Development of a complex anti-radiation protective drug

K Vagin1, T Gaynutdinov1, A Idrisov1, G Rakhatmatullina1, K Ishmukhametov1, M Gallyamova1, R Nizamov1, N Vasilevsky1 and V Semenov2,*

1Department of Radiobiology, Federal Center for Toxicological, Radiation and Biological Safety, Nauchny gorodok-2, 420075, Kazan, Russian Federation
2Department of Morphology, Obstetrics and Therapy, Chuvash State Agrarian University, 29 K. Marx Street, 428003, Cheboksary, Russian Federation

*E-mail: semenov_vg@edu.academy21.ru, https://orcid.org/0000-0002-0349-5825

Abstract. The article reports results of development of a drug for the prevention and treatment of radiation lesions in animals based on radio modified microorganisms E. coli PL-6 and B. bifidum 1. Aimed at target changing of metabolism, bacteria were exposed to gamma irradiation at doses from 1 to 20 kGy, studying the interaction of microorganisms in the consortium, as well as the safety of produced drugs. Irradiation of E. coli at a dose of 2 kGy led to the appearance of individual cells with polymorphism and having a length exceeding the initial size by 3-7 times. The irradiation of B. bifidum 1 at a dose of 4 kGy contributed to the formation of atypical, polychromic cells, multiple darkening of pigment granules and cell fragments as a result of destruction of microorganisms. The original E. coli PL-6 did not produce the enzymes superoxide dismutase and catalase, but the metabolites of radiomodified strains of E. coli PL-6 (R10) contained 0.97±0.09 m.c.M/g SOD and 27.38±0.59 mcat/g/ml of catalase activity. In the metabolites of radio-resistant bifidobacteria, as compared with the initial ones, a 1.45-fold excess of peroxidase was recorded.

1. Introduction
Depressive, allergic and immunodeficient states registered in humans and animals in recent decades are largely associated with the changing environmental situation in the world [1]. Environmental factors can cause pathological conditions in which the body cannot cope with the formed oxide radicals [2]. The further existence and development of animals is impossible without the use of therapeutic agents [3]. Chemical compounds were used in past years to protect animals from ecofactors that were toxic with a small dose increase and often had side effects. Recently, more effective [4], and non-toxic [5] medicinal substances of biological nature are being developed.

Microbial preparations in vivo change the functional activity of cells of organs and systems, increasing the body's radioresistance to radiation exposure [6], increasing number of factors and mediators, which stimulate the formation of antibodies, increasing resistance to exogenous infections due to the activation of the phagocytic function of cells of the phagocytic mononuclear system in blood and tissues [7].

Drugs based on substances of microbial origin in an irradiated organism stimulate the regeneration of hematopoietic tissue, spleen, and bone marrow [8]. There are attempts to treat animals with radiation sickness by using vaccines [9].
E. coli PL-6 and B. bifidum 1 in the process of vital activity produce antibacterial substances, enzymes, antigens, entero- and exotoxins, cytokines, which individually and in combination with each other have radioprotective properties [10].

Most probiotics have a positive effect on the host organism, modifying the metabolic processes occurring in the intestine and, thereby, providing antiallergic and antitoxic effects [11].

The hypothesis for the studies was the reports that in a mixed population of cultures of different species and genera of microorganisms, the mechanism of interaction with each other is different and can lead to an improvement in the biological parameters of the drugs being developed. An example of such an interaction is the drug Bifikol, created by co-cultivation of E. coli PL-6 and B. bifidum 1. This drug has multifunctional properties, increasing the body's resistance to infectious diseases (dysentery, colibacillosis) and non-infectious (radiation sickness) pathology. At the same time, the issue of the influence of ionizing radiation on the radioprotective properties of the components of the Bifikol drug: E. coli PL-6 and B. bifidum 1, both separately and as part of a consortium, remains unresolved [12].

Based on the foregoing, the aim of the research was to develop a complex drug for the prevention and treatment of radiation injuries to animals based on radio modified strains of microorganisms.

2. Materials and methods

Some series of experiments investigated the structural, functional and biochemical properties of native and exposed to increasing radiation effects of bacteria E. coli PL-6 and B. bifidum 1 studied the possibility of co-cultivation of two different taxonomic species of radio-modified microorganisms, the harmlessness of preparations made from bacteria and obtained consortia based on the original and radiomodified Escherichia and Bifidobacteria.

Industrial strains of E. coli PL-6 and lyophilized preparation ‘Bifidobacterin’ (B. bifidum 1) (Limited liability company firm ‘Ferment’, St. Petersburg, Russia) were used as biological models.

Blaurock solid and liquid media, meat-and-peptone broth (MPB) and meat-and-peptone agar (MPA) were used for the growth of microorganisms (Federal Center for Toxicological, Radiation and Biological Safety, Kazan, Russia).

Cultivation was carried out at 37 °C under aerobic conditions (E. coli PL-6), anaerobic (B. bifidum 1) and with partial oxygen content (consortium of microorganisms E. coli PL-6, B. bifidum 1).

To start the experiment, the initial culture strain of E. coli PL-6 was washed out of a test tube with slant agar with saline solution (Federal Center for Toxicological, Radiation and Biological Safety, Kazan, Russia) – 0.95% NaCl in H2O. Then the culture was applied to the surface of a mattress with MPA and cultured at 37 °C for 1 day. The grown colonies were washed off with saline into a separate container. Using a unipipette, 0.1 cm3 of cell suspension was transferred into the first well of a 12-well titrator containing 1.0 cm3 of saline to each well of a row. After mixing the suspension of cells in the first well, 0.1 cm3 of the suspensions were transferred to the next well. These procedures were performed sequentially up to the last well of the titrator. Using a light microscope with a Goryaev’s camera (limited liability partnership ‘Minimed’, Russia), the number of microorganisms in the last dilutions was counted and, using the calculation method, the content of microbes in a microliter of the matrix solution was determined. The number of microorganisms in the suspension was standardized to 107-108 m.c./ml by sedimentation or dilution. The obtained biomass was poured into five sterile vials (E-1, E-2, E-3, E-4, E-5), 50 ml for each vial.

Monoculture of bifidobacteria (B. bifidum 1) with a content of living microbial cells of at least 107-108 m.c./vial after rehydration under aerobic conditions in 5.0 ml of saline using a sterile syringe were equally distributed into 5 sealed (without oxygen access) vials (B-1, B-2, B-3, B-4, B-5) containing each 50 ml of Blaurocca’s liquid medium. Vials with a culture of bifidobacteria were placed in a thermostat at 37 °C for 4 days. After incubation process, the bacterial cells in the flasks were precipitated at 1.5 thousand rpm for 30 minutes, the supernatant was removed, the centrifugate was resuspended first in a small amount of saline and then, by tenfold dilution, selection and counting of cells in the Goryaev’s chamber, stabilized to 10^2-10^4 m.c./ml.
Radiation exposure was carried out using a stationary gamma device ‘Issledovatel’ (‘Baltiets’, Narva, Estonia) with the power of 60Co ionizing radiation sources – 1.82e-02 A/kg, in doses from 1 to 25 kGy, followed by sowing irradiated crops on nutrient media. *E. coli PL-6* and *B. bifidum 1* were exposed to radiation up to the inactivation limit at doses from 1 to 4 kGy (*B. bifidum 1*) and from 1 to 20 kGy (*E. coli PL-6*).

Each stage of irradiation, from outcome to irradiation, studied the cultural, morphological and biochemical properties of bacteria: *E. coli PL-6* irradiated at a dose of 7.5 kGy (E-1), 12 kGy (E-2), 18 kGy (E-3), 20 kGy (E-4) and *B. bifidum 1* irradiated at doses of 1-4 kGy (B-1, B-2, B-3, B-4). For this purpose, we took the samples of cell cultures, stained smears-prints according to Gram, and made bacterial preparations. Radiomodified Escherichia and Bifidobacteria, as well as their non-irradiated analogs, subsequently were used for their joint cultivation. Based on irradiated bacteria E-4 and B-4. A consortium of radiomodified bacteria (K-4) was prepared. Unirradiated bacteria E-5 and B-5 served as the basis for the formation of a consortium of unirradiated microorganisms (K-5). Microorganisms with the last dose of radiation were mixed in a consortium. For this purpose, 1 cm³ of the contents of vials E-4 and B-4 were placed with sterile syringes into vials with 50 ml of Blaurock's liquid medium with a partial oxygen content (K-4). A similar procedure was carried out with vials E-5 and B-5 (K-5). Vials with a consortium of native *E. coli PL-6* and *B. bifidum 1* (K-5) and irradiated bacteria *E. coli PL-6* and *B. bifidum 1* (K-4) in Blaurock's medium were placed in a thermostat at 37 °C on 4 days. After passage in a nutrient medium, the contents of the KB-4 and KB-5 vials were precipitated, the supernatant was removed, and the centrifugate was resuspended in a small amount of the primer medium.

The study of the expressing (enzymatic) activity of microorganisms was carried out by biochemical methods using the spectrophotometric complex ‘SP-46’ (Open joint stock company ‘LOMO’, St. Petersburg, Russia).

To determine the peroxidase activity (PA) according to Kushmanova used pyrogallol as an oxidizable substrate, which was reduced to purpurogallin in the oxygen binding reaction. The maximum absorption spectrum of the spectrophotometer was 430 nanometers. The test solution contained 0.8 ml of 0.006 molar sodium phosphate buffer with a pH of 6.8; 0.12 ml of enzymatic (bacterial) extract; 0.5 ml 0.15% H₂O₂; 1.1 ml H₂O and 0.5 ml 0.003 M pyrogallol. In the control group, the same amount of distilled water was added instead of 0.5 ml of peroxide. The measurement was carried out for 2-3 minutes. The enzyme activity was determined by the formula (1):

\[ A = \frac{(D_t - 2 - D_{t_1})}{(t_2 - t_1)c} \]  

where \( A \) – enzyme activity; \( D \) – the optical density; \( t \) – time (s); \( c \) – concentration.

Determination of catalase activity (CAT) according to Korolyuk was based on the ability of hydrogen peroxide to form a stable colored complex with molybdenum salts. To start the oxidation reaction, 0.03% peroxide solution was added to 1 ml of bacterial extract in Tris-HCl buffer (Research Center of Pharmacotherapy, St. Petersburg, Russia). Distilled water was added to the control tube instead of the sample. The reaction was stopped after 10 min by the addition of 4% ammonium molybdate (Biochem, France). The color intensity of the solution was measured with an SP-46 spectrophotometer (Open joint stock company ‘LOMO’, St. Petersburg, Russia) at a wavelength of 410 nm. Catalase activity was calculated using the formula (2):

\[ E = \left( A_k - A_{op} \right) V t K \left( m.kat / l / kg \right) \]  

where: \( E \) is the activity of catalase (m.kat/l); \( A_k \) and \( A_{op} \) are the activity of the experimental and control samples; \( V \) is the volume of the introduced sample (0.1 ml); \( t \) is the incubation time (10 minutes); \( K \) is the coefficient of millimolar extraction of hydrogen peroxide, equal to 22 10³ M⁻¹cm⁻¹.

The determination of the activity of superoxide dismutase (SOD) according to Paoletti [13] in the modification of our laboratory is based on the detection of the degree of inhibition by superoxide
dismutase of the reduction of colorless tetrazolium salts (NBT) by superoxide anion radicals, detected spectrophotometrically by the color of the formed formazan compounds. For the work used: 0.2 mM solution of EDTA-Na (Limited liability company ‘Shijiazhuang Jackchem Co’, China) and 0.05 M solution of tetramethylenediammine (limited liability company ‘Neva Reactiv’, St. Petersburg, Russia); 0.034 mM riboflavin solution (limited liability company ‘Neva Reactiv’, St. Petersburg, Russia); 0.85 mM solution of paranitrotetrazolium chloride (limited liability company ‘Neva Reactiv’, St. Petersburg, Russia); 0.1 M phosphate buffer (limited liability company ‘Neva Reactiv’, St. Petersburg, Russia) pH 7.8; 0.1 M phosphate buffer, pH 9.85; 1% KI solution; 0.9% NaCl solution (limited liability company ‘Neva Reactiv’, St. Petersburg, Russia), etc.

Nucleic acids were determined spectrophotometrically according to Spirin [14], which consists in determining the difference in refraction of suspensions of native and denatured DNA at different wavelengths (limited liability company ‘Samson-Med’, St. Petersburg, Russia). We used lyophilized sodium salt of calf thymus DNA, NaCl, HClO₄ 65%, H₂O (SQ) (LLC ‘Neva Reactiv’, St. Petersburg, Russia).

The selected samples native (K-5) and radiomodified cells (K-4), as well as, consortia were subjected to biological testing for harmlessness. The test substances were injected subcutaneously into the thigh area in mice in a volume of 0.1 cm³: group 1 – E-1, 2nd – E-2, 3rd – E-3, 4th – E-4, 5th – E-5, 6th – B-1, 7th – B-2, 8th – B-3, 9th –B-4, 10th – B-5, 11th – K-4, 12th – K-5. The animals of the 13th group were not injected with the drug (biological control group).

The animals were clinically observed for 10 days, noting their viability, behavioral reactions, and the presence of appetite.

After 10 days, the animals were bled from the heart during ethereal euthanasia. The number of formed elements (erythrocytes, leukocytes) was determined using a Goryaev’s camera and a microscope (Open joint stock company ‘LOMO’, St. Petersburg, Russia), hemoglobin – using a Salihemometer (Open joint stock company ‘LOMO’, St. Petersburg, Russia).

The results and the reliability of the results were assessed by the Student's criterion and described using the Microsoft Excel 2016 software included in the Microsoft Office 2016 software package. The obtained digital material was subjected to statistical processing using the computer program ‘Statistika 6’.

3. Results and discussion

Cell cultures: B. bifidum 1 (after rehydration, passing in the Blaurock medium, precipitation and separation of the supernatant), as well as E. coli PL-6 (after passing mesopatamia on agar and diluting with saline solution to a concentration of 100 million m.c./ml) were used for the production of Gram-stained microbial preparations E-5 (Escherichia) and B-5 (bifidobacteria), and were also selected as test microbial preparations for testing their harmlessness on animals. In addition, the original and subsequently radio-modified cultures were studied for their cultural, morphological and biochemical properties. The initial cultures of microorganisms were subjected to increasing irradiation with gamma quanta at doses from 1 to 25 kGy (E. coli PL-6) and from 1 to 4 kGy (B. bifidum 1) with their intermediate passaging on updated nutrient media. At each stage of the work, samples were taken for the above studies (E1-E4 and B1-B4).

During the research, it was found that E. coli PL-6 bacteria have rapid growth on simple and synthetic nutrient media at a temperature of 15 °C to 45 °C at pH of 7.2-7.4, the optimal growth temperature is 37-38 °C. On dense media, microbes formed rounded, convex colonies of medium size, moist, with a smooth, shiny surface with even edges. The growth of microorganisms in liquid media occurred in the form of intense uniform turbidity of the medium, the formation of a precipitate, which disintegrated during shaking, forming a homogeneous suspension. In the Endo environment, microbes formed colonies of red color, mainly with a metallic sheen.

Under microscopy, the bacteria were located singly or in the form of small conglomerates with sizes from 1-3 microns in length and 0.5-1.3 microns in width, with Gram –negative staining.
**B. bifidum** bacteria are strict anaerobes in vivo, but under laboratory conditions they have acquired the ability to develop in the presence of a small amount of oxygen and carbon dioxide. The optimal growth temperature ranged from 37 °C to 41 °C. The optimal pH value is 6-7. Bifidobacteria were grown on liquid and solid nutrient media, creating anaerobic conditions. In the liquid medium of Blaurocka, the culture grew in the form of filamentous colonies located in the lower part of the medium. Shaking the test tube in the nutrient medium led to uniform turbidity. On dense media, bifidobacteria formed colonies of various shapes from flat, hemispherical to shiny, rough with a darker center. The color of the colonies varied from white-gray to dark brown.

Microscopy revealed that bifidobacteria are polymorphic rods with unilateral or bilateral bifurcation, 4-5 microns long, 0.2-0.5 microns thick, arranged in clusters or individual cells. They form bifurcated structures when grown on liquid or nutrient-poor media; rods are formed on solid or nutrient-poor media, located separately or in the form of adhesions with each other.

Irradiated *E. coli PL-6* in doses from 1 to 18 kGy per MPB grew in the form of uniform turbidity of the medium. When the bacteria were irradiated at a dose of 20 kGy, the nutrient medium slightly opalesced. Irradiation at a dose of 25 kGy led to complete inactivation of microbes. The nutrient medium remains transparent.

*Escherichia*, subjected to gamma quanta at a dose of 20 kGy on Mesopotamia agar (MPA), give single colonies. Repeated passaging of cells on renewed media ensures continuous growth of the *E. coli* (R10) cell colony.

**B. bifidum** bacteria irradiated at doses of 1 and 2 kGy in the liquid medium of Blaurokk give typical filamentous colonies that visually fill the space of the nutrient medium. Irradiation at a dose of 3kGy suppresses the growth of microorganisms, there is a slowdown in growth, filamentous colonies do not fill the entire space of the medium. Irradiation at a dose of 4 kGy stably suppresses the growth of the culture, while the liquid medium of Blaurokk looks transparent.

Bifidobacteria exposed to radiation at a dose of 4 kGy in a semi-solid Blaurokk medium form single colonies, which, after repeated (R6) passaging in updated media, give a continuous growth of the microbial mass.

When studying microbial preparations, it was found that *E. coli PL-6* bacteria irradiated at doses of 2 kGy and higher are characterized by polymorphism. In this case, the individual cells have a length that exceeds the original size by 3-7 times. At a radiation exposure of 12 kGy, the average number of altered (modified) cells in 10 fields of view of the microscope (magnification of 10x100) is ≈1.7. At the same time, in some fields of vision, dense, physiologically inactive, smaller cells in size the dimensions of ≈0.3-0.5 µm were revealed. After irradiation at a dose of 18 kGy, the number of modified microorganisms increases to 2.8 and 20 kGy=5.1 units.

On microbial preparations made from bacteria irradiated at doses from 10 to 20 kGy, cells with the presence of darkening of the aggregating substance or, conversely, light spaces (vacuoles) caused by the disorganization of the bacterial content, are recorded. The amount of damage increases with increasing radiation dose. On these preparations, the number of viable cells is insignificant.

Studies have shown that under radiation exposure from 2 to 4 kGy on microbial preparations of bifidobacteria, multiple darkening of pigment granules and tissue fragments are recorded as a result of destructive changes in microorganisms. After irradiation of microorganisms at a dose of 4 kGy, atypical, increased in length, polychromic cells are revealed.

At the second stage of the experiment, the radiomodified bacteria *E. coli* (R10) and *B. bifidum* (R6) (E-4 and B-4) were co-cultivated in a liquid Blaurock medium with a partial presence of oxygen (K-4). A consortium of unirradiated bacteria *E. coli PL-6* and *B. Bifidum-1* (K-5) was studied as a control to compare the morphological properties of these cultures. The incubation lasted 4 days. At the same time, microbial preparations were prepared every day for the morphological assessment of microorganisms, and after 4 days – microbial preparations (K-4 and K-5) to determine the harmlessness of the microbial mass for animals.
A day after the start of incubation of consortia of irradiated bacteria and their unirradiated analogs (K-4 and K-5), a significant number of active Escherichiosis rods surrounded by single bifidobacteria and granules of destroyed B. Bifidum cells were found on microbial preparations. After 2 days, the number of Escherichia was significant; some of the E. coli cells were dense, physiologically inactive. Physiologically active bifidobacteria were present on the preparations. After 3 days, the number of active Escherichia was insignificant, destroyed cells were revealed on the preparations, part of the Escherichia was in the stage of statis. Many active bifidobacteriashave been identified. After 4 days, the number of bifidobacteria prevailed over the cells of Escherichia coli.

The consortia of radiomodified and non-irradiated bacteria did not differ in the characteristics of interaction, but the cellular reactions of the irradiated cells proceeded more intensively, which was characterized by an accelerated transition from aerobic culture (E. coli) to anaerobic culture (B. Bifidum).

Biochemical studies have shown that metabolites of E. coli PL-6 (R10) contain 2.51 times more deoxyribonucleic acid. The peroxidase activity was 1.67 times higher. The parent bacteria did not produce the enzymes superoxide dismutase (SOD) and catalase (CAT), but E. coli PL-6 (R10) acquired these properties. Escherichia metabolites contained 0.97±0.09 m.c.M/g SOD and 27.38±0.59 mcat/g/ml CAT.

In radio-resistant bifidobacteria, as compared to native bacteria, a 1.45 times higher peroxidase content was recorded in the culture liquid.

The prepared bacterial suspensions of native Escherichia and bifidobacteria (E-5, B-5) and radiomodified variants of E. coli (E-1, E-2, E-3, E-4) and B. bifidum (B-1, B-2, B-3, B-4), as well as their native (K-5) and radiomodified (K-4) consortia in the next series of experiments were subjected to biological testing for harmlessness. As a biological model for testing, 39 white mice with an average live weight of 20±2 g were used, divided according to the principle of analogs into 13 groups, three heads in each group.

The test preparations were injected subcutaneously into the area of the inner thigh in a volume of 0.1 cm³: group 1 – E-1, 2 – E-2, 3rd – E-3, 4th – E-4, 5-1st – E-5, 6th – B-1, 7th – B-2, 8th – B-3, 9th – B-4, 10th – B-5, 11th – K-4, 12th group – K-5. The animals of the 13th group were not injected with the drug – they served as a biological control.

It was found that the survival rate of mice during the use of microbial suspensions was 100%, and the animals of all groups during the entire period looked clinically healthy, were mobile, and retained their appetite. There were no differences in the behavioral responses of individuals who received different types of cells with different physical effects. All animals remained viable 10 days after the initiation of safety testing of the study drugs. The results of hematological studies are shown in figure 1 and table 1.

**Figure 1.** The content of erythrocytes (red line – left), leukocytes (blue – middle line), hemoglobin (brown – right) relative to the control (green line – horizontal line from above).
Table 1. Hematological parameters of animals 10 days after the use of the tested drugs.

| No. group | Used microbial drugs | Erythrocytes, $10^6, \mu l$ | Leukocytes, $10^3, \mu l$ | Hemoglobin, $10^{-1}, \mu l$ |
|-----------|----------------------|-----------------------------|---------------------------|--------------------------|
| 1         | E. coli PL-6 (E-1)   | 7.97±0.15                   | 7.27±0.18                 | 109±1.20                 |
| 2         | E. coli PL-6 (E-2)   | 9.67±0.23                   | 7.87±0.18                 | 132±1.73                 |
| 3         | E. coli PL-6 (E-3)   | 9.57±0.11                   | 7.77±0.25                 | 133±0.98                 |
| 4         | E. coli PL-6 (E-4)   | 8.37±0.29                   | 8.03±0.23                 | 122±2.11                 |
| 5         | E. coli PL-6 (E-5)   | 8.37±0.59                   | 7.37±0.39                 | 125±1.53                 |
| 6         | B. bifidum 1 (B-1)  | 8.30±0.46                   | 7.23±0.18                 | 127±1.34                 |
| 7         | B. bifidum 1 (B-2)  | 7.87±0.58                   | 7.40±0.46                 | 130±1.41                 |
| 8         | B. bifidum 1 (B-3)  | 8.30±0.49                   | 7.47±0.52                 | 123±2.32                 |
| 9         | B. bifidum 1 (B-4)  | 8.47±0.32                   | 6.73±0.50                 | 126±2.45                 |
| 10        | B. bifidum 1 (B-5)  | 8.63±0.15                   | 7.33±0.15                 | 137±3.19                 |
| 11        | E. coli PL-6-B. bifidum 1 (K-4) | 9.67±0.27 | 7.53±0.04 | 125±2.40 |
| 12        | E. coli PL-6-B. bifidum 1 (K-5) | 7.93±0.59 | 7.20±0.14 | 123±1.47 |
| 13        | No impact (NI)       | 8.57±0.32                   | 7.10±0.21                 | 119±1.72                 |

From these tables and the figure it follows that the studied microbial preparations did not have a significant effect on the hematological parameters of animals. There are also insignificant differences with biological control (BC) in animals of groups E-2, E-3 and K-4 an increase in the content of erythrocytes by 12-13% on the 10th day. In mice from groups E-1, E-2, E-3 and K-4 – leukocytes level by 11-13%, in mice from groups E-2, E-3 and K-4 – hemoglobin by 11-15%.

Thus, sequential irradiation of E. coli PL-6 and B. bifidum 1 cultures in increasing doses led to an increase in radioresistance with induction of enhanced synthesis of radioprotective enzymes superoxide dismutase, catalase, and peroxidase activity involved in the formation of the organism resistance to lethal effects of ionizing radiation.

At the next stage of the experiments, the obtained microbial substances were used to design radioprotective drugs.

The main purpose of the experiments was a directed change in the phenotype of bacteria of E. coli PL-6 and B. bifidum 1 strains towards an increase in the expression of some factors and mediators useful from the point of view of practice – stimulating metabolism, radioprotective, antioxidant, antitoxic and others, which include enzymes, antigens, entero-, exotoxins, cytokines [15].

The main tool for such an effect was ionizing radiation using a stationary gamma apparatus ‘Issledovatel’ with $^{60}$Co sources (‘Baltiets’ (Narva). Irradiation was carried out in the dose range from 1 to 25 kGy with a step of 1 kGy, then the irradiated culture was sown on nutrient media and was assessed by the growth of colony-forming units.

Used strain E. coli PL-6. Under irradiation at a dose of 20 kGy, changes in the cultural, morphological and biochemical properties of bacteria were noted. After irradiation at a dose of 25 kGy, all cells died. In this regard, bacteria with an irradiation dose of 20 kGy were used to form a microbial consortium. A similar situation was observed after irradiation of B. bifidum 1 bacteria, but the doses of gamma irradiation were significantly lower.

Irradiation of the initial E. coli PL-6 strain with successively increasing doses yielded a radioresistant E. coli PL-6 (R10) strain, which differed from the initial survival rate at a dose 2.14 times higher than the initial level, and a stable radioresistant variant of bifidobacteria B bifidum 1 (R6) strain, which differs from the initial one in high radioresistance and survives at a dose 2.5 times higher than the initial level.

According to S Bourdouuet al. [14] resistance to drying and radioresistance are similar, as they cause similar cellular damage. The authors found that microorganisms isolated from the Dry Valleys of Antarctica gave growth of cultured cells after exposure to ionizing radiation of 4 and 6 kGy. It was
shown that cooling bacteria to -79 °C increased the radiation resistance of microorganisms by 9 times [16].

It was found [17] that microorganisms living at extremely high temperatures of geysers and thermal springs have different mechanisms of adaptation to stress, which allow them to overcome numerous physical and chemical barriers to survival, such as DNA damage, oxidative explosions and protein damage. These bacteria have been used to isolate the radiation-resistant thermophiles Deinococcus geothermals, which are also resistant to desiccation and maintain their homeostasis through advanced DNA repair mechanisms, the reactive oxygen species (ROS) detoxification system, and the accumulation of compatible solutes.

Studied [18] cyanobacteria dominating in the most extreme arid places of hot and cold deserts. After irradiation of 10 different strains of Chroococcidiopsis at a dose of 2.5 kGy, the survival rate was 35-80%, with irradiation of the 4 most radioresistant bacteria at a dose of 5 kGy, the survival rate decreased by 1-2 orders of magnitude, but viable cells were restored after irradiation with 15 kGy, the dose 20 kGy was fatal [19] showed that radiation tolerance in Chroococcidiopsis is associated with a decrease in oxygen exposure upon drying.

A number of researchers have shown that the evolution of microorganisms includes the simultaneous spread of various beneficial mutations [20].

Experiments with the effect of low-energy electrons and gamma radiation on DNA molecules of prokaryotes are described [21]. It has been shown that radiation reduces the viability of cells, and DNA molecules are a target for radiation. DNA repair defects cause increased sensitivity to DNA damaging agents, accumulation of mutations in the genome, and, ultimately, the development of metabolic disorders [22]. However, cells have at least seven mechanisms for restoring the structural integrity of DNA, and in the case of irreparable damage, they trigger the mechanism of apoptosis [23].

The radioactive effect on bacteria Staphylococcus epidermidis and E. coli has been studied [24], and it has been shown that the growth of microorganisms in an aerobic environment generates reactive oxygen species (ROS), which leads to oxidative stress. Antioxidant enzymes (superoxide dismutases and hydroperoxidases) and DNA repair mechanisms provide protection against ROS. Acid stress is associated with the induction of Mn superoxide dismutase (MnSOD) in Lactococcus lactis and Staphylococcus aureus. In addition, these results were confirmed in Escherichia coli strains lacking both MnSOD and iron SOD (FeSOD), but expressing heterologous MnSOD from S. thermophilus. It has been found that in E. coli FeSOD does not provide the same protection as MnSOD, and hydroperoxidases are equally important in protecting cells from acid stress. These data explain the ability of some microorganisms to survive better in an acidified environment [25]. The key antioxidant in the metabolism of long-chain fatty acids (LCFA) in E. coli is the coenzyme benzoquinone [26]. With the help of external gamma radiation [27], as a result of directed evolution, an extremely stable strain of E. coli was created. Were obtained four populations of E. coli, each of which is specially adapted to survive under the influence of high doses of ionizing radiation. Several mutations in the RecA gene and deletion of the e14 prophage contributed to the creation of a new phenotype [28].

Based on the foregoing, the use of physical methods is a tool for changing the phenotypic properties of bacteria and the development of anti-radiation drugs for animals. Our data are consistent with the studies of other authors and can serve as a basis for further experiments.

4. Conclusion
The radiation stress caused by the bacteria E. coli PL-6 and B. bifidum 1 morphological changes in the appearance of atypical cells with polymorphism, with a length larger than the original size from 3 to 7 times the number is less than 1% of B. bifidum 1 after a dose of radiation exposure of 4 kGy; 1.7% with irradiation of E. coli PL-6 in a dose of 12 kGy and 2.8% after radiation effects on cells at a dose of 18 kGy and 5.1% after irradiation of E. coli PL-6 in a dose of 20 kGy.

Irradiation of E. coli bacteria at a dose of 25 kGy and B. bifidum 1 at a dose of 5 kGy led to complete sterilization of microorganisms.
The joint cultivation of *E. coli* PL-6 and *B. bifidum* bacteria in the consortium was expressed by the manifestation of cell antagonism, in which certain microorganisms prevailed in the most favorable conditions for each type of cell, namely: in the initial stage in the presence of oxygen (1-2 days) – *E. coli* PL-6 bacteria and later, in an airless environment (3-4 days) – *B. bifidum*.

Biochemical studies revealed a change in the morphological and expressing properties of radiomodified bacteria compared to their non-irradiated analogues in the form of an increase in the content of the amount of deoxyribonucleic acid in *E. coli* bacteria-by 2.51 times, an increase in peroxidase activity – by 1.67 times, the appearance in escherichia of the properties of the production of superoxide dismutase enzymes – 0.97±0.09 m.c.M/g, catalase – 27.38±0.59 mcat/g/ml; in *B. bifidum*, the increase in peroxidase expression was 1.45 times.

Preparations made from radiomodified cells did not have a negative effect on the tested laboratory animals, causing only minor changes in the picture of their blood – the content of the absolute number of red blood cells, white blood cells and hemoglobin.

References

[1] Agrawal S, Jaswal K, Shiver A L, Balecha H, Patra T and Chaba R 2017 A genome-wide screen in *Escherichia coli* reveals that ubiquinone is a key antioxidant for metabolism of long-chain fatty acids. *J. Biol. Chem.* 292(49) 20086 doi: org/10.1074/jbc.M117.806240

[2] Andreas M 2019 *Antioxidant Status, Diet, Nutrition, and Health* 1at ed. (Boca Raton: CRC Press) p 672

[3] Bald I and Denifl S 2019 The role of low-energy electrons in DNA radiation damage. *Low-Energy Electrons Fundamentals and Applications* eds O Ingolfsson (Singapore: Jenny Stanford Publishing) chapter 6 pp 1-56

[4] Burby P E and Simmons L A 2019 A bacterial DNA repair pathway specific to a natural antibiotic. *Mol. Microbiol.* 111(2) 338 doi: org/10.1111/mmi.14158

[5] Casero M C, Ascaso C, Quesada A, Mazur-Marzec H and Wierzchos J 2021 Response of endolithic chroococcidiopsis strains from the polyextremeatacama desert to light radiation. *Front. Microbiol.* 11 1 doi: org/10.3389/fmicb.2020.614875

[6] Catanzaro M, Corsini E, Rosini M, Racchi M and LanniC 2018 Immunomodulators inspired by nature: a review on curcumin and Echinacea. *Molecules.* 23(11) 2778 doi: org/10.3390/molecules23112778

[7] Ciorba M A, Hallemeier C L, Stenson W F and Parikh P J 2015 Probiotics to prevent gastrointestinal toxicity from cancer therapy: an interpretive review and call to action. *Curr. Opin. Support. Pa.* 9(2) 157 doi: org/10.1097/SPC.000000000000134

[8] Engelsöy U, Rangel I and Demirel I 2019 Impact of proinflammatory cytokines on the virulence of uropathogenic *Escherichia coli*. *Front. Microbiol.* 10 1051 doi: org/10.3389/fmicb.2019.01051

[9] Flagiarone C, Mosca C, Ubaldi I, Verseux C, Baque M and Wilmotte A 2017 Avoidance of protein oxidation correlates with the desiccation and radiation resistance of hot and cold desert strains of the cyanobacterium. *Chroococcidiopsis. Extremophiles.* 21 981 doi: org/0.1007/s00792-017-0957-8

[10] Gaynutdinov T R, Nizamov R N, Idrisov A M, Rakhmatullina G I and GuryanovaV A 2021 Obtaining radioactivated strains of microorganisms and studying their antiradiation efficiency. *IOP Conf. Ser.: Earth Environ. sci.* 723 042008 doi: org/10.1088/1755-1315/723/4/042008

[11] Lee C, Choi N, Bae M K, Choo K and Lee S J 2019 Transposition of insertion sequences was triggered by oxidative stress in radiation-resistant bacterium. *Deinococcus geothermalis. Microorganisms* 7(10) 446 doi: org/10.3390/microorganisms7100446

[12] Lumniczky K, Candeias S M, Gaip U S and Frey B 2018 Editorial: radiation and the immune system: current knowledge and future perspectives. *Front. Immunol.* 8 1933 doi: org/10.3389/fimmu.2017.01933
[13] Paoletti F and Macali A 1990 Determination of superoxide dismutase activity by purely chemical system based on NAD(P) Hoxidation. *Method. Enzymol.* **186** 209 doi: 10.1016/0076-6879(90)86110-H

[14] Bourdoux S, Li D, Rajkovic A, Devlieghere F and Uyttendaele M 2016 Performance of drying technologies to ensure microbial safety of dried fruits and vegetables. *Compr. Rev. Food. Sci. F.* **15** 1056 doi: org/10.1111/1541-4337.12224

[15] Mothibe M E, Kahler-Venter C P and Osuch E 2019 Evaluation of the in vitro effects of commercial herbal preparations significant in African traditional medicine on platelets. *BMC Complement. Altern. M.* **19** 1 doi: org/10.1186/s12906-019-2644-z

[16] Mun G I, Kim S, Choi E, Kim C S and Lee Y S 2018 Pharmacology of natural radioprotectors. *Arch. Pharm. Res.* **41**(11) 1033 doi: org/10.1007/s12272-018-1083-6

[17] Puggioni F et al. 2019 Immunostimulants in respiratory diseases: focus on Pidotimod. *Multidiscip. Respir. Med.* **14** 31 doi: org/10.1186/s40248-019-0195-2

[18] Quinteros M A, Cano Aristizabal V, Dalmasso P R, Paraje M G and Paez P L 2016 Oxidative stress generation of silver nanoparticles in three bacterial genera and its relationship with the antimicrobial activity. *Toxicol. In Vitro* **36** 216 doi: org/10.1016/j.tiv.2016.08.007

[19] Ranawat P and Rawat S 2017 Radiation resistance in thermophiles: mechanisms and applications. *World J. Microbiol. Biot.* **33**(6) 112 doi: org/10.1007/s11274-017-2279-5

[20] Riehl T E, Alvarado D, Ee X, Zuckerman A, Foster L, Kapoor V, Thotala D, Cicoria M A and Stenson W F 2019 *Lactobacillus rhamnosus* GG protects the intestinal epithelium from radiation injury through release of lipoteichoic acid, macrophage activation and the migration of mesenchymal stem cells. *Gut.* **68**(6) 1003 doi: org/10.1136/gutjnl-2018-316226

[21] Schultzhaus Z S, Schultzhaus J N, Romsdahl J, Chen A, Hervey Iv W J, Leary D H, and Wang Z 2020 Proteomics reveals distinct changes associated with increased gamma radiation resistance in the black yeast. *Exophialadermatitis. Genes-Basel.* **11**(10) 1 doi: org/10.3390/genes11101128

[22] Sheng D H, Wang Y X, Qiu M, Zhao J Y, Yue X J and Li Y Z 2020 Functional division between the RecA1 and RecA2 proteins in *Myxococcus xanthus*. *Front. Microbiol.* **11** 140 doi: org/10.3389/fmicb.2020.00140

[23] Suvorov A 2013 Gut microbiota, probiotics, and human health. *Bioscience of microbiota, Food and Health* **32**(3) 81 doi: org/10.12938/bmfh.32.81

[24] Vagin K N, Nizamov R N, Ishmukhametov K T, Shakurov M M and Vasilevsky N M 2021 Development of a radioprotective drug based on substances of plant, microbial, zoogenic and inorganic origin. *IOP Conf. Ser.: Earth Environ. Sci.* **723** 042007 doi: org/10.1088/1755-1315/723/4/042007

[25] Beilen J W and HellingwerF K J 2016 All Three endogenous quinone species of *Escherichia coli* are involved in controlling the activity of the aerobic/anaerobic response regulator ArcA. *Front. Microbiol.* **7** 1339 doi: org/10.3389/fmicb.2016.01339

[26] Wang W, Sun M, Zheng Y L, Sun L Y, and Qu S Q 2019 Effects of *Bifidobacterium infantis* on cytokine-induced neutrophil chemoattractant and insulin-like growth factor-1 in the ileum of rats with endotoxin injury. *World J. Gastroentero.* **25**(23) 2924 doi: org/10.3748/wjg.v25.i23.2924

[27] Wang Y, Wu Y, Wang Y, Xu H, Mei X, Yu D, Wang Y and Li W 2017 Antioxidant properties of probiotic bacteria. *Nutrients.* **9**(6) 521 doi: org/10.3390/nu9050521

[28] Xie X, He Z, Chen N, Tang Z, Wang Q and Cai Y 2019 The roles of environmental factors in regulation of oxidative stress in plant. *BioMed Res. Int.* **4** 1 doi: org/10.1155/2019/9732325