The Magnetic Isotopes Effect of Magnesium $^{25}\text{Mg}$ on the Physiological Properties of Bacteria $E. \text{coli}$

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Abstract. Magnetic isotope effects of magnesium $^{25}\text{Mg}$ on antibiotic resistance and biofilm formation of bacteria $E. \text{coli}$ were discovered. The increase in antibiotic resistance in the presence of the ion $^{25}\text{Mg}^{2+}$ was registered for $E. \text{coli}$ cells incubated with quinolones, indicating the inhibiting effect of the magnetic moments of nuclei $^{25}\text{Mg}$ on deoxyribonucleic acid (DNA) synthesis. It was discovered that the process of biofilm formation by the bacteria $E. \text{coli}$ is magnetosensitive. Magnetic magnesium isotope presence in nutrient media stimulated formation of biofilm compared with nonmagnetic isotopes $^{24,26}\text{Mg}$. As a result the enrichment of media by magnetic magnesium can be used to control the physiological properties of bacteria: biofilm formation and antibiotic resistance.

1. Introduction
Chemical elements present in living organisms have natural stable isotopes which are different in their mass and magnetic properties of their nuclei. Differences of mass values are the reason of isotope fractionation in biology: living organisms consume and use light and heavy isotopes of one and the same chemical element in different ways. Fractionation occurs in many biological processes, although effects are always insignificant. According to various data, reasons of this phenomenon are differences in values of response time or diffusion rates of light and heavy isotopes, plus differences in equilibrium constants [1]. The most interesting biological isotope effects are found for nuclei of hydrogen $^1\text{H}/^2\text{H}$. Among most interesting practical observations of isotope effects in biology, one can note the lethal action of deuterium for most of organisms; use of $^{12}\text{C}/^{13}\text{C}$ relations for determining of the biogenetic origin of carbon in ancient rocks and $^{32}\text{S}/^{34}\text{S}$ relations for determining of the time of sulphuric bacteria evolution [1]. It is shown in multiple researches that chlorophylls $a$ and $b$ present in cyanobacteria are different in the isotope composition of magnesium $^{24}\text{Mg}/^{26}\text{Mg}$ [2].

Apart from the atomic mass, isotopes differ from each other with their magnetic properties. Isotopes nuclei whereof possess magnetic moment and, accordingly, nuclear spin, are called magnetic, for example, $^{25}\text{Mg}$, $^{31}\text{P}$, $^{67}\text{Zn}$. All other stable isotopes of these elements are non-magnetic and their nuclei do not possess magnetic moments. For the first time, different influence of magnetic and non-magnetic isotopes on biological objects was demonstrated in experiences with the isolated phosphorylating ferment and isolated mitochondria [3-4]. The magnetic magnesium isotope $^{25}\text{Mg}$ presence in an active site of enzyme increased ATP yield compared to nonmagnetic isotopes $^{24,26}\text{Mg}$. Magnesium possesses three stable isotopes: $^{24}\text{Mg}$, $^{25}\text{Mg}$ and $^{26}\text{Mg}$ (the natural content: 78.60%, 10.11%, 11.29%, respectively); of them only $^{25}\text{Mg}$ has a magnetic nucleus (spin $I=5/2$). Thereafter a cycle of works was published devoted to magnetic-isotope effects of magnesium $^{25}\text{Mg}$, calcium $^{43}\text{Ca}$...
and zinc $^{67}$Zn in the synthesis of ATP and DNA [5-7]. At the same time, participation of the magnetic isotope stimulates the synthesis of ATP and inhibits the synthesis of DNA [5, 7].

In vivo experiments on E. coli bacteria showed that microorganisms are sensitive to presence of the magnetic moment of magnesium isotope nucleus. Adding of magnetic magnesium $^{25}$Mg into the medium led to increase of CFU, growth rate, ATP intracellular content, and influenced the bacteria’s element composition [8, 9]. It was proved that these changes are conditioned specifically by the magnetic nature of magnesium [8] and a strict theory was proposed [10]. Such a way of cultivation made it possible to enrich bacteria up to 98% with magnesium isotope [8].

The aim of this work is to investigate the influence of magnesium isotopes on the two most important physiological properties of E. coli bacteria – the process of biofilm formation and antibiotic resistance. The conducted research is relevant and practically in demand due to the increasing resistance of bacteria to external physical and chemical factors, including antibiotics.

2. Materials and methods

2.1. Cultivation conditions

The study object was the E. coli cells culture, viz., a museum strain K12 TG1 (from collection of Institute of Cellular and Intercellular Symbiosis, Ural branch of RAS, Orenburg, Russia), which was grown in minimum synthetic nutrient media M9: 37.4 mM NH$_4$Cl, 2.2 mM $^{24,25,26}$MgSO$_4$, 55.5 mM glucose, 84.5 mM Na$_2$HPO$_4$, 44.1 mM KH$_2$PO$_4$, 17.1 mM NaCl (Reachem, Moscow, Russia). For the preparation of agar M9 to this composition was added bacteriological agar. The media differed only by the isotope form of magnesium in the salt: nonmagnetic $^{24,26}$Mg and, magnetic $^{25}$Mg, and natural isotope Mg [9]. Magnesium isotopes were added to the media as sulphate MgSO$_4$. For the sulphates preparation, isotopically pure oxides were used: $^{24}$MgO, $^{25}$MgO, $^{26}$MgO (Combine “Electrochempribor”, Lesnoy, Russia) with extremely high isotope enrichment.

The museum strain of E. coli was pre-incubated in LB broth (Sigma-Aldrich, USA).

2.2. Determination of antimicrobial activity

Antibiotic resistance was evaluated by disk diffusion method on agar M9 in accordance with the guidelines 4.2.1890-04 [11]. To obtain inoculum whose density corresponds to 0.5 according to McFarland, we used LB-broth (Sigma-Aldrich, USA). Then, 2 mm of inoculum were added to Petri dishes with M9 medium containing magnesium isotopes. Disks with antibiotics of seven different groups (Pasteur Research Institute of Epidemiology and Microbiology, Russia) were applied 10 min after inoculation. Then, dishes were incubated for 24 h at 37°C, and the results were analyzed.

2.3. Determination of the ability to form biofilms

The ability to form biofilms was evaluated by the standard photometric procedure by the degree of binding of crystal violet [12]. After 48 h of incubation in a static magnetic field, the culture broth was carefully removed, and the wells were supplemented with 1.5 mL of 0.005% aqueous solution of crystal violet to stain the formed biofilms. Staining was performed for 60 min. Then, after complete removal of crystal violet solution from the tubes, the dye was extracted from the biofilm by incubation with 1 mL of 96% ethanol for 45 min at room temperature. Thereafter, the remains of biofilms and bacteria were pelleted by centrifugation at 9000 rpm for 7 min in a CM-50M centrifuge (ELMI, Latvia). Then, the samples (200 μL) were added to wells of a 96-well plate. The concentrations of crystal violet were measured by enzyme immunoassay (AIFR-01 UNIPLAN instrument, Picon, Russia, wavelength 530 nm).

2.4. Statistical Analysis

Data were expressed as mean ± SD. Student’s tests were used to determine statistical differences by Origin 8.0 software (Version 8.0; Microcal Software, Northampton, MA, USA). Differences between
groups were considered as statistically significant when \( p < 0.05 \). A sample size of each group was \( n=10 \), a degree of freedom was \( df=18 \).

3. Results and discussion

As a result of ten conducted experimental series, dependencies of optical density were obtained which characterize the ability to form biofilms by \( E. coli \) bacteria cultivated on the mediums with magnesium isotopes. The results are presented in Figure 1.

![Graph showing optical density vs magnesium isotope]

Figure 1. Formation of biofilms by bacteria \( E. coli \) cultured on M9 media containing magnesium. *The differences between the mean values for the magnetic magnesium isotope \( ^{25}\text{Mg} \) and other magnesium isotopes \( ^{24}\text{Mg}, \, ^{26}\text{Mg} \) and Mg are statistically significant \( p < 0.01 \) (\( n = 10, df=18 \)).

\( E. coli \) bacteria were better at forming biofilms in the case when they were cultivated on the medium with magnesium magnetic isotope \( ^{25}\text{Mg} \) in comparison with non-magnetic forms \( ^{24},^{26}\text{Mg} \). The found magnetic-isotope effect of is equal just to 7-8% and is statistically significant. The similar dependencies were earlier obtained for the growth rate of \( E. coli \) bacteria enriched by the magnetic magnesium isotope [9]. The formation of biofilms is one of properties of particular interest. Communities of microorganisms in biofilms are a complex structure consisting of the microbes themselves and the polymer matrix synthesized by them (proteins, polysaccharides, and nucleic acids). Biofilms protects bacteria from the action of external physicochemical agents: antibiotics, ultraviolet irradiation, mechanical impacts [13]. It is quite difficult to remove and inactivate developed and mature biofilms [13], which leads to undesirable problems, especially in medicine and industry.

Table 1 shows results of measuring of inhibition zones diameters of \( \text{Escherichia coli} \) bacteria, which were cultivated on M9 medium with magnesium isotopes \( ^{24}\text{Mg}, \, ^{25}\text{Mg}, \, ^{26}\text{Mg} \) or natural magnesium Mg. The zones were formed as a result of the antibiotic diffusion into the medium. Each value in the table is an average one obtained after statistical processing of 5 experimental series with two iterations in each.

The antimicrobial activity was determined for 10 antibiotics. Magnetic-isotope effects of \( ^{25}\text{Mg} \) were found for ciprofloxacin and nalidixic acid from the group of quinolones, for amikacin from the group of aminoglycosides, and for clindamycin from lincosamides. Resistivity to these antibacterial agents was different for those bacteria which were cultivated on a medium with the magnetic isotope...
of magnesium $^{25}\text{Mg}$ in comparison with non-magnetic $^{24,26}\text{Mg}$ and natural magnesium; it speaks for the specifically magnetic nature of the effect. Of interest is the result for lincomycin: *E. coli* bacteria enriched with the non-magnetic isotope of $^{26}\text{Mg}$ occurred to be most sensitive to its effect. Probably, it is connected with fractionating of magnesium isotopes.

### Table 1. Diameter of the growth inhibition zone of *E. coli* cultured on a nutrient medium containing one magnesium isotope $^{26}\text{Mg}$, $^{25}\text{Mg}$ or $^{26}\text{Mg}$ or natural magnesium Mg

| Antibiotics   | Concentration of drug, μg/disk | Diameter of growth inhibition zone, d± SD, mm |
|---------------|--------------------------------|-----------------------------------------------|
|               | medium with Mg | medium with $^{26}\text{Mg}$ | medium with $^{25}\text{Mg}$ | medium with $^{26}\text{Mg}$ |
| Cefazolin     | 30                | 26.0 ± 0.7 | 27.1 ± 0.2 | 27.3 ± 0.3 | 25.9 ± 0.5 |
| Ceftriaxone   | 30                | 32.2 ± 0.6 | 34.5 ± 0.5 | 33.7 ± 0.3 | 35.0 ± 0.5 |
| Meropenem     | 10                | 31.7 ± 0.8 | 31.0 ± 0.9 | 32.3 ± 0.9 | 30.7 ± 0.7 |
| Amoxicillin   | 20                | 17.7 ±0.7  | 18.3 ± 0.2 | 18.0 ± 0.5 | 18.6 ± 0.7 |
| Amikacin      | 30                | 19.3 ± 1.2 | 19.3 ± 1.2 | 16.0 ±0.6* | 18.5 ± 0.6 |
| Lincomycin    | 15                | 14.0 ±0.3  | 14.0 ± 0.5 | 13.5 ± 0.4 | 15.3 ±0.3** |
| Clindamycin   | 2                 | 13.7 ±0.8  | 12.6 ± 0.6 | 10.4 ±0.6* | 13.1 ± 0.8 |
| Tetracycline  | 30                | 20.3 ±0.4  | 21.3 ± 0.3 | 21.5 ± 0.4 | 22.1 ± 0.6 |
| Nalidixic acid| 30                | 20.7 ± 0.3 | 20.3 ± 0.2 | 22.2 ±0.4* | 19.5 ± 0.4 |
| Cyprofloxacin | 5                 | 32.0 ± 0.6 | 32.0 ± 0.1 | 34.3 ±0.7* | 32.0 ± 0.3 |

*The differences between the mean values for the magnetic magnesium isotope $^{25}\text{Mg}$ and other magnesium isotopes $^{24}\text{Mg}$, $^{26}\text{Mg}$ and Mg are statistically significant at $p<0.02$ ($n=10$, $df=18$).  
**The differences between the mean values for the nonmagnetic magnesium isotope $^{26}\text{Mg}$ and other magnesium isotopes $^{24}\text{Mg}$, $^{25}\text{Mg}$ and Mg are statistically significant at $p<0.01$ ($n=10$, $df=18$).

Analysis of the found magnetic-isotope effects of $^{25}\text{Mg}$ in antibiotic-related sensitivity of *Escherichia coli* bacteria allows concluding that in the case of the group of quinolones (nalidixic acid and ciprofloxacin) we observe potentiating of antimicrobial agents by the magnesium isotopes. The growth inhibition zone for bacteria which were incubated on a medium with presence of the magnetic magnesium isotope occurs to be larger by 9.5–13.5% for these antibiotics, as compared to *E.coli* cells incubated on the medium M9 with $^{26}\text{Mg}$, $^{26}\text{Mg}$ or natural magnesium Mg. The mechanism of nalidixic acid action is connected with inhibition of DNA replication through oppression of its polymerization, whereas ciprofloxacin inhibits DNA-gyrase, which causes killing of the bacteria [14]. It is reported in works [6, 7] that presence of the magnetic magnesium isotope in the DNA-polymerase active site blocks a direct reaction thus inhibiting DNA synthesis. That is, both antibiotics of the group of quinolones and the magnetic magnesium affect one and the same target, amplifying each other. It leads to a significant alteration of antibiotic-related sensitivity.

Resistance of bacteria is increased at enrichment of M9 media by the magnetic magnesium isotope for antibiotics of amikacin and clindamycin, the action mechanism whereof is connected with violation of protein synthesis stages and destruction of bacteria cytoplasmatic membrane [14]. Such magnetic-isotope effect can be connected with influence of magnetic moments of magnesium isotope $^{25}\text{Mg}$ nuclei on energetic processes in the cell, specifically on ATP synthesis [3, 4] and on duration of the adaptation phase of *E. coli* cells growth [8]. At the moment of application of disks with antibiotic on the M9 medium with inoculum, two processes are launched: diffusion of the antibiotic into agar and growth of the bacterial culture. The final result, that is, the diameter of growth inhibition zone will depend on bacteria growth rate. Bacteria enriched with the magnetic isotope of magnesium $^{25}\text{Mg}$ possess a lesser lag phase in comparison with bacteria enriched with non-magnetic isotopes, which means an earlier growth of the culture and, subsequently, the diameter of the growth oppression zone will be smaller. The revealed magnetic-isotope effects in resistance to amikacin and clindamycin confirm the higher growth power of bacterial cells enriched with such isotope, which was found
earlier [8]. The practical value of this result consists in increasing of probability of a rapid development in microorganisms and emerging of potentially pathogenic cultures at simultaneous antibacterial and isotope-enriched preparations.

Magnetic isotope effects of magnesium isotope $^{25}\text{Mg}$ on antibiotic resistance and biofilm formation of bacteria \textit{E. coli} were discovered. As a result the enrichment of media by magnetic magnesium can be used to control the physiological properties of bacteria: biofilm formation and antibiotic resistance.

Acknowledgments
This work was financially supported by the Council for grants of the President of the Russian Federation, the application of SP-225.2019.4.

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