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Combined action of low temperature and magnetic field of different intensities on growth of some bacterial species *in vitro*

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ABSTRACT

Growth dynamic of bacterial population after influence of magnetic field and cryoaction has been studied. *In vitro* experiments were performed on *Bacillus cereus*, *Staphylococcus aureus,* and *Lactobacillus delbrueckii* cultures. The most significant result was obtained in experiments with *Bacillus cereus*. When applied separately, cryoaction and alternating magnetic field were not able to kill this microorganism, whereas combined action of these factors led to the complete devitalization of bacterial culture.

Keywords: Magnetic Field Treatment; Cryotreatment; Bacillus Cereus

1. INTRODUCTION

We have presented here the novel combined method for devitalization of bacteria. Proposed combination brings together two well-known approaches, namely cryotreatment and magnetic treatment of microorganisms. When these effects are superposed the observed result often exceeds the influence of each treatment. So, synergetic action takes place.

Cryotreatment method is based on freezing and thawing of biological objects. Nowadays this approach is becoming very popular among researchers and physicians. As it was previously shown, the increased time of freezing was accompanied with higher degree of degraded cells and tissues [1]. From the other hand, the rate of thawing is experimentally proved to be an important destructive factor in cryotherapy. Phenomena mentioned above are the basis of cryosurgery techniques in medicine.

It is known that magnetic fields (MF) with varying intensity and character have different effects on living organisms. Various biological objects, including viruses, bacteria and bacterial spores, lower fungi, animal cells, respond adversely to MF action. In some experiments, stimulation effect on growth of several bacteria and fungi under MF action (e.g. with value of magnetic induction of 15 mT) was observed [2]. Water is generally accepted to be the basis of living matter. Therefore, effects of MF on living organisms should be primarily considered in terms of its influence on water. MF changes some physical properties of water (conductivity, dielectric permittivity, viscosity). Dissolving capacity of water after MF treatment was increased. So, carbonates of calcium and magnesium are better solved in magnetized water in comparison with non-treated water. It is noteworthy that oxygen solubility also elevates in MF-treated water with simultaneous enhancement of chemical activity of this gas. Bactericidal properties of magnetized water and its influence on kinetics of some chemical reactions are mainly explained by oxygen activation in MF-treated water [3].

This study is aimed to clarify how freezing/thawing procedure and MF application influence growth of some microorganism species. Results of such experiments could be useful for elaboration of certain biotechnological processes, solving of some environmental problems as well as development of cytotoxic intervention for treatment of pathologically changed cells of highly organized living organisms. The last one may have promising practical application in clinics.

In some cases, physiological state of human organism depends on presence or absence of pathogenic microorganisms in the environment. It is recognized to be an important part of global health care.

Results presented herein are reserved by the patents of Ukraine [4,5].

2. MATERIALS AND METHODS

Combined influence of low temperatures and magnetic field was performed with using of bacterial culture sus-

pensions. Representatives of the microflora, occurring in postoperative wounds with septically complicated healing, and normal microflora from human large intestine were used as test objects [6]. We carried out our experiments with two opportunistic cocci (*Staphylococcus aureus*, strain 202 and *Staphylococcus aureus*, strain *wild*) as well as nonpathogenic spore-forming microorganism, *Bacillus cereus*. Normal microflora was represented by *Lactobacillus delbrueckii*.

Bacteria were suspended in distilled water in compliance with all requirements of sterility with the exception of experiments with *Lactobacillus delbrueckii*, in which physical factors affected bacterial culture directly in liquid nutrient medium MRS. The value of magnetic field used in experiments was 50 mT for constant MF and 30 mT for alternating MF. Temperature range from –20˚C to –45˚C was used in the experiments *in vitro*. Magnetic field was applied at both freezing and thawing stages. Exposition of magnetic field applied was in the range of 2 - 15 min.

To affect microorganisms by MF, the samples of bacterial suspension (volume 0.5 - 1 ml) in Eppendorf tubes of total volume of 1.5 ml were placed between the poles of electromagnet. In case of control and in the experiments when cryoaction was studied only, electromagnet was switched off. Sterile applicator, which brings liquid nitrogen, was immersed in the sample to a depth of 10 mm measured from the top of the meniscus of bacterial suspension.

Cryoaction on bacterial suspensions consisted of their cooling to –45˚C and storing for 70 sec. Thawing procedure took place in free regime at room temperature and lasted approximately 10 min each time.

In case of separate action of MF on bacteria, two regimes of magnetization (120 sec or 15 min) were applied. Since there was no difference in results, exposition of both alternating and constant MF in further experiments was chosen to be 120 sec. In case of combined influence of MF and cryoaction, MF was applied only during freezing of the samples plus another 50 sec after thawing with gradual elevation of temperature inside the tube.

After thawing, samples exposed to cryoaction or combined influence of MF and deep freezing, were divided into aliquots and then suspensions of both stains of *Staphylococcus aureus* and *Bacillus cereus* were sown in standard Petri dishes onto solid nutrient medium (ribopeptone agar, RPA). With regard to *Bacillus cereus*, nonfrozen samples (control and samples exposed to MF only) were subjected to the same procedure. As a rule, results of experiments were calculated on a day after sowing of material, and if it is necessary to 7 consecutive days.

Staphylococci and *Bacillus* were cultivated at 37˚C, *Lactobacillus* was cultivated at 39˚C (cultivation was carried out on liquid manufactured nutrient medium MRS). The latter culture was diluted with distilled water at a ratio of 1:25 before spectrophotometrical measurement.

Growth rate of bacteria cultivated on RPA (by 4 standard grades: +, ++, +++, ++++) or absence of growth (–) was determined visually. In addition, we estimated character of growth, the average number of colonies per 1 cm^2 and average diameter of colonies (mm).

Growth of *Lactobacillus* was tested by changes in optical density of culture in liquid medium. Spectrophotometrical analysis at 440, 490 and 540 nm was used because difference between values of optical absorption of control and experimental samples measured at these wave lengths appeared to be the most informative.

3. DISCUSSION

Influence of alternating and constant magnetic field (MF), deep freezing and combined action of these factors on growth of some bacterial species were studied. It has been demonstrated that constant MF has no effect on growth of both strains of *Staphylococcus aureus* (202 and *wild*) as well as spore-forming microorganism *Bacillus cereus*. Similarly, all above-mentioned microorganisms were not affected by alternating MF (**Tables 1**-**3**).

Cryoaction in all studied cases caused growth retardation of both *Staphylococci* and *Bacillus cereus* during the period of 1 - 2 days with the following growth restoration of selected cryostable subpopulation. Since cultural peculiarities of microorganisms survived at rapid freezing have to be interrogated, we preliminarily concluded that such an unexpected selection should be taken into consideration during cryomagnetic therapy of patients.

Intense growth stimulation of *Staphylococcus aureus* (202) induced by combined action of constant MF and freezing in comparison with intact control has been shown. On the other hand, combined influence of alternating MF and cryoaction had no effect on *Staphylococcus aureus* (*wild*) compared with freezing action alone (**Tables 1** and **2**).

Combined treatment of cryoaction and alternating MF led to the complete loss of spore-forming microorganism (*Bacillus cereus*) (**Figure 1**). In contrast, cryoaction applied alone resulted in the selection of a very viable cryostable subpopulation (**Table 3**).

In experiments with representative of human normal microflora of large intestine, *Lactobacillus* delbrueckii, separate and combined action of two factors was applied to samples of lactic acid bacteria on the 3-rd day of cultivation. In other words, primary culture was divided into four parts on the 3-rd day of cultivation: control and 3 experimental parts (AltMF, Cryo and AltMF + Cryo).

We showed that cryoaction had no effect on further growth of culture, whereas alternating MF applied separately from freezing induced stimulation of bacterial growth in the late periods, starting from the 4-th day after

Table 1. Influence of constant magnetic field (MF), deep freezing and their combination on growth of *Staphylococcus aureus* (202).

Type of influence	Intensity of growth over 1 day	A number of colonies per 1 cm^2 of RPA surface	Colony diameter, mm
Intact control	++	127 ± 20	0.21 ± 0.02
Post-MF	++	117 ± 20	0.20 ± 0.01
Cryo	+	28 ± 6	0.69 ± 0.07
Post-MF + Cryo	Conjoint growth	Can not be counted	Can not be counted

Table 2. Influence of alternating magnetic field (MF), deep freezing (Cryo) and their combined action (AltMF + Cryo) on growth of *Staphylococcus aureus* (*wild*).

Type of influence	Intensity of growth over			A number of colonies per 1 cm^2 of RPA surface on the 7-th day	Colony diameter on the 7-th day, mm
	1 day	4 days	7 days		
Intact control	+++	+++	++++	74 ± 7	0.96 ± 0.05
AltMF	+++	+++	++++	77 ± 9	0.66 ± 0.07
Cryo	-	++	+++	27 ± 3	0.92 ± 0.09
AltMF + Cryo	-	++	+++	39 ± 3	0.84 ± 0.04

Table 3. Influence of alternating magnetic field (MF), deep freezing (Cryo) and their combined action (AltMF + Cryo) on growth of *Bacillus cereus.*

Type of influence	Intensity of growth over			A number of colonies per 1 cm^2 of RPA surface on the 7-th day	Colony diameter on the 7-th day, mm
	1 day	4 days	7 days		
Intact control	+++	+++	++++	8 ± 1	3.93 ± 0.54
AltMF	+++	+++	++++	9 ± 1	2.88 ± 0.49
Cryo	-	+	++	0.5 ± 0.03	10.00 ± 1.47
AltMF + Cryo	-	-	-	-	-

Figure 1. Influence of alternating magnetic field, deep freezing and their combined action on growth of *Bacillus cereus* culture: upper right quadrant is control; lower right quadrant-alternating MF; left lower quadrant-cryoaction; left upper quadrant-cryomagnetic action.

onset of action. In contrast, combined action of alternating MF and rapid freezing markedly suppressed growth of *Lactobacillus* as it was observed for sporulating microorganism *Bacillus cereus* (**Table 4**).

Slight decrease in optical density of the primary (control) culture *Lactobacillus delbrueckii* on the 4-th day can be due to partial physiological loss of microorganisms from the first generation. S-like bends of growth curve are common for many bacteria in the absence of fresh nutrient medium. Therefore, these features of growth curve can be considered as normal ones.

Variety of cell populations and their viability are considered to be the basis of their resistance to various external impacts. Obviously, complete loss of *Bacillus cereus* culture occurs because bacteria is appeared to be unable to adapt appropriately to the new combination of harmful environmental factors applied in the experiment. It is therefore concluded that obtained results can be regarded in aspect of theoretical potential of cryotreatment for elimination of other microorganism populations.

Table 4. Influence of alternating magnetic field (AltMF), deep freezing (Cryo) and their combined action (AltMF + Cryo) on growth dynamics of *Lactobacillus delbrueckii* according to optical density of culture.

λ, nm	MRS medium	Period of cultivation and type of sample								
		3 days, primary culture* ↓	4 days				7 days			
			Control	AltMF	Cryo	(AltMF + Cryo)	Control	AltMF	Cryo	(AltMF + Cryo)
440	0.092	0.630	0.610	0.623	0.620	0.580	0.857	0.925	0.860	0.640
490	0.053	0.518	0.500	0.512	0.500	0.490	0.710	0.750	0.720	0.530
540	0.030	0.440	0.420	0.437	0.420	0.410	0.596	0.630	0.600	0.420

4. CONCLUSIONS

1) It has been shown that neither alternating magnetic field (MF) (induction 52 mT, frequency 50 Hz) nor constant MF (induction 30 mT) applied separately from deep freezing have not induced any changes in further growth of both *Staphylococcus aureus* (202) and *Staphylococcus aureus* (wild) cultures.

2) Deep freezing (up to –45˚C) achieved within 70 sec has suppressed intensity of growth of *Staphylococcus aureus* (202), *Staphylococcus aureus* (wild) and *Bacillus cereus*. However, combined with alternating MF, this factor significantly stimulated ($P < 0.05$) growth of *Staphylococcus aureus* (202). Combined influence of cryoaction and alternating MF had no substantial effect on growth of *Staphylococcus aureus* (wild) compared with the action of deep freezing applied alone.

3) The most intriguing result has been obtained in experiments with *Bacillus cereus*. Separate influence of cryoaction or alternating MF has appeared to be unable to kill microorganisms of this species, whereas combination of alternating MF and cryoaction led to complete loss of bacterial culture. This observation provoked heightened interest because sporulating microorganisms are known to be the most resistant towards aggressive factors of environment.

4) It has been demonstrated that both *Staphylococcus aureus* (202) and *Staphylococcus aureus* (wild) respond to the impact of cryoaction by pronounced growth retardation followed by rapid increase in biomass increment of cryo-resistant clones selected during the experiment. Since cultural features and degree of pathogenicity of novel substrains were not specifically examined, it should be taken into consideration that cryogenic therapy in clinics may attend appearance of microorganisms with unidentified characteristics.

5) Alternating MF has promoted growth of *Lactobacillus delbrueckii* at rather remote period of cultivation, in particular, the most pronounced action of this factor was registered since three days after impact. Cryoaction had not any effect on the studied culture and superposition of cryoaction and magnetic influence has caused significant inhibition of culture growth.

Obviously, constant and alternating MFs affect bacteria in different ways. This fact can be explained by high resistance of procaryotic organisms towards influence of various surrounding factors and ability of quick adaptation to new environmental conditions. To summarize, our data are meant to stimulate further studying in this area and perspectives of their practical application.

REFERENCES

[1] Afanas'eva, N.I., Shevtsov, V.Ch. and Muzghychuk, A.V. (2002) Perspectives of application of cryo-surgery in oncology. *The International Medical Journal*, **3**, 156-159.

[2] Binhy, V.N. (2002) Magnetobiology: Underlying physical problems. Academic Press, San Diego.

[3] Vasil'ev, V.I. (1985) Chemistry-traditional and paradoxical. Leningrad State University Publishers, Leningrad.

[4] Raichenko, O.I., Mosienko, V.S., Chernenko, L.I., Yanish, Yu.V., Derev'yanko, O.V. and Karnaushenko, O.V. (2010) Combined device for devitalization of microorganisms. Ukraine Patent No. 48632.

[5] Raichenko, O.I., Mosienko, V.S., Chernenko, L.I., Yanish, Yu.V., Derev' yanko, O.V. and Karnaushenko, O.V. (2010) The method for devitalization of microorganisms in liquid media. Ukraine Patent No. 91475.

[6] Smirnov, V.V., Reznik, S.R. and Sorokulova, I.V. (1980) Methodical recommendations for isolation and identification of *Bacillus subtilis-mesentericus* bacteria group from human and animal organisms. Zdorov'e, Kiev.