SENSITIVITY OF *E. COLI* CELLS TO LOW STATIC MAGNETIC FIELDS

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Abstract. The aim of this work was to study low static external magnetic field effects 0-100 mT on the growth and development of *E. coli* bacteria. A large number of experimental points in this range, ongoing growth of bacteria in a magnetic field and the registration of changes in the physiological characteristics (CFU, growth rate) and biochemical parameters (ATP pool) guaranteed an obtainment reliable results. The colony-forming ability, growth rate constant and ATP pool in *Escherichia coli* bacteria are the magnetic-dependent characteristics of microorganism vital activity. They depend on the external static magnetic field and increase when its value lies in ranges 0-10 mT and 15-50 mT. The magnetic field rising from 50 to 100 mT leads to bacterial growth inhibition. All observed effects in bacteria *E. coli* are in good agreement with a theory of the enzymatic magnetosensitivity and determined by the spin dependent stages of intracellular enzymatic processes.
Key words: *E. Coli, magnetic field, ATP.*

INTRODUCTION

Living organisms are continuously exposed to static magnetic and variable electromagnetic fields, most of which are technogenic ones: power lines, cell phones, electronic equipment, and others. Numerous studies have shown that living organisms, including bacteria, respond in a different way to a magnetic field action [1-4]. The specific biological response of living cells depends on the type of microorganisms and the method of its registration [2]. The nature of the observed magnetic field effects at the cellular and molecular level is usually obscure and has not always reliable physic-chemical mechanism, in particular, due to the conflicting experimental results [4-5]. For example, the magnetic fields effect on *E. coli* bacteria can cause the increase and reduction of the cell division rate. It's depending on the physiological conditions of bacterial growth [6-7].

The experimental data accumulation and the hypotheses search about mechanisms of magnetic field effects in living cells is the beginning of the twentieth century [8]. During this time, the different parameters of the magnetic fields influence on living systems have been varied: magnetic field strength (from 10^{-6} to 10 T); exposure time (from several minutes to several days); types of studied systems (from molecular complexes to multicellular organisms) [4]. However, most of the researches are a blind attempt to find the patterns of response occurrence in living systems to the magnetic fields action. This is due to the lack of rigorous theoretical bases of biological magnetic field effects. There are various mechanisms of living organism magnetosensitivity [9-14]. Some of them could explain the magnetic orientation of birds and fishes [11-13]; the other – the magnetic field effects in biopolymers [14], etc. The universal mechanism of magnetosensitivity that would be true for all living systems, have not been suggested. The most likely candidate for the universal "receiver" of the external magnetic field in cells is spin-dependent enzymatic reactions, which have a strict theoretical basis [15-17] and experimental confirmations *in vitro* [18-19]. Magnetosensitive stages in these reactions are stages with one or more electrons transfer, which lead to the ion-radical pair's formation in the enzyme active site. An external magnetic field or the magnetic isotopes of chemical elements, such as ^{31}P, ^{25}Mg, ^{43}Ca having nuclear magnetic moment and participating in the reaction, can change the spin state of ion-radical pairs by inducing the singlet-triplet conversion. This leads to changes probabilities of direct and back electron transfer and, in turn, increases or decreases the reaction products yield.

The first spin-dependent enzymatic reactions were discovered in experiments with phosphorylating enzymes and magnesium isotopes: magnesium nucleus ^{25}Mg, having the magnetic moment (S=5/2), increased the ATP yield in the reaction of ATP synthesis compared with non-magnetic nucleus ^{24,26}Mg [20-21]. The proposed mechanism of this reaction involves electron transfer from the terminal phosphate group of ADP with the ion-radical pair formation in the enzyme active site. Later, similar effects in enzymatic ATP synthesis were first predicted and then obtained for the magnetic isotope of zinc ^{67}Zn and calcium ^{43}Ca [22-23]. Enzymatic reactions of DNA synthesis are also sensitive to the nuclear magnetic moment of magnesium ^{25}Mg, zinc ^{67}Zn and calcium ^{43}Ca isotope presence [24]. The effectiveness of magnetic isotopes in these reactions increased with an external magnetic field inclusion [25-27]. Obtained magnetic isotope and magnetic field effects in ATP and DNA synthesis have laid a reliable experimental foundation of the theory of living organism magnetosensitivity due to spin-dependent stages of enzyme reactions elementary acts presence. The strong theory, which illustrated the magnetic field dependence of the rate constant of enzyme reactions going with one or more electrons transfer, outlined in [15-17]. The main magnetosensitive stage of this process is the singlet-triplet conversion of ion-radical pairs in the enzymes active sites induced by Zeeman and hyperfine interactions (HFI) of electron and nuclear spins with the magnetic field. The products of these reactions "transform" the nuclear spin and the magnetic field effects into "biochemical response" of living organisms available for registration *in vivo*. The amplitude of magnetic field effects, in accordance with this theory, depends on the ratio between HFI constants of magnetic

isotope involved in the reaction and the rate constants of enzymatic reactions. Moreover, the strongest effects should be observed in a low magnetic field, the intensity of which does not exceed the values of the HFI constants 0-50 mT.

Magnetic field effects in living organisms, predicted by this theory, were not obtained previously due to used range of magnetic fields more than 0.1 T [4, 28], and also due to the limited number of experimental points and short magnetic field exposure. As a rule, the choice of specific values of the external magnetic field caused by experimental setup features or researchers preferences. The aim of this work was to study a low static external magnetic field effects 0-100 mT on the growth and development of *E. coli* bacteria. A large number of experimental points in this range, continuous growth of bacteria in a magnetic field and the registration of changes in the physiological characteristics (colony-forming units (CFU), growth rate) and biochemical parameters (ATP pool) allow us to make conclusions about the reliability and mechanism of the discovered effects.

MATERIALS AND METHODS

Bacteria cultivation and magnetic field exposure conditions. The study object was the *E. coli* cells culture, viz., a museum strain K12TG1 (from collection of Institute of Cellular and Intercellular Symbiosis, Ural branch of RAS, Orenburg. Russia), which was grown in in the LB broth (Sigma-Aldrich, St. Louis, USA). The density of the seed culture was monitored photometrically with the SOLAR (CM2203, SOLAR Technical Service, Minsk, Belarus) spectrofluorimeter (wavelength 620 nm, absorbance 0,61 + 0,01 rel. units). Then the cells of *E. coli* were re-sowed into the LB media. 200 µl of the medium with *E. coli* cells was added to each well of a 96-well plate (Apexlab, Moscow, Russia); volume of each well was 346 µl.

All the samples were placed in a static magnetic field (SMF), which was induced by the electromagnet with an iron yoke (TR-309, Takeda Riken, Tokyo, Japan) with the cooling system, and the temperature was continually monitored for 7 h to ensure a constant temperature of 37 °C. The scheme and photo of the experimental setup are presented in Fig. 1a-b. The electromagnet has two coils with an outside diameter of 28 cm and an internal diameter of 16 cm, the length of the coils 11 cm, and the distance between the coils 12 cm. The diameter of the pole pieces 8 cm. The current source allows adjusting the magnetic field strength from 0 to 1.5 T in the central region between the pole pieces. Coils have the total resistance 10 Ω. Long-term stability of the current and the magnetic field was less 10^{-3}. The direction of the magnetic field coincides with the direction of the Earth magnetic field. The combination of the coils and the iron yoke produce the inhomogeneous magnetic field inside a thermostated box with dimensions: length 68 cm, width 9 cm, height 8 cm.

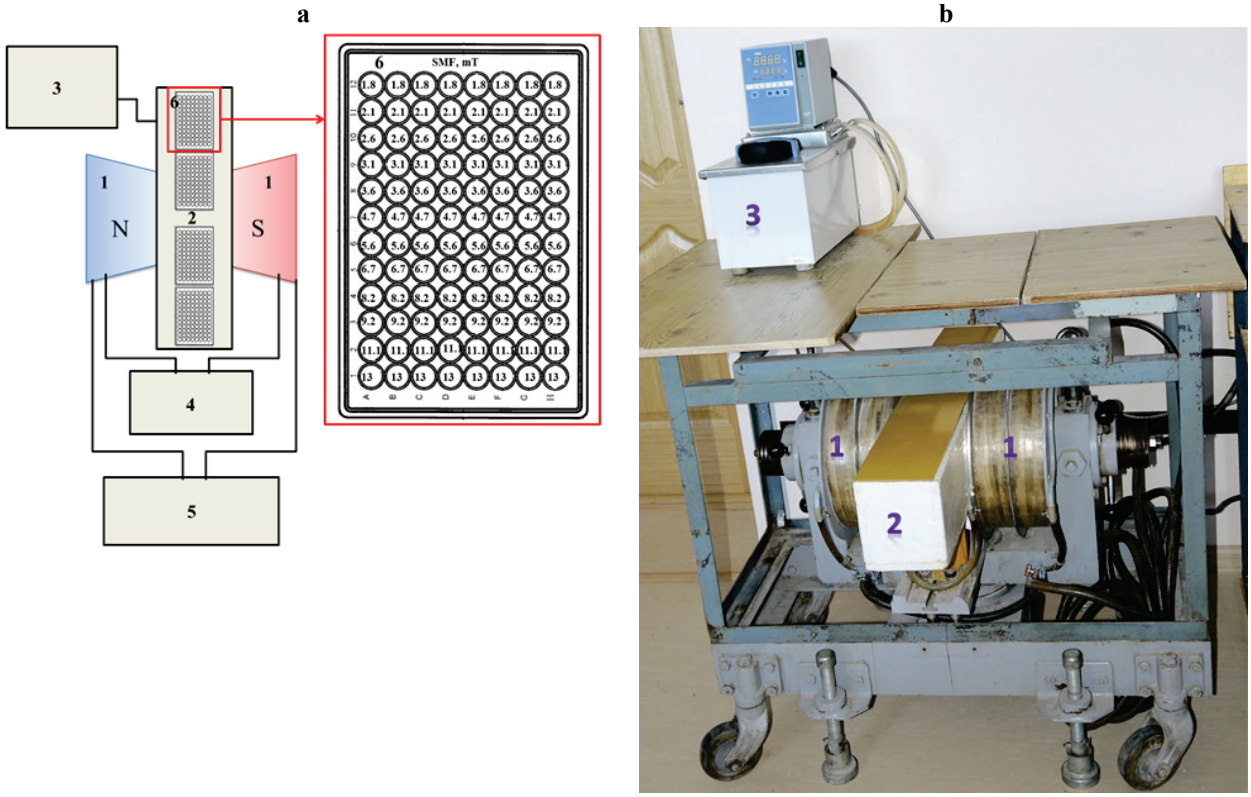

Figure 1. Scheme (a) and photo (b) of experimental setup: 1 – poles of electromagnet; 2 – thermostated box, in which 96-well plates with *E. coli* bacteria were placed for cultivation in static magnetic field, temperature of box was maintained at 37 °C; 3 – thermostat controlling temperature in box; 4 – magnet power supply; 5 – cooling system of electromagnet; 6 – example of scheme of static magnetic field points for 96-well plates, range of magnetic field for whole box for this example is from 1,8 to 100 mT

The 96-well plates with *E. coli* bacteria were placed in the thermostatic box for exposure to the magnetic field. The temperature in the box was maintained at 37 ± 0,4 °C using a circulating thermostat (LT-TWC/7, Labteh, Moscow, Russia). Aerobic cultivation conditions were provided by placing samples with growing bacterial culture on the ST-3 ELMI shaker (ST-3, ELMI, Riga, Latvia) every hour for 5 min. (rotation velocity of the platform 200 rpm). Thus the amount of oxygen was sufficient to ensure aerobic conditions of cultivation given that the shaking was performed every hour. However, the magnetic field in small wells of the 96-well plates can be thought as homogeneous one as far as it was averaged due to movement of bacteria and due to placing samples with growing bacterial culture on the shaker, which was independent from incubator. The magnetic field of the shaker was 0,30±0,05 mT that coincides with the laboratory background magnetic field.

The range of the chosen low magnetic fields was 0-100 mT. The bacteria were cultivated simultaneously in 96 control points corresponding to 22 stationary magnetic fields, whose values were measured by the milliteslameter (TP2-2U, Fela-conrol, St. Petersburg, Russia).The magnetic fields fluctuations at each point were monitored throughout the experiment and did not exceed 0,1 mT.

Growth curves. The growth curves (the dependencies of the suspensions optical density, which characterize the growth of the bacterial culture, on the cultivation time) were measured by the turbidimetric method every hour with the photometer UNIPLAN "Pikon" (ZAO "Pikon", Moscow, Russia) at 492 and 620 nm. The experimental growth curves recorded at the different wavelengths were qualitatively identical, so the data for one wavelength 492 nm is only shown.

The initial part of the curves (prior to the stationary phase, the first 5 h of cultivation) was processed using linear approximation adopted in biology and microbiology for determining the growth rate.

The linear portion of the growth curves, corresponding to the phase of active cell division, was approximated by the formula [29]:

$$D_{492}=D(0)+\mu t,$$

where $D(0)$ is the initial optical density, μ is the growth rate constant (in h^{-1}), also known as the reproductive potential of the bacterial culture.

CFU measurements. The colony-forming ability of bacteria, which was measured after 7 h of cell incubation in a static external magnetic field, was chosen as the main indicator of growth. The method of serial dilutions was applied for measuring CFU: three dilutions of the medium containing *E. coli* cells in the physiological solution [30].

The cell concentration in each sample was determined photometrically by using the SOLAR CM2203 spectrofluorimeter according to the calibration curve. Equal amounts of *E. coli* cells diluted in the corresponding concentrations were sowed on the solid nutrient medium LB agar in Petri dishes. The CFU values were calculated after 16 h of incubation at 37 °C.

Measurements of intracellular ATP. For the measurement of the intracellular ATP in the *E. coli* bacteria, the bioluminescence method was used. It was performed by the luminometer "LUM-01" (LUM-01, Lumtek, Moscow, Russia) – high sensitivity photon counter, which registers the intensity of luminescence in the range of 10 to 800 thousand pulse/s. The ATP concentrations were measured using the Lumtek set (measurement of the total concentration of ATP in extracts of cells and tissues) (Lumtek, Moscow, Russia). The set includes 4 solutions: the ATP-reagent "Lumtek", lyophilized [31]; the solution for reconstruction of the ATP-reagent; ATP-control, lyophilized; the solution for cell destruction. For measuring the ATP pool the ATP-reagent reconstruction was carried out by adding 4 ml of solution for reconstruction (solution aged for 0,5-1 h at room temperature). Bacterial cells were destroyed using the appropriate solution (DMSO) to release the ATP (0,05 ml homogenate of bacteria was added to 0.45 ml of DMSO). 0,1 ml of the extract was transposed into the luminometer cuvette. Then 0,02 ml of the ATP reagent was added into the cuvette. The bioluminescent intensity of the received signal I_{max} was compared with the signal intensity of the ATP control I_{Contr}. To measure the intensity of the ATP control a 1 ml solution for cell destruction was placed in a bottle and the ATP control added. The ATP concentration of the bacterial population was calculated by the formula:

$$[ATP]_{cells} = 10*10^{-8}((I_{max})/ (I_{Contr})), \text{ mol/l.}$$

To calculate the intracellular ATP pool in single bacteria the ATP concentration measured by using the bioluminescent method was divided by the number of bacterial cells measured using the CFU method.

Statistical Analysis. Data were expressed as mean ± SD. The Shapiro-Wilk normality test was used to determine whether experimental data have been drawn from a normally distributed population. Student's tests were used to determine statistical differences by the Origin 8,0 software (Version 8,0; Micro-cal Software, Northampton, USA). The differences between groups were considered as statistically significant when $P < 0,05$.

RESULTS

As the result of performing series of six identical experiments (in each series, at least three replicates were performed), the cell growth curves for *E. coli* were obtained. The magnetic field dependencies of the growth rate constants, which were calculated after linear approximation of bacteria growth curves, are presented in figure 2. These dependencies reflected the different influence of the low magnetic field on the microorganisms' growth.

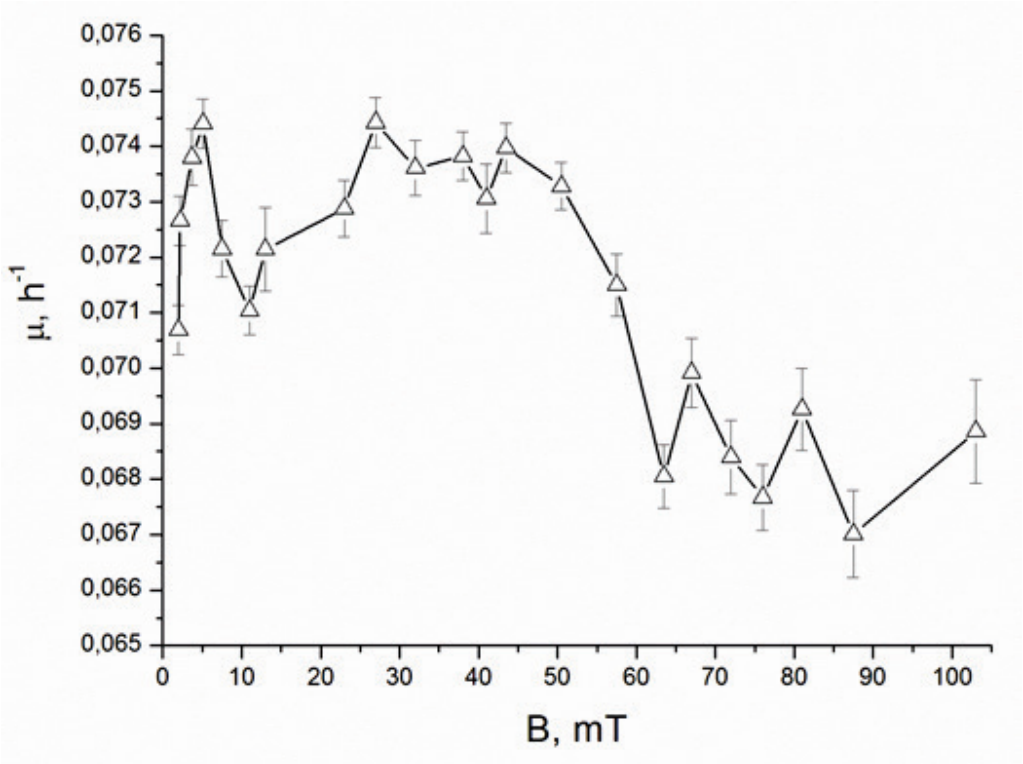

Figure 2. Magnetic field dependence of growth rate constants of *E. coli* cells. Rate constants were determined using various linear approximations of growth curves. Results were expressed as means ± SD. Significant difference was set at $P < 0.05$ ($n = 18$)

Magnetic field effects of the growth rate constant were observed for the microorganisms cultivated in the ranges 0-10 mT and 25-55 mT. When the magnetic field intensity increased from 0 to 6.5 mT, growth rate constant increases in 7 % and reaches the maximum value throughout the studied range. The magnetic field intensity increase from 55 to 100 mT leads to a significant inhibition of bacterial growth. The growth rate constant reduced by 11% compared with its maximum value.

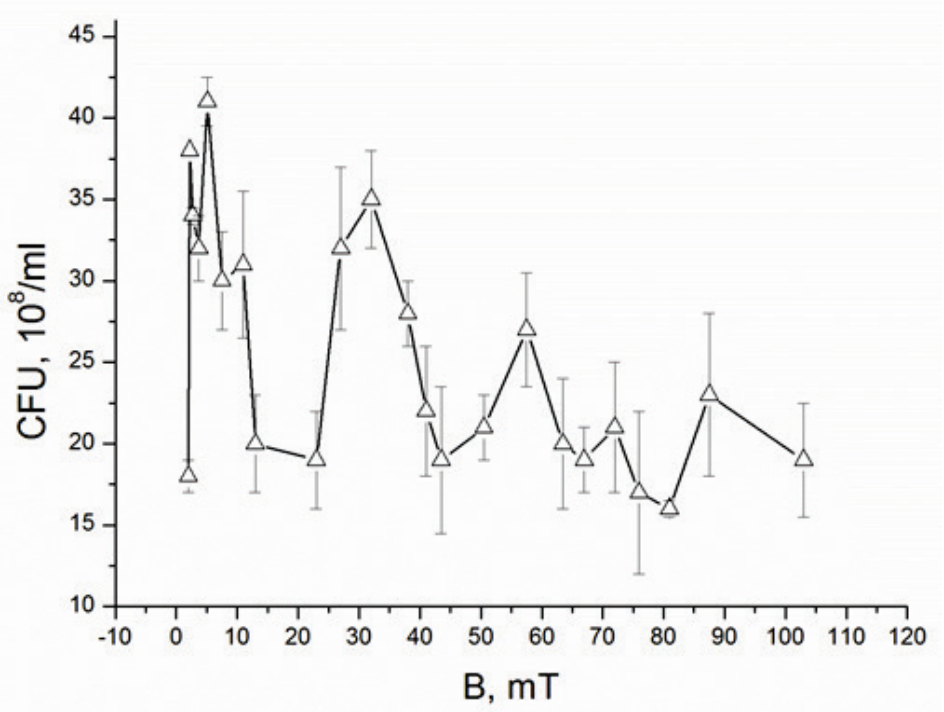

Figure 3. Magnetic field dependencies of *E. coli* cells CFU. Results were expressed as means ± SD. Significant difference was set at $P < 0.05$ ($n = 18$)

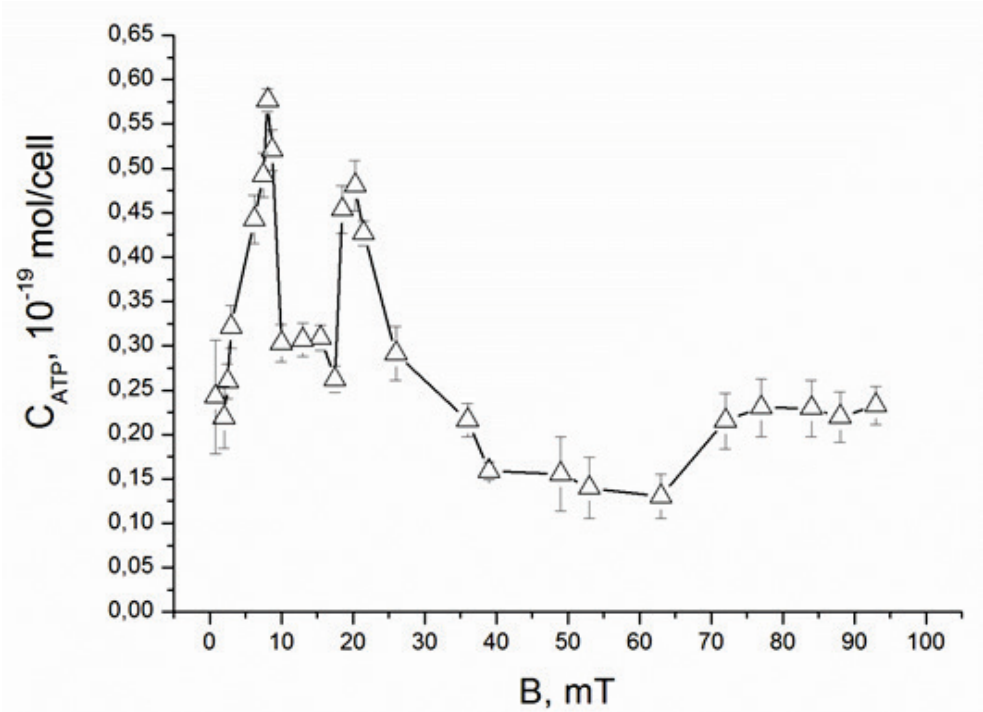

Figure 4. Magnetic-field dependence of ATP pool in *E. coli* bacteria cultivated in LB medium. Results were expressed as means ± SD. Significant difference was set at *P < 0,05 (n = 12)

Another important microbiological indicator of the bacterial culture's growth is it's the colony forming ability. The similar magnetic field dependence was observed for CFU of bacterial cells grown in the nutrient-rich medium LB. This dependence is presented in figure 3. When the magnetic field intensity increased from 0 to 7 mT, the colony-forming ability increases by 2-2.5 times and reaches the maximum value. The next range of magnetic fields where number of CFU increased was 23-40 mT. No reliable differences between the CFU of the bacteria subjected to the static external magnetic field in the range 43-100 mT were observed.

The dependence of the intracellular ATP concentration in the bacteria *E. coli* on the external magnetic field is presented in figure 4. There were 6 experimental series of which each was repeated twice or three times. The average ATP content in single bacterial cell in the selected growth conditions was 10^{-19} mol, which was consistent with the literature data [32]. It was observed three characteristic ranges of the magnetic-field dependencies of the ATP pool in *E. coli*: 0-10, 15-26, 30-100 mT. In the first range the ATP pool reaches the maximum value, 2 times higher than ATP concentration for bacteria cultivated in geomagnetic field. The external static magnetic field influence of the second range also promotes intracellular ATP concentration. A further increase of an external magnetic field to 100 mT (third range) leads to inhibition of the ATP synthesis and hydrolysis in bacterial cells. The value of bacterial ATP pool in this range is less than in previous ones.

DISCUSSION

The magnetic field effects in the physiological characteristics (CFU, growth rate) and biochemical parameters (ATP pool), which were obtained in the range 0-10 mT, are the most interesting effects accordingly to the theoretical predictions [15-17]. These fields are attractive because the HFI constants of the most organic radicals containing magnetic nuclei of natural isotopes ^{1}H and ^{13}C are in the range of 0,1-4 mT [33]. It is important to note that magnetic fields dependences of CFU, growth rate constants and ATP pool are correlated.

A large number of studies wrongly focused on the detection of magnetic field effects in strong magnetic fields, much larger than the Earth's magnetic field [34-36]. The effects usually detected in such cases cannot explain the magnetic sensitivity of living organisms in the low magnetic field of the Earth (about 0.5 10^{-2} mT) and their reaction to variations of this field. Moreover, those results can hardly be used to understand a mechanism of intracellular enzymatic process magnetic control. The joint effects of external magnetic fields and magnetic moments of atomic nuclei can be observed and registered exactly in low magnetic fields, the strength of which is less than the value of hyperfine interactions constants [17]. The increase of the ATP pool, colony-forming ability and growth rate constant in all bacteria in the range of 0-10 mT registered in our experiments confirms the theoretical conclusions. In this range we expected the bright magnetic-field effects in living organisms according to the theory of enzymatic magnetosensitivity, where the primary receiver of an external magnetic field is a spin-dependent stage of elementary acts of enzymatic processes. The magnetosensitivity of these stages is due to the participation of particles with nuclear magnetic moments, such as magnetic isotopes ^{1}H, ^{13}C, ^{39}K and their hyperfine interaction with the electronic spin and the external magnetic field [15, 17]. A required condition of occurrence of such magnetosensitive enzyme reactions is the electron transfer and the formation of an ion-radical pair. The nuclear magnetic moments of stable isotopes and an external static

magnetic field are to induce the transition of ion-radical pairs from the initial singlet state into a triplet one. The probabilities of the forward and reverse electron processes depend on the total spin state of such pair. For example, the singlet-triplet conversion of the pair "magnesium ion $^{25}Mg^{+}$ - ADP radical" in the active site of ATP-synthase [18, 21] leads to increase the probability of a direct reaction (ATP formation).

The detected magnetic field effects in the growth rate constant, colony-forming ability and intracellular ATP concentration for all studied *E. coli* bacteria in the range 0-10 mT indicates validity of the theoretical predictions and the presence of magnetically sensitive enzymatic reactions occurring in the ion-radical mechanism.

The magnetic field effects, which were registered for the growth rate, CFU and ATP pool in the second range from 15 to 50 mT, may be related to the enzymatic reactions involving ion-radicals stages with high hyperfine interactions constants, for example, ^{23}Na, ^{31}P. On the other hand, these effects may be appeared due to magnetic fields influence on the intracellular processes with paramagnetic ions, Fe, Mn, etc.

The magnetic field increase from 50 to 100 mT leads to bacterial growth inhibition. This is manifested as the growth rate and the CFU number decrease and inhibition of ATP production by cells.

CONCLUSION

The colony-forming ability, growth rate constant and ATP pool in *Escherichia coli* bacteria are the magnetic-dependent characteristic of microorganism vital activity. It depends on the values of the external static magnetic field. It was obtained that two ranges of low magnetic fields 0-10 mT and 15-50 mT can affect the bacterial growth. All observed magnetic field effects in bacteria *E. coli* are determined by the spin dependent stages of intracellular enzymatic processes; synthesis of ATP is the most probable one.

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