The study of antibacterial, fungicidal and cytotoxic properties of antagonist microorganisms

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Abstract. Microorganisms isolated from natural sources that have antagonistic properties are the objects of the research in this paper. The main objective of the study was to screen and characterize the antagonistic properties of microorganisms isolated from natural sources in connection with the creation of new pharmaceutical substances. Methods of cultivating strains of microorganisms, chromatographic methods, spectrometric, electrophoretic methods of analysis, and methods of cryopreservation of accumulative cultures were applied. In the course of the experiment, nutrient media for cultivating strains of antagonist microorganisms were optimized, and the physiological and biochemical properties of lactic acid bacteria and other antagonist microorganisms were studied. It is proved that lactic acid bacteria and other antagonist microorganisms show antimicrobial properties on a solid medium. The antibiotic resistance of lactic acid bacteria and other antagonist microorganisms was studied, the biocompatibility of lactic acid bacteria and other antagonist microorganisms was proved. During the investigation, the antibacterial properties of the isolated peptide fractions were determined, the fungicidal properties of the isolated peptide fractions were established, and low-molecular protein compounds with antibacterial and antifungicidal activity were identified; the toxicity indicators of identified bacteriocins and fungicides were studied in vitro. The novelty of this work consists in the development of scientifically based approaches to screening and characterization of antagonistic properties of microorganisms isolated from natural sources, as well as to the cultivation of antagonist microorganisms in connection with the creation of new pharmaceutical substances of antimicrobial action.

1. Introduction
In medical institutions over the past few years, many microorganisms of various types that are not affected by traditional antibiotics have been isolated [1–5]. Even today, more than 50 people in Germany have become victims of intestinal infection caused by an antibiotic-resistant hemorrhagic strain of E. coli [6–9]. In this regard, the search for new antimicrobial drugs with a wide spectrum of action and a lower level of microbial resistance to them has both scientific and practical significance.
For several decades, the attention of researchers has been drawn to antimicrobial peptides-bacteriocins, which are produced mainly by gram-positive bacteria [3, 10–13]. Bacteriocins and bacteriocin-like substances are mainly complex antibacterial substances of protein nature [4, 14–18]. Bacteriocins differ in their spectrum of activity, mode of action, genetic control, and biochemical properties [8, 19, 20]. Many bacteriocins act against closely related species of bacteria, but sometimes have a fairly wide range of actions, which is especially characteristic of gram-positive bacteria. The peculiarity of bacteriocins is that they are not toxic and they are usually resistant to bacteria [6, 7, 21]. Lactic acid bacteria are regarded as promising strains producing bacteriocins [3, 9, 22–24].

Lactic acid bacteria acquired special significance after the discovery of the ability to produce fungicidal substances [25]. Obtaining new strains of lactic acid bacteria with antibacterial and fungicidal activity can solve the problem of not only food spoilage, but also the treatment of a number of infectious diseases in the future [26, 27]. Fungicidal substances formed by Lactobacillus bacteria have been well studied [28], while the presence of fungicidal activity among Lactococcus bacteria is a very rare and poorly studied property, and almost nothing is known about the nature of fungicidal substances [2].

In this regard, the isolation, study of the properties and synthesis of new antibacterial and fungicidal antibiotics formed by lactic acid bacteria and other antagonist microorganisms, as well as the exploring the prospects for their use in the pharmaceutical industry is of fundamental and practical interest [13].

Promising sources of microorganisms with antibiotic properties are natural sources: soils, reservoirs, plants, and food products: fermented meat and dairy products [16].

2. Research methods and materials

The objects of research were lyophilized cultures of lactic acid strains presented in Table 1. The table also shows the optimal cultivation conditions recommended by the supplier (All-Russian National Collection of Industrial Microorganisms - VKPM), as well as their morphological features and source of production.

Table 1. Strains of lactic acid bacteria.

| № Sequ./No. | Name | Origin | Application | Medium and t°C | Cultural and morphological properties |
|-------------|------|--------|-------------|----------------|---------------------------------------|
| 1.          | Bacillus subtilis (B-7918) 339 | From soil | Antibacterial and fungicidal agent. Producer of proteolytic enzymes | LB 37°C | Gram+ fixed small rods, located mostly singly. Colonies are flesh-colored, shiny, rounded, with an even edge |
| 2.          | Bacillus pumilus (B-7886) 122 | From compost | Antimicrobial agent | LB 30°C | Cells - spore-bearing rods with rounded ends, arranged singly. Colonies are grayish-white in color, rounded in shape, with a slightly wavy edge. Cells - fixed spore-bearing rods, located singly, in pairs. Colonies are rounded, |
| 3.          | Bacillus pumilus (B-1133) | BKM B-508 | Type strain | LB 30°C | |
| 4.          | Bacillus coagulans (B-9868) | DSM1, ATCC 7050 | Type strain | LB 40°C | |

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|   | **Geobacillus sterothermophilus** (B-4320) (B-11336) NCA 26 | ATCC 7953, DSM 5934, NRRL B-1102 | Sterility control, gas and steam sterilization control | LB 55°C |
|---|---|---|---|---|
| 5. |   |   |   | flesh-colored, and opaque. |
|   | **Geobacillus sterothermophilus** (B-5814) | BKM B-510 | Type strain | LB 60°C |
| 6. | **Leuconostoc lactis** (mesenteroides subsp. dextranicum) (B-2405) РД-5 | From fermented milk product | Lactic acid producer. | MRS 25°C |
| 7. | **Leuconostoc mesenteroides** subsp.mesenteroides (B-9280) | DSM 20240, ATCC 10830a | A dextran, isomaltulose | MRS 30°C |
| 8. | **Leuconostoc mesenteroides** subsp.mesenteroides (B-6243) | The rotting olive, ATCC 8293, DSM 20343 | Type strain | MRS 30°C |
| 9. |   |   |   | The cells - elongated cocci, arranged in pairs in the form of short chains. |
| 10. | **Pediococcus pentosaceus** (B-8894) PDP-106 (PDP-23) | From raw cured French sausage | Producer of lactic acid, bacteriocin, antagonist of E. coli, Salmonella, Protea | MRS 37°C |
| 11. | **Pediococcus pentosaceus** (B-7537) | BKM B-1951, CFN 150 |   | MRS 37°C |
| 12. | **Pediococcus damnosus** (B-11332) | ATCC 29358; DSM 20331 | Type strain | MRS 26°C |
| 13. | **Pediococcus damnosus** (B-10908) | ATCC 29358; DSM 20331 | Type strain | MRS 26°C |
| 14. | **Lactobacillus plantarum** (B-5466) K 9 | Corn silage | Probiotic, sourdough for silage, an antagonist of intestinal bacteria, phytopathogenic fungi, putrefactive and purulent bacteria | MRS 28°C |
|   |   |   |   | Cells - rods of different lengths, round-edged, arranged singly, in pairs, in short chains. Colonies are white, convex, shiny, rounded with an even edge. |
|   |   |   |   | Cells - rods of different lengths, arranged singly, in pairs, less often in short chains. Colonies are white, small, and rounded. |
When performing the work, generally accepted, standard and original research methods were used. The Bacillus and Geobacillus microorganisms were cultured on a common LB medium of the following composition (g / l): trypton - 10, yeast extract - 5, NaCl - 10. To obtain a solid medium, bacteriological agar was additionally added in an amount of 2%.

The Leuconostoc, Pediococcus, Lactobacillus microorganisms were cultured on the MRS medium (g/l): bactopepton - 10, ammonium limmonic acid – 2, meat extract – 10, sodium acetic acid – 5, yeast extract - 5, MgSO4×7H2O - 0.1, glucose – 20, MnSO4×5H2O - 0.05, twin 80 – 1, Na2HPO4 – 2. The pH of the medium was adjusted to 6.5 by adding 1 M hydrochloric acid solution using a Mettler Toledo pH meter (USA). The finished media were autoclaved in a Tuttnauer 2340 mk autoclave (Israel) at a temperature of 121°C for 20 minutes at a pressure of 0.5 ATM. Media were stored for no more than a month at a temperature of 4°C and were not used if color changes and precipitation occurred. Cultivation was carried out in a shaker-incubator firm at the optimal temperature for the strains.

For growing the Aspergillus flavus and Aspergillus niger fungi, The Chapek mineral medium was used, (g / l): NaN03 – 2, KN2P04 – 1, Mdso4h7n20 – 0.5, KS1 – 0.5, Fe2 (S04) 3h7n20 – 0.01. The pH of the finished medium was adjusted to 5.2 by adding 1 M hydrochloric acid solution using a pH meter Mettler Toledo (USA). The finished media were autoclaved in a Tuttnauer 2340 mk autoclave (Israel) at a temperature of 121°C for 20 minutes at a pressure of 0.5 ATM. To obtain a solid medium, an additional 2% bacteriological agar was added to the composition.

A cuvette spectrophotometer from BioRad (USA) was used for the measurement. To do this, daily cultures of bacteria grown in a liquid nutrient medium were transferred to flat-bottomed flasks in a volume of 50 ml to achieve an optical density of 0.01 at a wavelength of 600 nm. Then they were cultured at constant stirring of 120 rpm in a shaker-incubator for 18 hours for the genera Bacillus and Geobacillus and for 30 hours for the bacteria of the genera Leuconostoc, Pediococcus, and Lactobacillus.
After that, the aliquot of the culture was selected and its optical density was re-measured relative to the control, which served as a medium for the growth of the microorganism. The optimal growth was considered to be the achievement of an optical density higher than 0.5 by the culture during the specified time.

The antibacterial activity of the obtained metabolites was measured by the disco-diffusion method. Gram-positive bacteria Bacillus pumilus and gram-negative Escherichia coli were used to test the antimicrobial activity of the metabolites. The suspensions of night crops grown on a standard liquid medium of LB with a titer of 0.5 were used. The number of microorganisms (titer) in the suspension was determined by the optical density at a wavelength of 595 nm. The pathogen culture was applied by a drop method in the amount of 200 µl to a Petri dish with a diameter of 90 mm and then rubbed on the surface with a sterile spatula using the lawn method. They were left for 20 minutes under laminar drying with the lid slightly open. Next, sterile filter discs with a diameter of 0.5 cm were placed on a Petri dish in the radial direction, soaked in the studied metabolites and dried at room temperature for 10 minutes. After that, Petri dishes were left for 30 minutes at room temperature, then incubated in a thermostat at 37°C for 12 hours. The results were taken into account by the presence and size of a transparent zone of no bacterial growth around the disk. The antibiotic ampicillin at a concentration of 5 mg/ml was used as a positive control, and a disk impregnated with a liquid medium was used as a negative control.

The fungicidal activity of the obtained metabolites was measured by the disco-diffusion method. To test the fungicidal activity of metabolites, the Aspergillus flavus and Aspergillus niger mold fungi were used. The seeding density for testing fungicidal activity was 6x10⁷ conidia per 1 ml of medium. The duration of cultivation was 7 days. The results of the disk filtering method were analyzed at intervals of time (3, 9, 12, 24, 48, 72 h, etc.) and by the phases of fungi growth (stationary, accelerated, logarithmic), i.e. during periods of increased cell development in geometric progression, reduced growth rate, death or autolysis. At the end of the incubation, the zone of inhibition of growth around the disc in mm was measured, the largest of which is estimated by the degree of biocenotic connection, or its lack. Samples with filters impregnated with a medium were used as a negative control, and the pharmaceutical preparation Irunin ® (JSC Veropharm, Russia), which contains the active substance of Itraconazole, was used as a positive control.

The cytotoxicity of compounds in the in vitro system was studied by the MTT test. To do this, cells of the NEK293 line were placed in a 96-well tablet in the amount of 104 cells per well. The tested substances were added to the tablet in 5 different concentrations: 0.05 mg / ml, 0.15 mg/ml, 0.3 mg/ml, 0.6 mg / ml, 1 mg / ml. As a control, a buffer was taken in which the compounds were dissolved. The cells with the compounds were incubated for 72 hours in a CO2 incubator, after which the medium with the compounds was replaced with a working solution of MTT (5 mg/ml in a PBS buffer), consisting of 20 µl of runoff and 80 µl of the medium for cell growth. After 2 hours in the incubator, the medium with MTT was replaced with 100 ml of lysing buffer (10% SDS in 1:1 water mixture:DMFA, pH 4.7 reduced by acetic acid) and incubated for 8 hours. until the formazane crystals were dissolved.

After that, the indicators were taken with a spectrophotometer at a wavelength of 570 nm and calculated using the following formula:

\[ V = \frac{(D_s-D_g)}{(D_{std}-D_g)} \times 100\%, \]

Where \( V \) – viability (viability of experiment cells relative to control);
\( D_s \) – the optical density of the experimental wells (pilot samples);
\( D_g \) – the optical density of the medium;
\( D_{std} \) – the optical density of control wells (control samples).

3. Findings

To select optimal conditions for cultivation, the growth kinetics of microorganisms was studied using a cuvette spectrophotometer manufactured by BioRad (USA). To do this, daily cultures of bacteria grown in a liquid nutrient medium were transferred to flat-bottomed flasks in a volume of 50 ml to achieve an
optical density of 0.01 at a wavelength of 600 nm. Then they were cultured at constant stirring of 120 rpm in a shaker-incubator for 18 hours for the Bacillus and Geobacillus bacteria and for 30 hours for the Leuconostoc, Pediococcus, and Lactobacillus bacteria. After that, the culture aliquot was selected and its optical density was re-measured. As an optimal growth, we considered that the culture achieved an optical density above 0.5 during the specified time. The experiment was performed in 3 repetitions. The results of the experiment are shown in table 2.

Table 2. The results of culturing strains of lactic acid bacteria in classical media.

| № Seq./No. | Strain                                      | Optical density of the crop at the end of the cultivation period | Result             |
|------------|---------------------------------------------|-----------------------------------------------------------------|--------------------|
| 1.         | Bacillus subtilis (B-7918) 339              | 1.43±0.04                                                       | Optimal growth     |
| 2.         | Bacillus pumilus (B-7886) 122               | 1.25±0.03                                                       | Optimal growth     |
| 3.         | Bacillus pumilus (B1133)                    | 1.01±0.05                                                       | Optimal growth     |
| 4.         | Bacillus coagulans (B-9868)                 | 1.22±0.04                                                       | Optimal growth     |
| 5.         | Geobacillus sterilthermophilus (B-4320) (B-11336) NCA 26 | 0.78±0.02                                                        | Optimal growth     |
| 6.         | Geobacillus sterilthermophilus(B-5814)      | 0.64±0.07                                                       | Optimal growth     |
| 7.         | Leuconostoc lactis (mesenteroides subsp. dextranicum) (B-2405) RD-5 | 0.13±0.02                                                        | Moderate growth    |
| 8.         | Leuconostoc mesenteroides subsp.mesenteroides (B-9280) | 0.85±0.03                                                        | Optimal growth     |
| 9.         | Leuconostoc mesenteroides subsp.mesenteroides (B-6243) | 0.41±0.04                                                        | Moderate growth    |
| 10.        | Pediococcus pentosaceus (B-8894) PDP-106 (PDP-23) | 0.96±0.01                                                        | Optimal growth     |
| 11.        | Pediococcus pentosaceus (B-7537)            | 0.73±0.05                                                       | Optimal growth     |
| 12.        | Lactobacillus plantarum (B-5466) K 9        | 0.99±0.08                                                       | Optimal growth     |
| 13.        | Lactobacillus casei (B-4627)               | 0.26±0.06                                                       | Moderate growth    |
| 14.        | Lactobacillus casei (B-2873) 326            | 0.59±0.04                                                       | Optimal growth     |
| 15.        | Lactobacillus sakei (B-8936) LSK-104        | 0.32±0.01                                                       | Moderate growth    |

According to the results presented in the table, the Bacillus and Geobacillus bacteria have an optimal growth rate in the classical LB environment at the temperature recommended by the supplier. The Leuconostoc lactis Lactic acid bacteria (mesenteroides subsp. dextranicum) (B-2405) RD-5, Leuconostoc mesenteroides subsp.mesenteroides (B-6243), Lactobacillus casei (B-4627), Lactobacillus sakei (B-8936) LSK-104 showed moderate growth in the classical MRS medium and the optimization of the biomass production rate is required.

Strains of Pediococcus damnosus (B-11332), Pediococcus damnosus (B-10908), and Lactobacillus sakei (B-8952) 306(1) did not show growth in the classical MRS medium, so their growth rate indicators could not be analyzed.

To optimize the conditions of lactic acid bacteria cultivation, nutritional components, pH, and aeration conditions were varied.

The pH range was from 5 to 8 in increments of 0.5. The pH level was adjusted to the required values using 1M HCl and 1M NaOH. The results for strain growth are shown in table 3.

Table 3. The dependence of the growth rate of lactic acid bacteria on the pH value.

| Strain                                      | pH Value of the Medium |
|---------------------------------------------|------------------------|
|                                             | 5         | 5.5       | 6.0       | 6.5       | 7.0       | 7.5       | 8.0       |

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The redoses can have a significant effect on them. Various aeration modes were analyzed. For this purpose, the mixing range on the shaker changes in the aeration medium. It was decided to leave the pH of the medium at 6.5. For strains of Leuconostoc lactis (mesenteroides subsp. dextranicum) (B-2405) RD-5, Leuconostoc mesenteroides subsp. mesenteroides (B-6243), Lactobacillus casei (B-4627), Lactobacillus sakei (B-8936) LSK-104, Lactobacillus sakei (B-8952) 306(1), Pediococcus damnosus (B-11332) and Pediococcus damnosus (B-10908) strains show no growth in the MRS environment at the selected pH range. The Lactobacillus sakei (B-8936) strain LSK-104 reaches the optimum growth at a pH of 7.5. For strains of Leuconostoc lactis (mesenteroides subsp. dextranicum) (B-2405) RD-5, Lactobacillus casei (B-4627), the change in the pH of the medium was insufficient to achieve optimal growth, but it was decided to use the optimized pH in further experiments. The Leuconostoc mesenteroides subsp. mesenteroides strain (B-6243) shows similar growth in the pH range of 5.5-7.5, so it was decided to leave the pH of the medium at 6.5.

The Lactobacillus, Leuconostoc and Pediococcus bacteria are facultatively anaerobic organisms, so changes in the aeration modes can have a significant effect on them. Various aeration modes were analyzed. For this purpose, the mixing range on the shaker-incubator was set from 40 to 120, and the air flow was limited due to the use of Parafilm® sealing laboratory film. The results are presented in Table 4.

**Table 4.** The dependence of the growth rate of lactic acid bacteria on aeration modes.

| Strain                                      | Aeration parameters |
|---------------------------------------------|---------------------|
|                                             | 40 rpm              | 80 rpm              | 120 rpm             |
|                                             | Standard Parafilm® M| Standard Parafilm® M| Standard Parafilm® M|
| Leuconostoc lactis (mesenteroides subsp. dextranicum) (B-2405) RD-5 | 0.09±0.07 | 0.12±0.3 | 0.13±0.3 | 0.14±0.07 | 0.16±0.04 | 0.14±0.04 |
| Leuconostoc mesenteroides subsp. mesenteroides (B-6243) | 0.29±0.05 | 0.35±0.02 | 0.32±0.05 | 0.39±0.02 | 0.43±0.03 | 0.53±0.03 |
| Lactobacillus casei (B-4627), pH 7.5 | 0.25±0.07 | 0.21±0.06 | 0.28±0.03 | 0.25±0.05 | 0.36±0.06 | 0.38±0.05 |
| Lactobacillus sakei (B-8952) 306(1) | No growth | No growth | No growth | No growth | No growth | No growth |
| Pediococcus damnosus (B-11332) | No growth | No growth | No growth | No growth | No growth | No growth |
| Pediococcus damnosus (B-10908) | No growth | No growth | No growth | No growth | No growth | No growth |
According to the results of table 4, strains of Lactobacillus sakei (B-8952) 306 (1), Pediococcus damnosus (B-11332), and Pediococcus damnosus (B-10908) show no growth in the MRS environment under the presented aeriation parameters. Changes in the aeriation parameters of mixing modes do not affect the growth of Leuconostoc lactis (mesenteroides subsp. dextranicum) (B-2405) RD-5. Leuconostoc mesenteroides subsp. mesenteroides (B-6243) and Lactobacillus casei (B-4627) demonstrate the maximum increase in biomass growth with limited oxygen access and at 120 rpm mixing mode.

To optimize the growth of crops, the media composition was also modified. In type 1, trypton/casein was added to the MRS medium as an additional source of amino acids and trace elements in the amount of 5 g/l. In type 2, milk agar was used in the composition: meat extract (g / l) – 5.00, yeast extract – 3, dry milk -1. In option 3, an additional 5 g/l of NaCl was added to the medium. In type 4, an alternative composition of MRS medium salts was used: sodium acetic acid 3-water-5, ammonium limmonic acid-2, K2HPO4×3H2O-2.6, MgSO4×7H2O-0.1, MnSO4×4H2O-0.05, cysteine chloride 1- water – 0.5. The results are presented in table 5.

Table 5. The dependence of the growth rate of lactic acid bacteria on the nutritional components.

| Strain | Medium type №1 | Medium type №2 | Medium type №3 | Medium type №4 |
|--------|----------------|----------------|----------------|----------------|
| Leuconostoc lactis (mesenteroides subsp. dextranicum) (B-2405) PD-5 | 0.14±0.02 | 0.13±0.05 | 0.21±0.01 | 0.10±0.05 |
| Lactobacillus casei (B-4627) | 0.38±0.05 | 0.42±0.06 | 0.50±0.09 | 0.49±0.02 |
| Lactobacillus sakei (B-8952) 306(1) | No growth | No growth | No growth | No growth |
| Pediococcus damnosus (B-11332) | No growth | No growth | No growth | No growth |
| Pediococcus damnosus (B-10908) | No growth | No growth | No growth | No growth |

The results presented in table 5 indicate that the optimal growth medium for the specified parameters for Leuconostoc lactis (mesenteroides subsp. dextranicum) (B-2405) RD-5 is the variant of medium №3, although the strain after optimization of cultivation demonstrates a moderate growth rate. For Lactobacillus casei (B-4627), the optimal media composition is type 3 and 4. In the future, we will use the medium type №4. Strains of Lactobacillus sakei(B-8952) 306 (1), Pediococcus damnosus (B-11332), and Pediococcus damnosus (B-10908) do not grow on the selected media types, which most likely indicates that the crops are not viable. Further work with these strains was not conducted.

Thus, the media and conditions for growth of Leuconostoc lactis (mesenteroides subsp. dextranicum) (B-2405) RD-5, Lactobacillus mesenteroides subsp.mesenteroides (B-6243), Lactobacillus casei (B-4627), Lactobacillus sakei (B-8936) LSK-104 strains were optimized. For Leuconostoc lactis (mesenteroides subsp. dextranicum) (B-2405) RD-5, the growth optimum is pH 7.5, with a standard aeration mode and a mixing speed of 120 rpm. The optimal medium for the growth rate of the type №3 medium, which is a milk agar with the addition of sodium chloride. Leuconostoc mesenteroides subsp. mesenteroides (B-6243) will be cultivated on a pH 6.5 medium, under partially aerobic conditions at a mixing speed of 120 rpm. For Lactobacillus casei (B-4627), the optimal growth is type №4 medium, which is a modified version of the MRS medium with a pH of 7.5, under partially aerobic conditions at a mixing speed of 120 rpm. Lactobacillus sakei (B-8936) LSK-104 demonstrated a good growth rate under the following conditions: pH of the MRS medium equals 7.5.

The study of the antibacterial activity of conditioned medium metabolites after cultivation of lactic acid bacteria strains was carried out. At this stage it was possible to determine whether the strains...
produce antibiotic substances in the medium, as well as whether they lose their properties after drying the medium and freezing it at -20°C for long-term storage. The results of the strain study are presented in Table 6.

Table 6. The results of the study of the antibacterial activity of medium metabolites after lactic acid bacteria cultivation.

| № Seq./No. | Antagonist | Pathogen lysis zone (mm) |
|-------------|------------|--------------------------|
|             |            | B. pumilus | E. coli |
| 1.          | Bacillus subtilis (B-7918) 339 | 2 | 1 |
| 2.          | Bacillus pumilus (B-7886) 122 | 0.5 | 0 |
| 3.          | Bacillus pumilus (B-1133) | 1 | 2 |
| 4.          | Bacillus coagulans (B-9868) | 1 | 0.5 |
| 5.          | Geobacillus sterothermophilus (B-4320) (B-11336) NCA 26 | 1 | 0.2 |
| 6.          | Geobacillus sterothermophilus (B-5814) | 1 | 1 |
| 7.          | Leuconostoc lactis (mesenteroides subsp. dextranicum) (B-2405) RD-5 | 0 | 0 |
| 8.          | Leuconostoc mesenteroides subsp. mesenteroides (B-9280) | 0.5 | 0.2 |
| 9.          | Leuconostoc mesenteroides subsp. mesenteroides (B-6243) | 0.1 | 0.3 |
| 10.        | Pediococcus pentosaceus (B-8894) PDP-106 (PDP-23) | 0 | 0.2 |
| 11.        | Pediococcus pentosaceus (B-7537) | 2 | 2 |
| 12.        | Lactobacillus plantarum (B-5466) K 9 | 1 | 0 |
| 13.        | Lactobacillus casei (B-4627) | 1 | 0 |
| 14.        | Lactobacillus casei (B-2873) 326 | 1 | 1 |
| 15.        | Lactobacillus sakei (B-8936) LSK-104 | 0.2 | 0.2 |

According to the results of the experiment, it was proved that the medium metabolites after cultivation of Leuconostoc lactis (mesenteroides subsp. dextranicum) (B-2405) RD-5 do not have an antagonistic effect on the strains of B. pumilus and E. coli. Also, this strain has a moderate growth rate, so it will not participate in further studies.

Lactobacillus plantarum (B-5466) To 9, Lactobacillus casei (B-4627) show activity against B. pumilus and do not show activity against E. coli. Pediococcus pentosaceus (B-8894) PDP-106 (PDP-23) shows no activity against B. pumilus and shows little activity against E. coli.

Lactobacillus sakei (B-8936) LSK-104, Leuconostoc mesenteroides subsp. mesenteroides (B-6243) show moderate activity against both types of strains. The bacteria Bacillus subtilis (B-7918) 339, Bacillus pumilus (B1133), Pediococcus pentosaceus (B-7537), Lactobacillus casei (B-2873) 326, and Geobacillus sterothermophilus (B-5814) have a pronounced antagonism against both strains. Expressed antagonism against B. pumilus and weak antagonism of E. coli are the strains of Geobacillus sterothermophilus (B-4320) (B-11336) NCA 26 and Leuconostoc lactis (mesenteroides subsp. dextranicum) (B-2405) RD-5. The strain of Leuconostoc mesenteroides subsp. mesenteroides (B-9280) shows medium antagonism against B. pumilus and weak antagonism against E. coli.

Thus, all strains with the exception of Leuconostoc lactis (B-2405) produce antibacterial compounds in the medium and remain active after drying and freezing, which makes it possible to use these strains as producers of substances that have antagonistic properties.

The analysis of the sensitivity of the ICD strain to the action of antibiotics showed the presence of a bactericidal effect in 6 cases out of 15 (40%), a bacteriostatic effect in 13 cases out of 15 (86%) and the manifestation of a combined effect in 2 of 15 definitions (13%). Statistical indicators of the activity of antibiotic substances in relation to the strain are presented in Table 7.
Table 7. The results of determining the sensitivity of lactic acid bacteria to antibiotics on the agarized substrate of LB and MRS.

| Antibiotic       | Absence zone. Growth retardation | \( \Delta \) Lim. mm | CV, %\(^6\) | \( x \pm S. \) mm |
|------------------|----------------------------------|----------------------|-------------|------------------|
| Rifampicin       | 6-31                             | 33.3                 | 23.2+ 9.5   |
| Gentamicin       | 6-8                              | 33.3                 | 7.0+ 1.0    |
| Streptomycin     | 6-8                              | 13.3                 | 7.0+ 1.0    |
| Benzylpenicillin | 0                                | 0                    | 0           |
| Ristomycin       | 6-16                             | 26                   | 12.3+4.5    |
| Oleandomycin     | 6-30                             | 33.3                 | 22.2+8.4    |

\(^6\)CV, % - a statistical indicator of the antibiotic substances activity directed to lactic acid bacteria strains (%).

Table 8 shows the results of determining the antibiotic resistance of lactic acid bacteria strains.

According to the data obtained, the strains of Leuconostoc lactis (mesenteroides subsp. dextranicum) (B - 2405), Leuconostoc mesenteroides subsp.mesenteroides (B-6243), Lactobacillus sakei (B-8936) have resistance to the studied types of antibiotics.

Table 8. The results of research on antibiotic resistance of lactic acid bacteria strains.

| № Seq./No. | Object name                                      | Antibiotics            |
|------------|--------------------------------------------------|------------------------|
|            |                                                  | Rifampicin | Gentamicin | Streptomycin | Benzylpenicillin | Ristomycin | Oleandomycin |
| 1.         | *Bacillus subtilis* (B-7918)                     | -          | -          | -            | -               | -          | -           |
| 2.         | *Bacillus pumilus* (B-7886)                      | +          | +          | +            | -               | +          | +           |
| 3.         | *Bacillus pumilus* (B-1133)                      | -          | -          | -            | -               | -          | -           |
| 4.         | *Bacillus coagulans* (B-9868)                    | +          | -          | -            | -               | +          | -           |
| 5.         | *Geobacillus stercothermophilus* (B-11336)       | +          | +          | -            | -               | +          | +           |
| 6.         | *Geobacillus stercothermophilus* (B-5814)        | +          | -          | -            | +               | +          | +           |
| 7.         | *Leuconostoc lactis* (mesenteroides subsp. dextranicum) (B-2405) | +          | +          | +            | -               | -          | +           |
| 8.         | *Leuconostoc mesenteroides subsp.mesenteroides* (B-9280) | -          | -          | -            | +               | +          | -           |
| 9.         | *Leuconostoc mesenteroides subsp.mesenteroides* (B-6243) | -          | -          | -            | -               | -          | -           |
10. *Pediococcus pentosaceus* (B-8894) 

   *Pediococcus pentosaceus* (B-7537) 

11. *Lactobacillus plantarum* (B-5466) 

12. *Lactobacillus casei* (B-4627) 

13. *Lactobacillus casei* (B-2873) 

14. *Lactobacillus sakei* (B-8936) 

*Lactobacillus casei* (B-2873), *Lactobacillus casei* (B-4627), *Pediococcus pentosaceus* (B-8894), *Lactobacillus plantarum* (B-5466), *Bacillus pumilus* (B-1133), *Bacillus subtilis* (B-7918).

The Bacillus pumilus strain (B-7886) had a bactericidal effect of the rifampicin and ristomycin antibiotics, a bacteriostatic effect was produced by gentamicin and streptomycin, and a mixed effect – oleandomycin. Geobacillus sterothermophilus (B-5814) also showed no growth when exposed to rifampicin and ristomycin, a bacteriostatic effect was observed when using the gentamicin and oleandomycin antibiotics. In the case of the bacterial strain of Geobacillus sterothermophilus (B-11336), the bactericidal effect with antibiotics was detected in the case of ristomycin, the bacteriostatic effect was identified with gentamicin, oleandomycin and ristomycin.

Against Bacillus coagulans (B-9868), the bactericidal effect was demonstrated by rifampicin and ristomycin, bacteriostatic effects were not detected. Leuconostoc mesenteroides subsp. mesenteroides (B - 9280) was resistant to bactericidal action, the bacteriostatic effect was recorded with the following antibiotics: rifampicin, gentamicin, streptomycin, oleandomycin. *Pediococcus pentosaceus* (B-7537) was also resistant to bactericidal action, the bacteriostatic effect was detected with such antibiotics as: rifampicin, gentamicin, oleandomycin.

The MTT assay, which is widely used in experimental studies to assess cellular cytotoxicity, was applied in this research. This test is based on the ability of a group of enzymes that catalyze redox reactions such as mitochondrial and cytoplasmic dehydrogenases of living metabolically active cells to convert and restore tetrazolium derivatives – colorless water-soluble 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (synonym MTT, yellow tetrazol) to formazan. Formazan crystallizes inside the cell in the form of violet-blue crystals. Conversion of crystals of formazan in the solution is carried out by using suitable organic solvents. Subsequent spectrophotometry of this solution makes it possible to accurately compare the change in optical density relative to the control with the change in the number of viable cells, and in cytotoxic studies to assess the specific cell death induced by a particular cytotoxic agent.

The research has shown that some of the identified bacteriocins and fungicides have weak cytotoxic properties and on average, at a maximum concentration of 1 mg/ml of the test substance, the cell viability is 90% of the control cells in most of the studied compounds. Connections 9868_10, 9868_11, 8936_9, 7537_9, 8894_15, 8894_17 demonstrate a lack of cytotoxic activity within the limits of statistical progressiveness.

Thus, as a result of the carried out study, the antibacterial, fungicidal and cytotoxic properties of lactic acid bacteria and other antagonist microorganisms were established and investigated.

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