the incubation atmosphere. The atmosphere of reduced partial pressure of oxygen and increased partial pressure of carbon dioxide (the microaerophilic conditions) reproduces, to a certain extent, the conditions of lactobacilli *in vivo*.

The application efficiency of the agents is determined also by the ability of lactobacilli probiotic strains to gain a foothold in human epithelial cells and propagate themselves before mucosal layer cells are renewed. For the experimental investigation results on adhesive properties of *Lactobacillus spp.*, see Table 1.

According to the obtained data, the strains *L. plantarum* and *L. rhamnosus* under aerobic conditions of cultivation have average adhesive abilities. The adhesive abilities of the strain *L. acidophilus* are worse (the MOAI is under 2.5).

Under microaerophilic culture conditions, the adhesive activity of all lactobacilli strains gets better. The MAI increases by 1.2 to 1.3 times ($p < 0.05$), the AC for *L. rhamnosus* and *L. plantarum* strains increases, on the average, by 1.2 times ($p < 0.05$) (Fig. 1).

Thus, a reduced partial pressure of oxygen stimulates the lactobacilli ability to gain a foothold in eukaryotic cells.

According to the evaluation results on the antagonistic activity of lactobacilli picked in different ecoiniches, all the studied lactobacilli strains cannot suppress the growth of yeast-like fungi of *Candida* genus under both aerobic and microaerophilic cultivation conditions.

The amount of antagonistic properties of *Lactobacillus spp.* towards *C. diphtheriae* and *S. aureus* depends on the primary residence range of the antagonistic strains.

Table 1. Adhesion indicators of lactobacilli strains under different conditions of cultivation

<i>Lactobacillus spp.</i>	Adhesion indicators ($M \pm m$)	Cultivation conditions	
		aerobic	microaerophilic
<i>L. plantarum</i>	MAI	$1.77 \pm 0.11^*$	$2.15 \pm 0.14^*$
	AC, %	$63.3 \pm 0.67^*$	$73.3 \pm 1.76^*$
	MOAI	2.79 ± 0.16	2.95 ± 0.24
<i>L. rhamnosus</i>	MAI	$1.49 \pm 0.09^*$	$1.98 \pm 0.16^*$
	AC, %	$52.0 \pm 2.00^*$	$61.2 \pm 1.84^*$
	MOAI	2.87 ± 0.23	3.04 ± 0.23
<i>L. acidophilus</i>	MAI	$1.09 \pm 0.08^*$	$1.42 \pm 0.16^*$
	AC, %	$51.3 \pm 4.81^*$	58.0 ± 2.00
	MOAI	2.13 ± 0.06	2.44 ± 0.20

* $p < 0.05$.

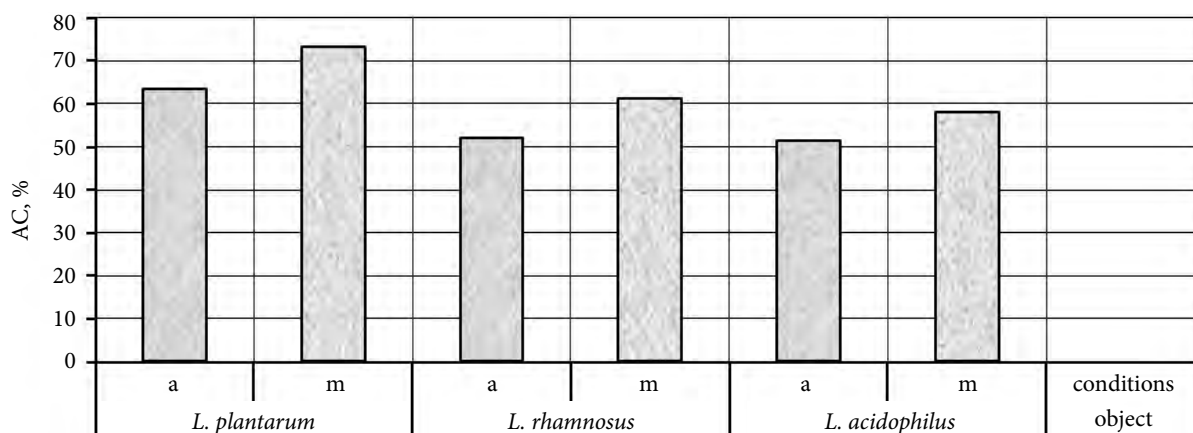


Fig. 1. AC changes in *L. plantarum*, *L. rhamnosus* and *L. acidophilus* lactobacilli cultivated under aerobic (a) and microaerophilic (m) conditions

Table 2. Antagonistic activity of *L. plantarum* picked in the gut of bees towards pathogens under different culture conditions

Functional indices	Pathogens	Culture conditions	
		aerobic	microaerophilic
Growth dynamics, mm ($M \pm m$)	<i>C. diphtheriae</i>	$1.71 \pm 0.71^{**}$	$8.14 \pm 1.18^*$
	<i>S. aureus</i>	0.0^{**}	$2.67 \pm 0.40^*$

* $p < 0.001$ compared to aerobic culture conditions; ** $p < 0.001$ compared to probiotic strains.

In 100 percent of cases, the greatest anti-diphtheria activity is offered by *L. plantarum* strain picked in the gut of bees. Lactobacilli strains picked in humans mostly show moderate antagonistic properties. On the average, 24.7% (aerobic) and 76.7% (microaerophilic) of the pathogens and the standard probiotic strains (*L. rhamnosus* and *L. acidophilus*) did not affect the growth of the test cultures in all the aerobic cultivation conditions and were 86.96% ineffective.

The ability to suppress the growth of golden staphylococcus strains under the aerobic and microaerophilic cultivation conditions is shown by all the lactobacilli strains examined. Circulating strains of lactobacilli, no matter whether picked in humans or bees, suppress the growth of about a third of the *S. aureus* crops studied, and the probiotic strains do not possess antagonistic properties towards more than 90% of the test crops. As with diphtheria, an increase in the antagonistic activity was observed under microaerophilic culture conditions.

The reason is that lactobacilli are microaerophils, i.e. the environment with an increased partial pressure of carbon dioxide and reduced oxygen is more favourable for their growth.

For the further research, *L. plantarum* strain picked in the gut of bees is chosen as having pronounced antagonistic properties towards *C. diphtheriae* and *S. aureus* strains. The investigation results are shown in Table 2.

As a result, the diameter of the growth retardation zone of *C. diphtheriae* strain under the microaerophilic cultivation conditions increased significantly: by 4.8 times ($p < 0.05$) as compared to the aerobic conditions, and in *S. aureus* strain from 0 to 2.67 mm, respectively ($p < 0.05$).

Among the most significant factors that influence the nature of inter-microbial relationships, namely the development of other members of biocenosis, are the effects of bacteria-produced substances. The effect of exometabolites of *L. plantarum* strain on the growth dynamics and biofilm formation of *C. diphtheriae* and *S. aureus* is illustrated in Table 3.

A statistically significant inhibitory effect on the growth properties of pathogenic bacteria is exerted by the addition of exometabolites of strain *L. plantarum* in the amount of 0.1 and 0.3 ml per 1.0 ml of the nutrient medium. The exometabolites of the candidate strain in the amount of 0.1 ml suppresses, on the average, the growth dynamics of patho-

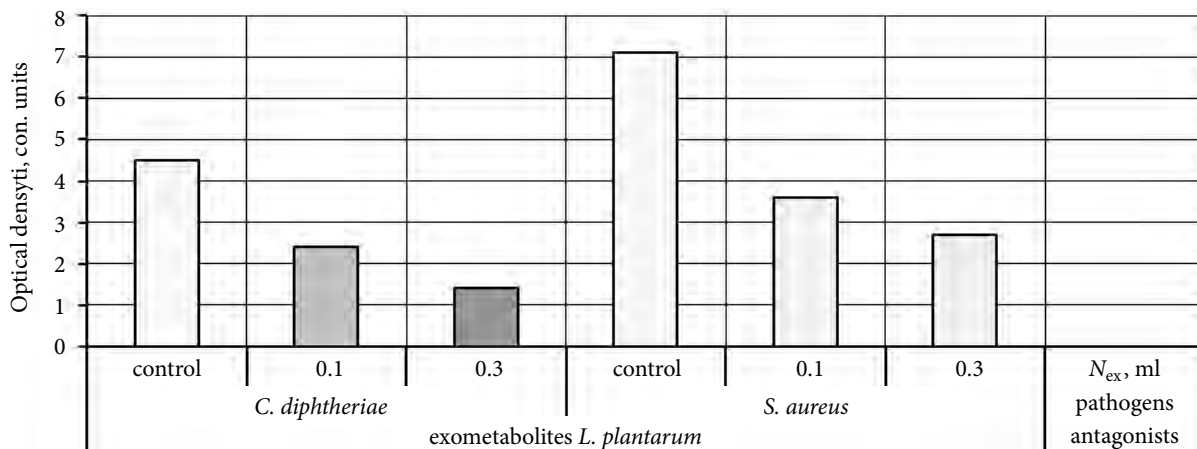


Fig. 2. Change of biofilm formation in pathogenic test cultures of *C. diphtheriae* and *S. aureus* as a result of the antagonistic activity of exometabolites of strain *L. plantarum* (N_{ex} is the number of exometabolites in 1.0 ml of the medium)

Table 3. Effect of *L. plantarum* exometabolit on growth dynamics and biofilm formation of *C. diphtheriae* and *S. aureus*

Functional indices	Pathogens	Exkzometabolit quantity in 1.0 ml of medium		
		control	0.1 ml	0.3 ml
Optical density, con. units ($M \pm m$)				
Growth dynamics	<i>C. diphtheriae</i> ($n = 7$)	2.1 \pm 0.3	1.6 \pm 0.3*	0.9 \pm 0.2*
	<i>S. aureus</i> ($n = 15$)	6.9 \pm 0.4	5.7 \pm 0.4*	3.9 \pm 0.4*
Biofilm formation	<i>C. diphtheriae</i> ($n = 9$)	4.5 \pm 0.6	2.4 \pm 0.2*	1.4 \pm 0.1*
	<i>S. aureus</i> ($n = 18$)	7.1 \pm 0.7	3.6 \pm 0.3*	2.7 \pm 0.2*

* $p < 0.05$.

genic corynebacteria by 1.3 times ($p < 0.05$), and of golden staphylococcus by 1.2 times ($p < 0.05$) compared to the control one. By increasing the dose of exometabolites in the medium, the pathogen growth dynamics is inhibited by 2.3 and 1.8 times ($p < 0.05$), respectively.

The addition of exometabolites in the amount of 0.1 ml to strains of pathogenic corynebacteria decreases the biofilm formation on the average by 1.9 times ($p < 0.05$), and in golden staphylococcus — by almost 2.0 times ($p < 0.05$). The addition of a 0.3 ml dose inhibits the biofilm formation in *C. diphtheriae* on the average by 3.2 times ($p < 0.05$), in *S. aureus* — by 2.6 times ($p < 0.05$) (Fig. 2).

It should be noted that the inhibition of the corynebacteria ability to form biofilms is directly proportional to the concentration of exometabolites in the medium.

According to the WHO recommendations with regard to the identification of candidate strains to be put into production, they should not inhibit the host normal flora. Investigations were carried out

on the effect exometabolites of candidate strain *L. plantarum* exert on the bioplane formation ability in clinical strains *Lactobacillus spp.* The result is a moderately decreased biofilm formation as opposed to pathogenic bacteria with suppression significant.

In addition, the effect of exometabolites of *L. plantarum* strain on enzymatic and phagocytic activity was investigated. The inhibition of invasion enzymes in *S. aureus in vitro* plasmocoagulase and lecithinase strains [21] has been established. A stimulation of phagocytosis values in neutrophilic leukocytes of donors was noted, including phagocytic activity, oxygen metabolism of neutrophils and opsonization of bacteria [22].

It has been shown that exometabolites of *L. plantarum* strain can inactivate or partially degrade an enzyme of aggression, such as diphtheria toxin. The determination of the toxicity of diphtheria toxin was carried out on laboratory animals (guinea pigs weighing 350...400 g) [23].

The analysis of the obtained results suggests that the candidate strain *L. plantarum* picked in the

gut of bees can be recommended as a production strain in the development of potential methabiotic complexes for therapeutic and prophylactic purposes. The suppression of the functional properties of pathogenic bacteria by co-culturing them with lactobacilli is the basis of the subsequent search for alternative and safe ways to suppress persistent pathogens.

Next, the use of EM and ultrasonic technologies for targeted modification of the functional indices of lactobacilli will be considered.

Microwave radiation is known to affect the electrostatic relationships in the bacterial agent-host cell system and to change the adhesive properties on the surface of bacteria. The previous experimental works revealed a possibility to modify adhesive properties of pathogenic bacteria *C. diphtheriae* [24]. In the present research, normophlora representatives are exposed to EM fields and the ultrasound.

The electromagnetic field exposure of lactobacteria *L. plantarum*, *L. rhamnosus*, and *L. acidophilus* was held in the 42.2 and 61.0 GHz frequency bands under aerobic cultivation conditions for 3 hours. Table 4 illustrates the corresponding changes in the adhesive activity.

As a result, for *L. plantarum* and *L. rhamnosus* strains, the average number of microbes attached to one erythrocyte decreases on the average by 1.7 and 3.8 times ($p < 0.05$), respectively, when irradiated in the 42.2 GHz band. The percentage of erythrocytes having adhesive microorganisms on their surface decreases on the average by 1.3 and 2.0 times ($p < 0.05$), and the adhesion

index of the microorganisms decreases by 1.3 and 1.9 times ($p < 0.05$) (Table 4). The *L. acidophilus* strain, in contrast, exhibits an increase in the MAI and MOAI, on the average, by 1.4 and 1.2 times, respectively ($p < 0.05$), whereas the AC tends to be suppressed.

The use of the millimeter waves in the 61.0 GHz band leads to the stimulation of the adhesive process in *L. plantarum* and *L. acidophilus* strains. The MAI increases on the average by 1.2 ($p < 0.05$) and 2.1 times ($p < 0.05$) and the AC — on the average, by 1.3 times ($p < 0.05$) in both cases. As for the MOAI, *L. acidophilus* shows its average increase by 1.6 times ($p < 0.05$), whereas *L. plantarum* shows little change in the adhesion index. *L. rhamnosus* strain irradiation in the 61.0 GHz band results in the MAI suppression by 2.2 times ($p < 0.05$) on the average and the MOAI — by 2.0 times ($p < 0.05$) (Fig. 3).

Thus, the frequency dependence of electromagnetic effect efficiency on the functional state of probiotic strains of lactobacilli cultured under aerobic conditions is observed. In most cases, the exposure in the 42.2 GHz band inhibits the adhesive properties, and in 61.0 GHz band stimulates them.

The exposure to the ultrasound was held in the 18.0 and 60.0 kHz frequency bands for 1 hour. In the result, the lactobacilli adhesion significantly decreases in all probiotic strains (Table 4). At the 18 kHz frequency band exposure, the MAI decreases by 1.8...7.5 times ($p < 0.05$), the AC — by 1.2...2.9 times ($p < 0.05$), and the MOAI — by 1.3...2.5 times ($p < 0.05$). The exposure to the 60 kHz band ultrasound reduces the average number of microbes attached to one erythrocyte (MAI)

Table 4. Effect of low-intensity EM field and ultrasound on the functional indices of the adhesion of probiotic strains at the cultivation in aerobic conditions

<i>Lactobacillus</i> spp.	Adhesion indices ($M \pm m$)	Control	Effect mode			
			EM field		ultrasound	
			42.2 GHz	61.0 GHz	18.0 kHz	60.0 kHz
<i>L. plantarum</i>	MAI	1.77 ± 0.11	1.05 ± 0.02*	2.05 ± 0.06*	0.8 ± 0.03*	0.73 ± 0.07*
	AC, %	63.3 ± 0.67	50.0 ± 1.2*	79.3 ± 1.76*	53.33 ± 3.5*	50.7 ± 4.67*
	MOAI	2.79 ± 0.16	2.11 ± 0.04*	2.78 ± 0.08	1.5 ± 0.04*	1.45 ± 0.03*
<i>L. rhamnosus</i>	MAI	1.49 ± 0.09	0.39 ± 0.02*	0.67 ± 0.04*	0.2 ± 0.02*	0.41 ± 0.02*
	AC, %	52.0 ± 2.0	25.3 ± 0.67*	48.0 ± 3.46	18.0 ± 3.05*	28.6 ± 1.33*
	MOAI	2.87 ± 0.23	1.53 ± 0.07*	1.41 ± 0.02*	1.13 ± 0.07*	1.44 ± 0.02*
<i>L. acidophilus</i>	MAI	1.09 ± 0.08	1.54 ± 0.16*	2.36 ± 0.17*	0.61 ± 0.02*	0.42 ± 0.01*
	AC, %	51.3 ± 4.81	46.67 ± 2.7	68.7 ± 1.76*	36.7 ± 0.67*	28.7 ± 1.33*
	MOAI	2.13 ± 0.06	3.28 ± 0.17*	3.43 ± 0.16*	1.65 ± 0.02*	1.52 ± 0.06*

* $p < 0.05$.

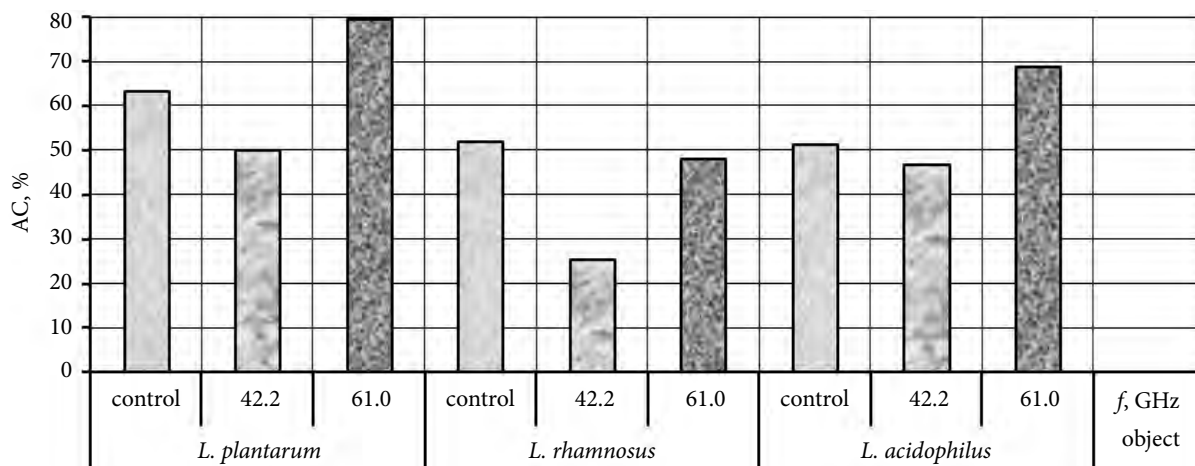


Fig. 3. Changes in AC of lactobacteria *L. plantarum*, *L. rhamnosus* and *L. acidophilus* cultivated under aerobic conditions and exposed to EM fields

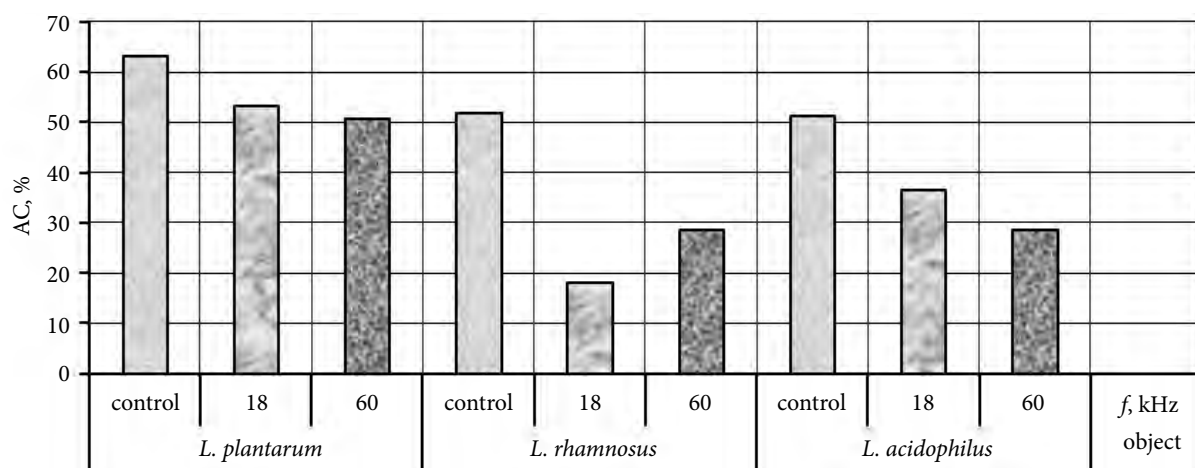


Fig. 4. Changes in AC of lactobacteria *L. plantarum*, *L. rhamnosus*, and *L. acidophilu* cultivated under aerobic conditions and exposed to the ultrasound

by 2.4...3.6 times ($p < 0.05$). The percentage of erythrocytes having adhesive lactobacilli (AC) on their surface decreases by 1.2...1.9 times ($p < 0.05$) and the adhesion index of microorganisms decreases by 1.4...2.0 times ($p < 0.05$) (Fig. 4).

Thus, the ultrasound exposure is a factor that can lead to a change in the state of the objects under study, which is evident, in particular, in the suppression of the adhesive properties of *Lactobacillus spp.* under aerobic conditions of the cultivation. Also, the frequency dispersion dependence can be seen in the effect magnitude, with general behavioral trends retained.

One of the tasks was to increase the ability of *Lactobacillus spp.* strains to suppress the growth of *C. diphtheriae* and *S. aureus* test cultures due to the EM field and ultrasound exposures.

For this purpose, *L. plantarum* strain was exposed to the EM field in the 42.2 and 61.0 GHz frequency bands for 3 hours. The antagonistic activity of lactobacilli after the co-cultivation in aerobic and microaerophilic conditions was determined by the change of the growth retardation zone diameter of pathogenic strains.

The obtained data analysis (Table 5) suggests a significant increase in the diameter of the growth retardation zones of the test crops when they are co-cultured with irradiated *L. plantarum* strains compared to the control (non-irradiated) ones.

After the *L. plantarum* treatment in the 61.0 GHz frequency band, the antagonism of lactobacilli rises by 3.3 times ($p < 0.05$) compared to the strains of *C. diphtheriae*. For *S. aureus*, the diameter of the growth inhibition zone increases from 0 to

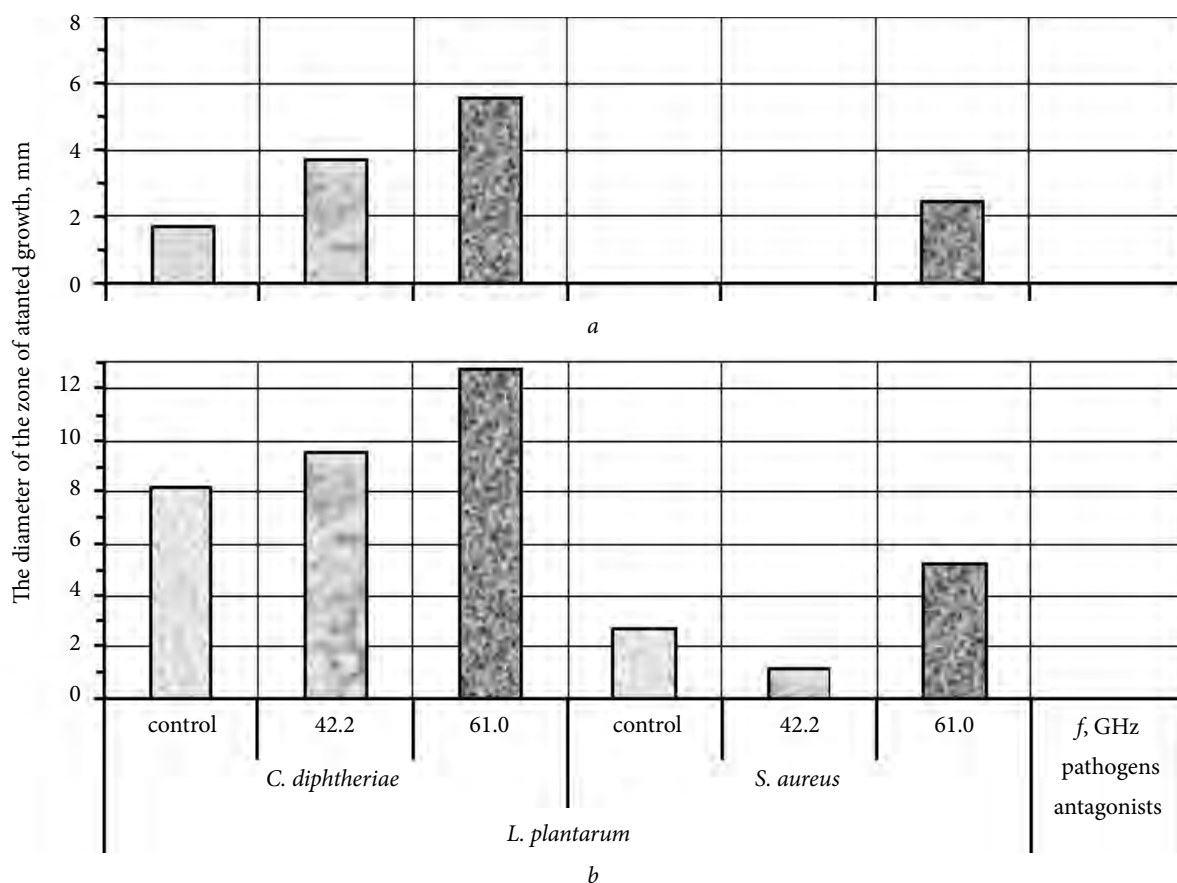


Fig. 5. Changes in the growth properties of the pathogenic *C. diphtheriae* and *S. aureus* strains as a result of the antagonistic activity of the *L. plantarum* strain due to the EM field exposure at the co-cultivation in aerobic (a) and microaerophilic (b) conditions

Table 5. Influence of low-intensity EM field on the antagonism of *L. plantarum* strains towards pathogens under different conditions of cultivation

Pathogens functional indices	Pathogens	Cultivation conditions					
		aerobic			microaerophilic		
		control	42.2 GHz	61.0 GHz	control	42.2 GHz	61.0 GHz
Growth dynamics, mm ($M \pm m$)	<i>C. Diphtheriae</i> ($n = 7$)	1.71 \pm 0.71	3.71 \pm 0.36*	5.58 \pm 0.7*	8.14 \pm 1.18	9.57 \pm 1.25	12.7 \pm 0.84*
	<i>S. aureus</i> ($n = 15$)	0.0	0.0	2.47 \pm 0.47*	2.67 \pm 0.40	1.13 \pm 0.41*	5.27 \pm 0.28*

* $p < 0.05$.

2.47 mm (Fig. 5) under aerobic conditions of the co-cultivation.

At the lactobacillus exposure to the EM field in the 42.2 GHz band, a double increase of the growth inhibition zones was observed only for *C. diphtheriae* strains ($p < 0.05$). The *S. aureus* test cultures sensitivity to the irradiated antagonist strain does not change.

Under the microaerophilic conditions, the EM field exposure of *L. plantarum* in the 61.0 GHz

band increases the antagonistic properties towards *C. diphtheriae* by 1.5 times ($p < 0.05$) and towards *S. aureus* – by 2 times ($p < 0.05$). The irradiation in the 42.2 GHz frequency band yields an opposite effect. The growth retardation zone of *S. aureus* gets 2.4 times less ($p < 0.05$). For *C. diphtheriae*, this effect is not observed (Fig. 5).

Thus, the irradiation of *L. plantarum* strains changes their antagonistic properties towards the *C. diphtheriae* and *S. aureus* test cultures.

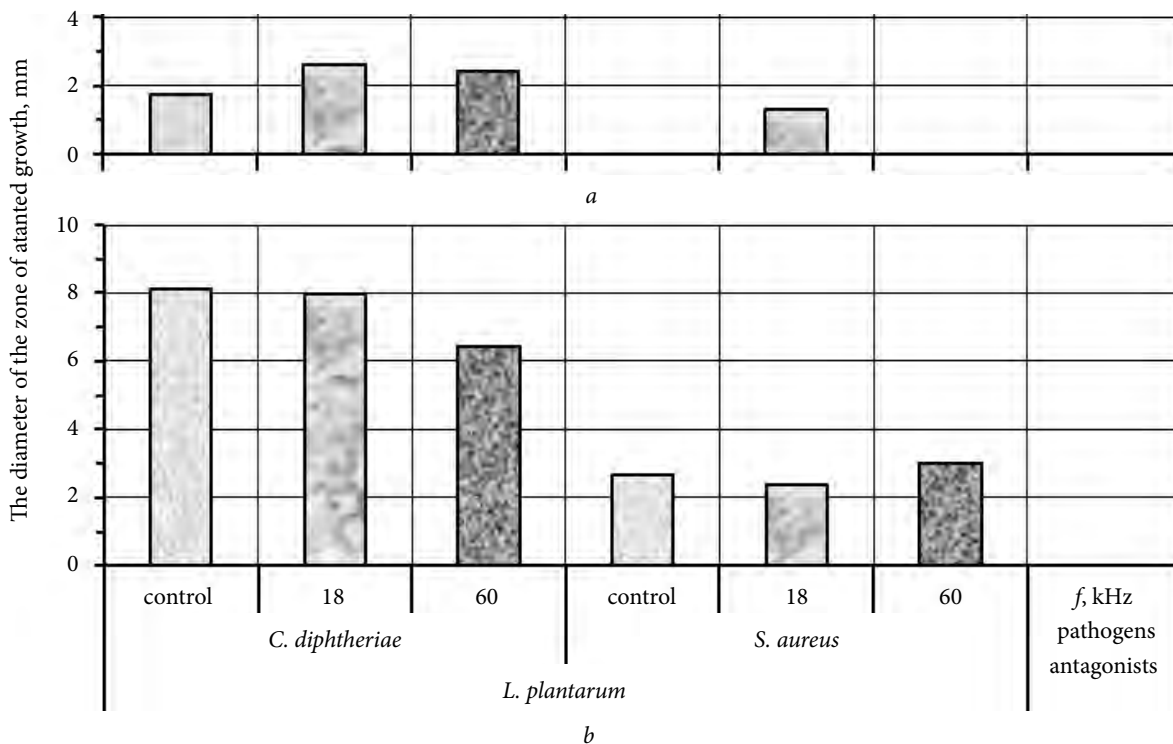


Fig. 6. Changes in the growth properties of *C. diphtheriae* and *S. aureus* pathogenic strains due to the antagonistic activity of *L. plantarum* strain after the ultrasound exposure for the co-cultivation in aerobic (a) and microaerophilic (b) conditions

Table 6. The ultrasound exposure effect on the antagonistic activity of *L. plantarum* strains towards pathogens in view of different co-cultivation conditions

Pathogens functional indices	Pathogens	Cultivation conditions					
		aerobic			microaerophilic		
		control	18.0 kHz	60.0 kHz	control	18.0 kHz	60.0 kHz
Growth dynamics, mm (M ± m)	<i>C. diphtheriae</i> (n = 7)	1.71 ± 0.71	2.57 ± 0.53	2.42 ± 0.48	8.14 ± 1.18	8.0 ± 1.05	6.43 ± 1.56
	<i>S. aureus</i> (n = 15)	0.0	1.27 ± 0.41*	0.0	2.67 ± 0.40	2.4 ± 0.48	3.0 ± 0.35

* p < 0.05.

This effect exhibits the radiation frequency dispersion. Namely, the 61.0 GHz exposure raises the antagonism of lactobacilli in all the cases. Yet, the exposure to the 42.2 GHz field brings an opposite result, which in turn depends on pathogenic bacteria strains and conditions of their co-cultivation.

There is a dependence of the antagonistic activity of the irradiated lactobacilli with non-irradiated pathogenic strains on conditions of the co-cultivation. Under the microaerophilic (*in vivo*) and aerobic (*in vitro*) co-cultivation conditions, a biological response to the EM field exposure may have an opposite result.

The ultrasound exposure was in the 18 and 60 kHz bands and lasted for 1 hour.

The results of the lactobacilli antagonistic activity towards pathogens after the ultrasound exposure and in view of different cultivation conditions are shown in Table 6.

Under the aerobic culture conditions, the ultrasound exposure at 18 kHz is effective, with the growth retardation zone diameter increase from 0 to 1.27 mm for *S. aureus*. For the microaerophilic conditions, no significant changes are observed (Fig. 6).

Conclusions. It has been found that among lactobacilli strains taken from standard probiotic preparations and picked in alternative econiches, i.e. from humans and bees, those selected from the gut of healthy bees, *L. plantarum*, have the greatest antagonistic activity. Exometabolites of candidate

strain *L. plantarum* are able to suppress enzymes of aggression and invasion of pathogenic corynebacteria and golden staphylococcus, reduce their growth potential and ability to form biofilm, and do not inhibit human normal flora. The use of *L. plantarum* strain is promising as a production one in the development of potential methabiotic complexes for therapeutic and prophylactic purposes.

The effect of the environmental atmosphere gas composition on the viability of microorganisms when co-cultivated with other strains under aerobic and microaerophilic conditions has been shown. Microaerophilic cultivation conditions that imitating the *in vivo* residence of bacteria have been prioritized.

A possibility has been found to modify properties of microorganisms by their irradiation with the ultrasound and a low-intensity EM field in the UHF range, implying, in particular, the adhesive

and antagonistic properties of lactobacilli towards *C. diphtheriae* and *S. aureus* test cultures.

There is a dispersion dependence of the effect on the radiation frequency of the EM field and the ultrasound. The exposure in the 61.0 GHz frequency band increases adhesiveness and activates the lactobacilli antagonism. For the exposure in the 42.2 GHz band, the adhesion is suppressed, and the antagonistic abilities weaken. The ultrasound exposure in the 18.0 and 60.0 kHz bands, leads to the suppression of the adhesive properties. A comparison of the two physical factors, i.e. the EM field and the ultrasound, shows that the electromagnetic field exposure exerts more effect.

Individual features of different pathogenic strains have been studied to find out that the *C. diphtheriae* test cultures are more susceptible to the irradiated antagonistic strains than those of *S. aureus* ones.

REFERENCES

1. Electromagnetic fields and human health. In: *Proc. 2nd Int. Conf. Problems of human electromagnetic safety. Fundamental and applied research. EM field normalization: philosophy, criteria and harmonization*. Moscow, Russian Federation, 20–24 Sept. 1999 (in Russian). Moscow, 1999.
2. Kolbun, N.D., Bessonov, A.E., and Volynyuk, R.E., 1993. *Information and wave therapy: scientific-practical guide*. Kiev: Ukr. Enycl. Publ. (in Russian).
3. Kravkov, G.A., 2006. *Effect of non-thermal (informational) effects of electromagnetic radiation of extremely high frequency on biological objects and humans*. Part 1. Kiev. 123 p. (in Russian).
4. Zelentsov, V.I., Perel'muter, Ya.M., Cha, V.A., Fal'kovich, V.M., Votoropin, S.D., 2007. Biological effects of low intensity millimeter electromagnetic radiation. *Millimeter Waves in Biology and Medicine*, 2(46), pp. 42–49 (in Russian).
5. Perlmutter, D., Loberg, K., 2015. *Brain Maker: The Power of Gut Microbes to Heal and Protect Your Brain – for Life*. Publisher: Little, Brown Spark.
6. Valyshev, A.V., Gilmudtinova, F.G., 2006. Microbial Ecology of the Human Digestive Tract. In: O.V. Bukharin, ed. 2006. *Ecology of Human Microorganisms*. Yekaterinburg: Institute of Cellular and Intracellular Symbiosis Publ., pp. 169–290 (in Russian).
7. Volodin, N.N., Degtyareva, M.V., 2000. Role of pro- and anti-inflammatory cytokines in immune adaptation of newborn babies. *Int. J. Immunorehabilit.*, 2, pp. 175–184 (in Russian).
8. Kartashova, O.L., Usvyatsov, B.J., 2006. Human skin microflora. In: O.V. Bukharin, ed. 2006. *Ecology of Human microorganisms*. Yekaterinburg: Institute of Cellular and Intracellular Symbiosis Publ., pp. 61–102 (in Russian).
9. Wenner, M., 2008. Going with his gut bacteria. *Sci. Amer.*, 299(1), pp. 90–92.
10. Yankovsky, D.S., Dyment, G.S., 2008. *Microflora and Human Health*. Kiev: Chervona Ruta-Tours Publ. (in Ukrainian).
11. Kalinichenko, S.V., Korotkykh, O.O., Tishchenko, I.Yu., 2013. Current state of development and use of probiotic, prebiotic and sinbiotichny medicines (review). *Annals of Mechnikov Institute*, 3, pp. 5–12 (in Ukrainian).
12. Soloveva, I.V., Tochilina, A.G., Novikova, N.A., Belova, I.V., Ivanova, T.P., Sokolova, K.Ya., 2010. Study of biological properties of new lactobacillus strains. *Vestnik of Lobachevsky State University of Nizhni Novgorod*, 2(2), pp. 462–468 (in Russian).
13. Bengmark, S., 2003. Synbiotic treatment in Clinical Praxis. In: *Host Microflora Crosstalk. Old Herborn University Seminar*, 16, pp. 69–82.
14. Shenderov, B.A., 2005. Probiotics, prebiotics and synbiotics. General and selected sections of the problem. *Food ingredients. Raw materials and additives*, 2, pp. 23–26.
15. Maslyanko, R.P., 1999. *Principles of Immunobiology*. Lviv: Vertikal' Publ. (in Ukrainian).
16. *Standardization of preparation of microbic suspensions: Information letter on innovations in health care system*. 163. Kyiv: Ukrmedpatentinform, 2006. 10 p. (The normative document. Ministry of Health of Ukraine; Ukrainian center of scientific medical information and patent and license work. Information letter) (in Ukrainian).
17. Brilis, V.I., Brilene, T.A., Lentsner, H.P., Lentsner, A.A., 1986. Technique of studying of adhesive process of microorganisms. *Laboratory business*, 4, pp. 210–212.
18. Yegorov, N.S., 1965. *Microbes-antagonists and biological methods of determining antibiotic activity*. Moscow: Higher School Publ. (in Russian).

19. Borshch, S.K., Seredyuk, N.M., Kutsik, G.V., 2004. Studying of antagonistic activity of the lactobacilli entered into probiotic medicine laktobakterin, etiologicheskyy factors of an intestinal dysbiosis, pyoinflammatory processes and probiotic strains of microorganisms. *Galician Med. J.*, 3, pp. 16–19 (in Ukrainian).
20. Bendat, J., Pirsol, A., 1989. *Applied random data analysis*. Translated from English by V.E. Privalsky, A.I. Kogubinsky. Moscow: Mir Publ. (in Russian).
21. Popov, M.M., Kalinichenko, S.V., Chausovska, T.A., Babich, E.M., Korotky, A.A., Kivva, F.V., Kovalenko, O.I., Balak, A.K., 2016. Influence of electromagnetic radiation and gas composition of the atmosphere cultivation on ability of stafilocokk and korinebakteriya to bioplivkoutvorenniya. *Biomedical and biosocial anthropology*, 26, pp. 45–49 (in Ukrainian).
22. Kalinichenko, S.V., 2005. Kalinichenko, S.V., 2005. Influence of millimeter waves on an allosteric regulation of enzymes of the corynebacteria associated with a cycle of trikarbonovy acids. *Problems of uninterrupted medical training and science*, 2, pp. 81–83 (in Ukrainian).
23. Kalinichenko, S.V., Babych, E.M., Kivva, F.V., Ryabovol, E.V., Gorbunov, L.V., Ryzhkova, T.A., Sklyar, N.I., Kovalenko, O.I., Katrych, V.A., Zhdamarova, L.A., Bobyryeva, I.V., Kalinichenko, E.O., 2011. The influence of physical and physico-chemical factors on immunobiological properties of diphtheria toxin. *Annals of Mechnikov Institute*, 4, pp. 316–320 (in Ukrainian).
24. Kalinichenko, S.V., Babich, E.M., Kuchma, I.Yu., Volyansky, Yu.L., Sklyar, N.I., Krestetska, S.L., Drach, M.I., Kolokolova, O.B., Rudenko, L.M., Kivva, F.V., Kovalenko, O.I., 2007. *Method for modulation of microorganisms adhesive properties*. Ukraine. Pat. 79848 (in Ukrainian).

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ЕЛЕКТРОМАГНІТНІ ТА АКУСТИЧНІ ТЕХНОЛОГІЇ В РОЗРОБЦІ АНТИБАКТЕРІАЛЬНИХ ПРЕПАРАТІВ

Предмет і мета роботи. Розглянуто можливості застосування хвильових технологій в розробці альтернативних антибіотикам способів пригнічення розвитку патогенної мікрофлори. Мета — дослідити конкурентні можливості штамів лактобацил, виділених з різних еконіш, щодо патогенних штамів, провести цілеспрямовану модифікацію адгезивних і антагоністичних властивостей лактобацил шляхом впливу на них низькоінтенсивних електромагнітних полів (ЕМП) та ультразвуку (УЗ).

Методи і методологія роботи. Робота є експериментальною. Досліджувалися лактобацили з різних еконіш: 1) стандартні штами з пробіотичних препаратів, 2) циркулюючі штами, виділені від людей і бджіл. Для опромінення ЕМП використовувалися генератори Г4-141 і Г4-142, для ультразвукового впливу — генератори ГЗ-109 і ГЗ-Ф. Оцінювалися адгезивні показники та антагоністична активність штамів *Lactobacillus spp.* щодо *C. diphtheriae*, *S. aureus* і дріжджоподібних грибів роду *Candida* в аеробних і мікроаерофільних умовах культивування. При обробці й аналізі результатів використовувалися статистичні методи.

Результати роботи. Встановлено, що штам *L. plantarum*, виділений з кишечника здорових бджіл, володіє найбільш вираженими конкурентними властивостями по відношенню до патогенів. Показано, що пріоритетними для лактобактерій є мікроаерофільні умови культивування, що імітують перебування їх *in vivo*. Встановлено можливість модифікації властивостей мікроорганізмів за допомогою їх опромінення УЗ і низькоінтенсивними ЕМП у вузьких смугах НЗВЧ-діапазону. Спостерігається дисперсійна залежність ефективності впливу від частоти. Встановлено індивідуальні особливості для різних патогенних штамів.

Висновок. Отримані результати відкривають перспективу розвитку електромагнітних і акустичних технологій у створенні альтернативних і безпечних засобів, здатних протистояти персистуючим патогенам та підвищувати стійкість до них організму людини.

Ключові слова: електромагнітне поле, ультразвук, лактобактерії, дифтерія, золотистий стафілокок, адгезивна та антагоністична активність, модифікація.