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# ANTIBIOTIC RESISTANCE OF LACTIC ACID BACTERY LEAVEN "VIVO PROBIOYOGURT"

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Lactic acid bacteria play a key role in human microecology and biotechnology — form organoleptic characteristics of products, increase the nutritional, including biological value of functional foods. Natural resistance to antibiotics is one of the important factors that determine the probiotic properties of lacto- and bifidobacteria.

*Aim*. Study of the antibiotic resistance of functionally active probiotic cultures of «VIVO probioyogurt» leaven to determine the possibility of using a fermented milk product, which is prepared on its basis, during antibiotic therapy to maintain and restore normal intestinal microflora.

*Methods*. Pure cultures of lactic acid bacteria (LAB) were selected for the study: (*Lactobacillus delbrueckii ssp., L. acidophilus, L.casei, L. rhamnosus, L.paracasei, Streptococcus thermophilus, Bifidobacterium lactis* (2 strains), *B. infantis*), which are part of leaven „VIVO probioyogurt" the quality of which is confirmed by certificates of the International Organization for Standardization ISO 9001: 2008, as well as ISO 22000: 2005. The method of the experiment consisted of the following stages: preparation of nutrient media („Lactobacagar", „Bifidoagar", glucose-peptone medium), working solutions of antibiotics; working suspension of LAB; suspensions of cultures (lacto- and bifidobacteria), cultivation LAB on elective nutrient media with the addition of antibiotics and evaluation of research results. Determination of antibiotic resistance of LAB was performed by the method of double dilutions.

*Results*. The use of this technique enabled to establish the minimum inhibitory concentration (MIC) of antibiotics of different groups relative to the LAB. The results of the research were processed using a licensed computer program Microsoft Excel.

*Conclusions*. Evaluation of the results of studies to determine the MIC of antibiotics — benzylpenicillin, azithromycin, lincomycin, gentamicin sulfate, ceftriaxone, norfloxacin, amoxil, streptomycin, tetracycline, erythromycin in relation to IBD; fermented milk product, which was prepared on the basis of this starter culture, it was advisable to use during antibiotic therapy to restore and maintain normal intestinal microflora.

***Key words:*** antibiotic resistance, lactic acid bacteria, minimum inhibitory concentration, yeast, probiotics.

In recent years, with the global environmental problems exacerbated by pollution and chemical preparations in wide use, human microecology has been strongly disrupted. This causes pathological changes in the digestive and immune organ systems. The problem became ever more urgent during the COVID-19 pandemics, as the doctors assign antibiotics to treat pneumonia. Antibiotics are the choice treatment for a suspected bacterial infection; to protect the gut microbiome during such therapy, it is recommended to use probiotics or high-quality fermented dairy products, which help the normal gut flora of the patients recover [1–5].

Currently, the functional nutrition is widely accepted, e. g., the systematic consumption of foodstuffs exhibiting regulatory effects on the human organism or its specific systems and organs.

Functional nutrition foods include products with governable properties depending on the aim they are supposed to serve. The foods to be regularly consumed by

all population subgroups should support and improve health. These foods should also lower the risk of nutrition-related diseases through constituent functional components that efficiently moderate physiological functions and metabolic reactions in the human organism [6–10].

In 1984, Japan started the first State project whose main goal was to create a system of functional nutrition. In 1991, the system was officially recognized by law as the Food for specified health use (FOSHU). That was when the concept of foodstuffs usable to support the population's health was developed [11–13].

The USA market for functional foods is the largest, amounting to 35–50% by different estimates. The high level of functional foods consumption in the country is due to the liberal nutritional law, market freedom, and the public attention to the innovations in nutrition and health protection.

In 1992, Ukraine and 159 other countries of the world approved the World Declaration and Plan of Action on Nutrition, committing to prevent the chronic shortage of the necessary vitamins, microelements, and other substances in the people's nourishment [11].

Nowadays, an unresolved problem is introducing dairy products containing probiotics apathogenic for the human body but antagonistic to the relatively pathogenic microbes and opportunistic infections. Such properties help preserve and recover normal gut microbiota [7–8]. The most common dairy products are kefir, yogurts, bioyogurts, ryazhenka, etc.

In 2020, Ukraine initiated the reform of the school catering system. Several Ministries (of Science and Education, of Economy, and of Health) joined the task beginning with comprehensively studying how the service was provided. As a result, the Plan for Measures to Reform School Catering (Cabinet of Ministers Decree on August 5, 2020) was developed and approved as part of the abovementioned reforms; the schoolchildren's daily menu now should include 150 mL of a dairy product (kefir, bioyogurt, or ryazhenka) [14].

The main requirements to be met by the probiotic preparations are bile tolerance, temperature tolerance, antimicrobial activity, and resistance to the most common antibiotics. The list suggests that antibiotic resistance is a major requirement for the microbial cultures selected for the probiotics or the fermented foods on their basis.

Many authors [14–22] have researched lactic acid bacteria (LAB) strains and probiotic preparations. For example, Kitaevskaya [16] established by the disk diffusion test that pure LAB cultures (*L. casei*, *L. fermentum*, *L. acidophilum*, *L. brevis*, etc.), except for *L. fermentum,* are highly susceptible to benzylpenicillin (for *L. casei*, the inhibition zone was 24 mm). Also, three strains, of *L. casei*, *L. plantarum*, and *L. brevis,* are sensitive to lincomycin (for *L. casei*, the inhibition zone is 3 mm). LAB cultures are *L. casei*, *L. fermentum* and *L. bavaricus*.

Studying the genetics of the antibiotic resistance of LAB, the authors of [16–18] proved that LAB have chromosomal resistance to many antibiotic substances depending on the species and the strain. The genetic basis of the phenomenon was confirmed to be a result of either own DNA mutations or incorporation of DNA of other microbes. The heredity of LAB resistance to numerous antibiotics is a consequence of mutations or determined by the acquired plasmids [15].

The author of [17] used serial dilutions to find out the MIC of antibiotics for bifidobacteria. The bacteria were resistant to third-generation antibiotics. The MIC for ceftazidime and cefepime was approximately 4 μg/mL.

With this in mind, studying antibiotic resistance of functionally active strains of LAB, which promise a lot for producing a wide range of fermented drinks, is an urgent task.

We aimed to study the resistance to antibiotics of the functionally active probiotic cultures of the "VIVO probioyogurt" starter to find out whether the product based on it is a valid choice to consume to support and recover the gut microbiome during antibiotic therapy.

## Materials and Methods

For this research, we chose pure cultures of lactic acid bacteria (LAB) (*Lactobacillus delbrueckii* ssp., *L. acidophilus, L. casei, L. rhamnosus, L. paracasei, Streptococcus thermophiles, Bifidobacterium lactis,* and *B. infantis*), of which the "VIVO probioyogurt" starter is composed. The starter (produced by the Food Resources Institute of the National Academy of Agrarian Sciences of Ukraine) was selected as a source of the pure cultures of lactobacilli and bifidobacteria because it meets the European standards of quality, certified by the International Organization for Standardization (ISO 9001:2008 and ISO 22000:2005).

LAB were cultured on the Lactobacagar selective culture medium, g/L: fermentative

peptone — 15.0, glucose — 5.0, microbiological agar — 10.0, sodium acetate — 4.0, yeast extract — 2.8, potassium dihydrogen phosphate — 1.5, ammonium citrate — 1.5, magnesium chloride — 0.1, ascorbic acid — 0.04, manganese sulfate — 0.04, and distilled water — 1.0 L, pH of 6.8–7.0. Bifidobacteria were cultured on the Bifidoagar selective medium, g/L: fermentative peptone — 2.3, glucose — 7.5, yeast extract — 5.25, sodium chloride — 5.0, lactose — 2.5, bacteriological agar — 0.75, sodium acetate — 0.5, cysteine hydrochloride — 0.5, ascorbic acid — 0.5, magnesium chloride — 0.5, and distilled water — 1.0 L, with pH of 6.8–7.0. The cultures were kept for 48 hr at 37 °C. The working bacterial suspension of the LAB was prepared on the glucose-peptone accumulation medium, g/L: peptone — 5, glucose — 10, sodium chloride — 5, and distilled water — 1 L. The culture media were sterilized at 0.5 atm for 30 min.

We tested such antibiotics: benzylpenicillin, azithromycin, lincomycin, gentamicin sulfate, ceftriaxone, norfloxacin, amoxil, streptomycin, tetracycline, erythromycin.

The working dilution of the antibiotics was 200 μg/mL:

- Sample No. 1 — benzylpenicillin (Pen) (produced at PJSC Kyivmedpreparat, Ukraine, Kyiv);
- Sample No. 2 — azithromycin (Az) (PJSC Chervona Zirka, Chemical & Pharmaceutical Plant, Ukraine, Kharkiv);
- Sample No. 3 — lincomycin (Lin) (PJSC Kyivmedpreparat, Ukraine, Kyiv);
- Sample No. 4 — gentamicin sulfate (Gen) (Galychfarm, Ukraine, Lviv);
- Sample No. 5 — ceftriaxone (Cef) (PJSC Kyivmedpreparat, Ukraine, Kyiv);
- Sample No. 6 — norfloxacin (Nor) (Zdorovye LLC, Ukraine, Kharkiv);
- Sample No. 7 — amoxil (Am) (PJSC Kyivmedpreparat, Ukraine, Kyiv);
- Sample No. 8 — streptomycin (Str) (PJSC Kyivmedpreparat, Ukraine, Kyiv);
- Sample No. 9 — tetracycline (Tetr) (JSC Vitaminy, Ukraine, Uman);
- Sample No. 10 — erythromycin (Er) (PharmaLife LTD, Ukraine, Lviv).

The antibiotics were diluted aseptically, adding sterile distilled water. To determine MIC, the following concentrations were prepared and tested by adding to the Lactobacagar and Bifidoagar culture media, μg/mL: 100; 50; 25; 12.5; 6.25; 3.13; 1.56; 0.78. The range was chosen based on data in the literature [15, 16,18].

In the bacteriological practice, two methods to determine MIC of antibiotics are the disk diffusion test and the serial dilution one [19, 23]. The latter yields more accurate quantitative data. Testing the LAB resistance to antibiotics was done by the double dilution method [19, 23]. The resistance to antibiotics in this way is characterized by the substance's activity relative to the LAB. The method allows establishing the MIC of the antibiotic in the semi-liquid (Bifidoagar) and solid (Lactobacagar) culture media.

To establish the LAB antibiotic resistance, we prepared a homogeneous working suspension based on the saline solution and the "VIVO probioyogurt" starter: the contents of the vial (0.5 g) were aseptically re-suspended in 10 mL 0.85% NaCl. The suspension was added to the two selective culture media to obtain the lactobacilli and bifidobacteria from the starter's consortium, which is the common practice [18]. For this, the biomaterial from the selective culture media (Lactobacagar and Bifidoagar) was transferred by a loop to test tubes with the glucose-peptone medium and cultured for 4–5 hr to obtain a $1 \cdot 10^5$ cell/mL suspension. Cell density was standardized by diluting the suspension with the medium or the saline solution. The optical density was measured using the DEN-1 densitometer, for which the operating range is 0.0–6.0 McFarland Units [24].

The morphology of the isolated LAB was studied by microscopy using the Microscope Digital Eyepiece DCM-800 (8.0M pixels, CMOS) at 1000× magnification after staining the samples with methylene blue.

The results were treated using Microsoft Excel software. The relative error meets the $P < 0.05$ condition. The bacteriological parameters (LAB titers depending on the antibiotic concentration) are presented on the graphs logarithmically.

## Results and Discussion

The probiotics market in Ukraine boasts numerous starters for fermented dairy products recommended for daily use. We chose one, the "VIVO" starter (manufactured in Ukraine) to determine whether the probiotic yogurt is worthwhile in augmenting antibiotic treatment.

To study the antibiotic resistance of the probiotic cultures of the "VIVO probioyogurt" starter, we tested not the individual strains but the antibiotic sensitivity for the *Lactobacillus* and *Bifidobacterium* genera since fermented

dairy products are made from starters using pure LAB cultures in symbiosis.

The obtained bacterial suspension was identified and studied morphologically. The microscopy results are presented in Fig. 1.

According to the studied morphological features of the lactobacilli and bifidobacteria, the species composition of the "VIVO probioyogurt" was as stated: the rod-shaped lactobacilli formed short chains either single or paired, the bifidobacteria were Y- or V-shaped rods. By using the standard double solution method [19, 23], we found the MIC for such antibiotics: benzylpenicillin, azithromycin, lincomycin, gentamicin sulfate, ceftriaxone, norfloxacin, amoxil, streptomycin, tetracycline, erythromycin, for the lactobacilli and bifidobacteria of the "VIVO probioyogurt" starter (Table 1). The least antibiotic concentration inhibiting LAB growth (visually identified as no colony growing on the Petri dishes and the test tubes containing the culture medium remaining transparent) was taken for the substance's minimal inhibiting concentration (MIC) for the studied cultures. It fully inhibited LAB growth.

By their susceptibility to antibiotics, LAB can be graded susceptible (MIC does not exceed 8 μg/mL), conditionally resistant, and resistant. As for the antibiotic MIC of the lactobacilli and bifidobacteria of the starter (Table 1), the test tubes filled with the culture medium were transparent, without visible signs of opalescence. On the Petri dishes, the antibiotics used in noted concentrations (Table 1) inhibited colony growth, characterizing the MIC for these antibiotic groups. The results show that the bacteria were most resistant to

*Table 1*. **LAB sensitivity to various antibiotics, P < 0.05**

| Sample No. | Antibiotic | Antibiotic MIC for the LAB, μg/mL | |
|---|---|---|---|
| | | *Lactobacilli* | *Bifidobacteria* |
| 1 | Pen | 12.5 | 6.25 |
| 2 | Az | 6.25 | 25 |
| 3 | Lin | 3.13 | 6.25 |
| 4 | Gen | 6.25 | 25 |
| 5 | Cef | 50 | 50 |
| 6 | Nor | 50 | 25 |
| 7 | Am | 25 | 50 |
| 8 | Str | 100 | 100 |
| 9 | Tetr | 100 | 50 |
| 10 | Er | 25 | 25 |

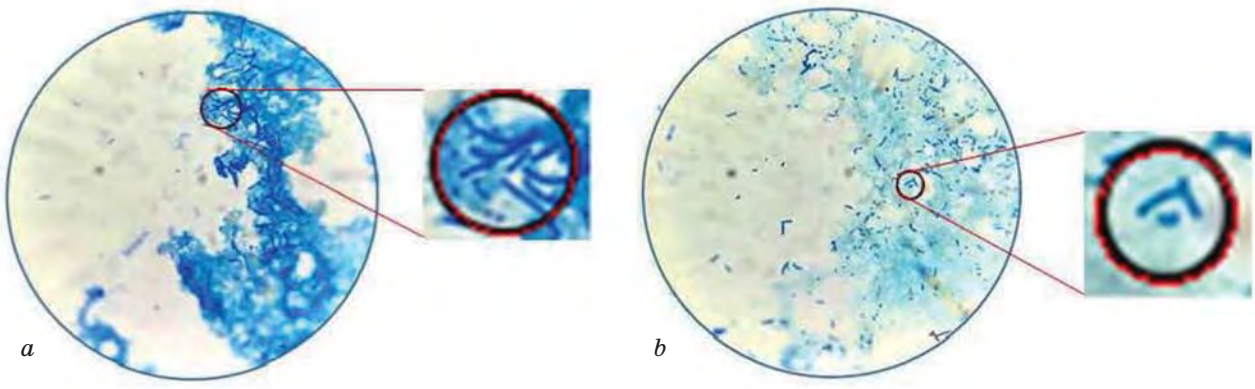


*Fig. 1*. **Microscopy of the studied suspension samples, at 1 000× magnification:**
*a* — lactobacilli; *b* — bifidobacteria

streptomycin, tetracycline, erythromycin, and ceftriaxone; the MIC for these antibiotics were 25, 50 and, 100 μg/mL. The bacteria were the least resistant to lincomycin, gentamicin sulfate, and benzylpenicillin (MIC range 3.13–12.5 μg/mL). The bifidobacteria were the most susceptible to benzylpenicillin and lincomycin. The LAB titer in the control samples (without antibiotics) was $1.5 \cdot 10^7$ CFU/mL (lg = 7.17) for the lactobacilli and $2 \cdot 10^7$ cells/mL (lg = 7.3) for bifidobacteria.

To compare and evaluate the results, we studied the susceptibility of reference strains of the lactobacilli and bifidobacteria (*L. lactis* 4/1, *L. casei* 5/4, *L. delbrueckii* 39/2, *L. acidophilus* 31/2, *L. plantarum* 17/2, *B. longum* PXN 30, *B. breve* PXN 25, *B. bifidum* PXN 23)) to the same antibiotics (Table 2).

Table 2 shows the susceptibility of reference LAB strains (*L. lactis* 4/1, *L. casei* 5/4, *L. delbrueckii* 39/2, *L. acidophilus* 31/2, *L. plantarum* 17/2, *B. longum* PXN 30, *B. breve* PXN 25, *B. bifidum* PXN 23) to the following antibiotics: benzylpenicillin, azithromycin, lincomycin, gentamicin sulfate, ceftriaxone, norfloxacin, amoxil, streptomycin, tetracycline, erythromycin. The reference strains have the bacteria's typical physiological, morphological, and biochemical properties. The studied reference strains of the lactobacilli and bifidobacteria were the most susceptible to benzylpenicillin (No.1) except for *L. casei* 5/4. All the studied reference strains of the lactobacilli were highly susceptible to azithromycin (MIC 3.13; 6.25 μg/mL). The most resistant to this antibiotic were the reference strains of bifidobacteria *B. longum* PXN 30, *B. breve* PXN 25, and *B. bifidum* PXN 23, which to our thought, is caused by antibiotic resistance genes. Many authors [17, 18] consider the genus *Bifidobacterium* to be naturally resistant to some antibiotics, which is also confirmed by our results (Tables 1, 2). Among the lactobacilli reference strains, the most resistant to the tested antibiotics was *L. casei* 5/4. *L. lactis* 4/1 was the most sensitive among the lactobacilli reference strains to the preparations (benzylpenicillin (η 1), azithromycin (η 2), lincomycin (η 3), ceftriaxone (η 5), and amoxil (η 7)). Among the representatives of the *Bifidobacterium* genus, the most resistant to the tested antibiotics was *B. lactis* PXN 30, sensitive to benzylpenicillin (No. 1).

A comparison of MIC values for the lactobacilli and bifidobacteria of the "VIVO probioyogurt" starter and the reference LAB strains (*L. lactis* 4/1, *L. casei* 5/4, *L. delbrueckii* 39/2, *L. acidophilus* 31/2, *L. plantarum* 17/2, *B. longum* PXN 30, *B. breve* PXN 25, and *B. bifidum* PXN 23) shows that the LAB consortium of the starter is sufficiently resistant to several antibiotic preparations (Table 1). The MIC for the starter is comparable to the MIC for the reference strains (Table 2).

We determined the numbers of LAB cultured on Lactobacagar (Fig. 2) and Bifidoagar (Fig. 3) at antibiotics concentrations below the MIC (0.78 to 50 μg/mL). The results are statistically significant at $P < 0.05$.

The study results (Fig. 2) show that adding antibiotics No. 5–10 (ceftriaxone, norfloxacin, amoxil, streptomycin, tetracycline, erythromycin) at 0.78–1.56 μg/mL do not significantly inhibit the lactobacilli compared

*Table 2.* **Susceptibility of the LAB reference strains to various antibiotics, $P < 0.05$**

| Strain | MIC of the antibiotic (1–10) for the reference strains of lactobacilli and bifidobacteria, μg/mL | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| *L. lactis* 4/1 | 6.25 | 3.13 | 1.6 | 12.5 | 3.13 | 12.5 | 6.25 | 25 | 50 | 12.5 |
| *L. casei* 5/4 | 25 | 6.,25 | 6.25 | 6.25 | 50 | 50 | 25 | 100 | 100 | 25 |
| *L. delbrueckii* 39/2 | 12.5 | 6.25 | 3.13 | 12.5 | 25 | 25 | 12.5 | 100 | 100 | 25 |
| *L. acidophilus* 31/2 | 12.5 | 3.13 | 1.56 | 3.13 | 50 | 100 | 12.5 | 100 | 100 | 12.5 |
| *L. plantarum* 17/2 | 6.25 | 6.25 | 6.25 | 3.13 | 12.5 | 12.5 | 12.5 | 50 | 50 | 12.5 |
| *B. lactis* PXN 30 | 6.25 | 25 | 6.25 | 50 | 50 | 25 | 50 | 100 | 25 | 25 |
| *B. breve* PXN 25 | 3.13 | 12.5 | 3.13 | 25 | 50 | 12.5 | 25 | 100 | 12.5 | 12.5 |
| *B. bifidum* PXN 23 | 3.13 | 12.5 | 6.25 | 50 | 50 | 25 | 25 | 100 | 50 | 12.5 |

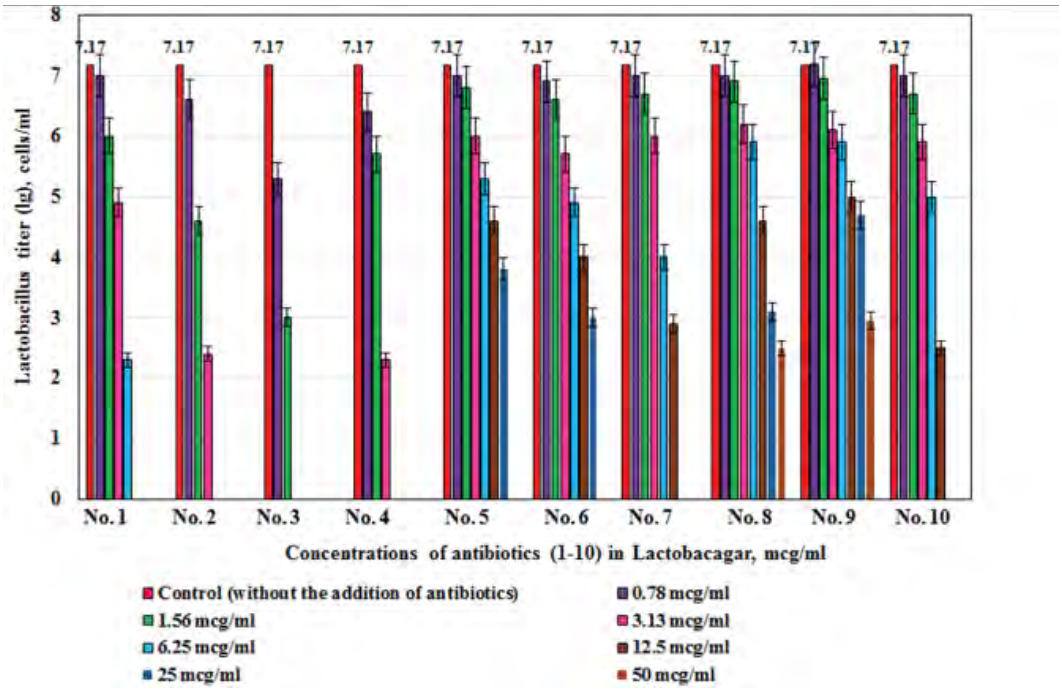


*Fig. 2.* **Lactobacilli numbers on Lactobacagar with antibiotics:**
1 — benzylpenicillin; 2 — azithromycin; 3 — lincomycin; 4 — gentamicin sulfate;
5 — ceftriaxone; 6 — norfloxacin; 7 — amoxil; 8 — streptomycin; 9 — tetracycline; 10 — erythromycin

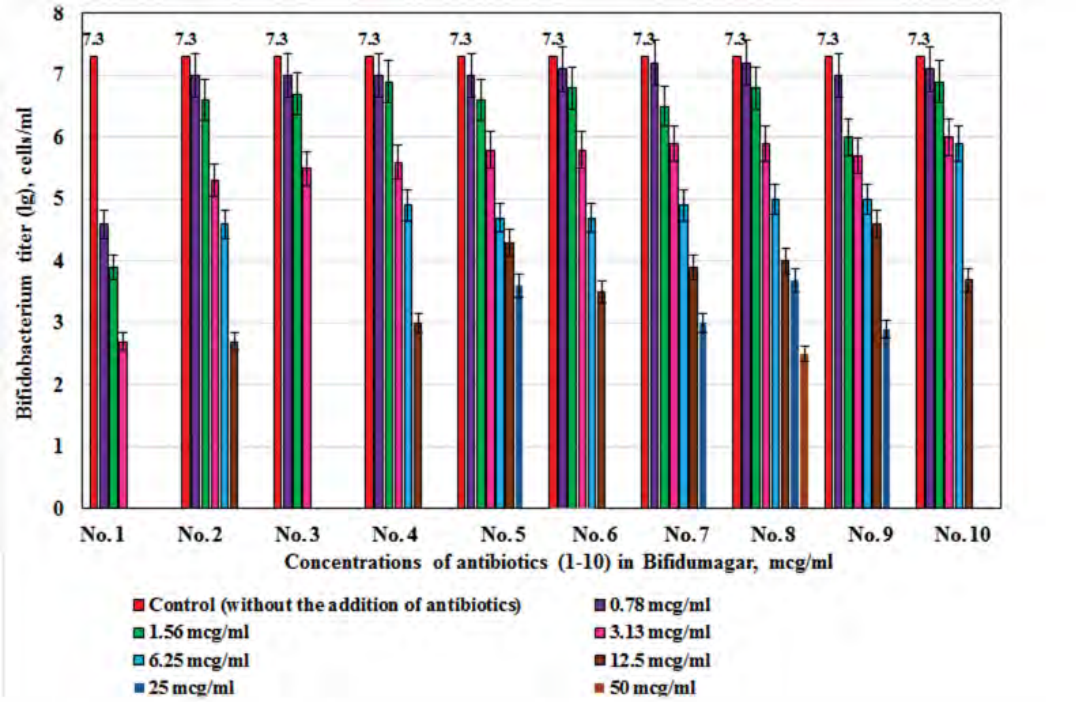


*Fig. 3.* **The number of bifidobacteria on the Bifidoagar with antibiotics:**
1 — benzylpenicillin; 2 — azithromycin; 3 — lincomycin; 4 — gentamicin sulfate;
5 — ceftriaxone; 6 — norfloxacin; 7 — amoxil; 8 — streptomycin; 9 — tetracycline; 10 — erythromycin

to the control (the change is within 8%). A significantly lower lactobacilli titer was seen at adding antibiotics No. 1, 5–10 (benzylpenicillin, ceftriaxone, norfloxacin, amoxil, streptomycin, tetracycline, erythromycin) at 12.5 μg/mL and above.

The lactobacilli were most susceptible to such antibiotics as lincomycin, gentamicin sulfate, and azithromycin (MIC of 3.13–6.25 μg/mL).

The bifidobacteria were most susceptible to benzylpenicillin (No. 1), with MIC of 6.25 μg/mL. At 0.78 μg/mL benzylpenicillin, the bifidobacteria titer fell by 37% compared to the control, while for all other antibiotics, the titer decreased by 8% or less. Experimentally, bifidobacteria are the most resistant to streptomycin and tetracycline (MIC 100 μg/mL), while for ceftriaxone, norfloxacin, and amoxil, the MIC was 50 μgmL. Bifidobacteria were the most sensitive to benzylpenicillin (No. 1) and lincomycin (No. 3). If we compare the antibiotic MIC for the lactobacilli and bifidobacteria, the latter appear more resistant for the following antibiotics: azithromycin (No. 2), lincomycin (No. 3), gentamicin sulfate (No. 4), amoxil (No. 7). Notably, both bacteria groups are sensitive to all tested antibiotics, although the MIC are different.

The antibiotics' effect (No. 1–10) on the titer of the lactobacilli and bifidobacteria compared to the control is shown in Fig. 4 and 5.

According to the presented results (Fig. 4), adding benzylpenicillin (No. 1), azithromycin (No. 2), ceftriaxone (No. 5), norfloxacin (No. 6), amoxil (No. 7), streptomycin (No. 8), tetracycline (No. 9) and erythromycin (No. 10) to Lactobacagar at 0.78 μg/mL decreases the bacteria titer by 2–8%. For gentamicin sulfate (No. 4), it was 11%, and for lincomycin (No. 3) 28%.

At concentration of 1.56 μg/mL, the decrease in titer below 8% was seen for antibiotics No. 5–10. The lowest lactobacilli titer was found for the antibiotic No. 3 — lincomycin (58.3% compared to control). For the media with antibiotics No. 5–10 at 313 μg/mL, the lactobacilli decreased by 17–20%.

The concentration of 6.25 μg/mL was found to be the MIC for antibiotics No. 2–4 (azithromycin, lincomycin, gentamicin sulfate). In other samples with various antibiotics the viable lactobacilli decreased by 18–68%.

The concentration of 12.5 μg/mL was the MIC for benzylpenicillin, while for antibiotics No. 5–10 the lactobacilli decreased by 36–65%.

At 25 μg/mL, the number of viable lactobacilli colonies decreased compared to control by 40–50% for the following antibiotics: No. 9 — tetracycline, No. 5 — ceftriaxone, No. 8 — streptomycin, No. 6 — norfloxacin.

Adding antibiotics to Bifidoagar (Fig. 5) at 0.78 μg/mL decreased bifidobacteria titer by 2–4% for samples No. 2–10, and for benzylpenicillin (No. 1), the bifidobacteria titer decreased by 37%. At twice the concentration (1.56 μg/mL), antibiotics No. 2–8 inhibited the viable cell titer by 5.5–11%, and benzylpenicillin did that by 47%.

At 3.13 μg/mL benzylpenicillin, the sample titer decreased by 63% compared to control. For other tested samples (antibiotics No. 2–10), the titer decreased by 19–27%.

The concentration of 6.25 μg/mL was the MIC for benzylpenicillin. For other antibiotics, the bifidobacteria titer decreased by 31–49.3%.

12.5 μg/mL was the MIC for sample No. 10 (erythromycin); for samples No. 2–9, the bacteria titer decreased by 37–63%.

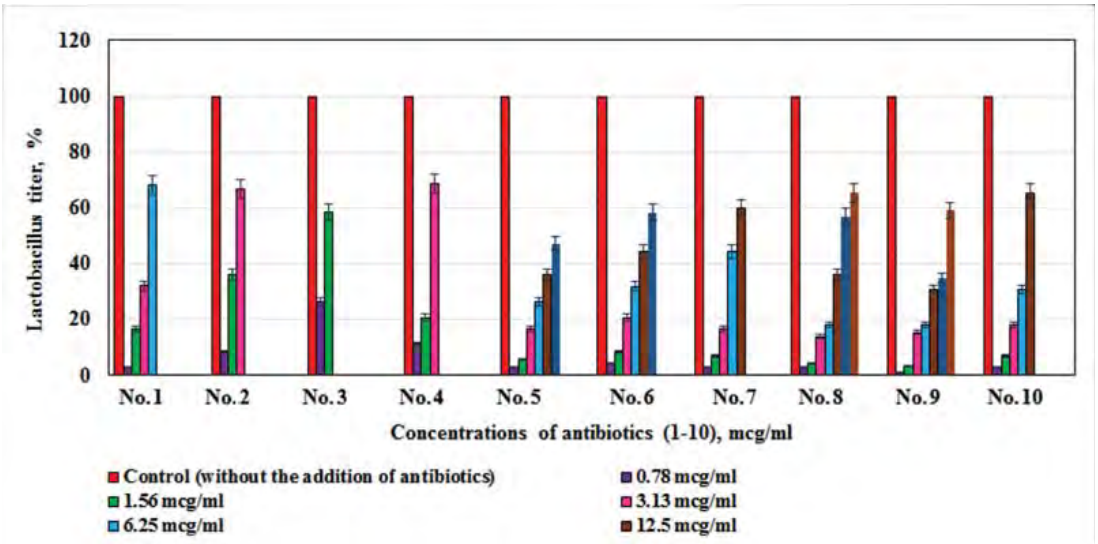


*Fig. 4.* **A comparison of lactobacilli titer (%) of the control sample (medium without antibiotics) o antibiotic-augmented medium samples:**
1 — benzylpenicillin; 2 — azithromycin; 3 — lincomycin; 4 — gentamicin sulfate; 5 — ceftriaxone; 6 — norfloxacin; 7 — amoxil; 8 — streptomycin; 9 — tetracycline; 10 — erythromycin

25 μg/mL was the MIC for samples No. 2–4 (azithromycin, lincomycin, and gentamicin sulfate); for samples No. 5–9 the titer decreased by 49.3–60.3%.

At 50 μg/mL streptomycin, the bifidobacteria titer decreased by 65.8% compared to control. This decrease was 65.3% for the lactobacilli. The antibiotic's MIC for both groups was 100 μg/mL.

Natural resistance to antibiotics is an essential factor determining the probiotic properties of lactobacilli and bifidobacteria, especially during antibiotic therapy. This is a most urgent problem, as the lactobacilli and bifidobacteria are included in the fermented dairy products and preparations they are based on. Thus, using the probiotic microbes for starters to obtain fermented dairy products for daily use or recommended during/after antibiotic therapy would prevent the concomitant intestine dysbacteriosis.

Comparing the MIC for antibiotics for the two groups of microbes showed that the bifidobacteria were more resistant than the lactobacilli against the following antibiotics: azithromycin, lincomycin, gentamicin sulfate, amoxil. Notably, both the lactobacilli and the bifidobacteria were susceptible to all studied antibiotics but at different levels. The results suggest that overall, the tested LAB cultures' antibiotics susceptibility is either intermediate (MIC above 8 μg/mL) for almost all tested substances or slight (MIC not below 25 μg/mL) for some of them (norfloxacin, ceftriaxone, erythromycin, tetracycline, streptomycin), and so using the functional fermented milk drinks on their basis to support the gut microbiome is a reasonable measure during antibiotic therapy.

Adding antibiotics at 0.78 and 1.56 μg/mL to the culture medium somewhat lowered the LAB titer (within 8%), while the near-MIC levels caused sharp declines in the lactobacilli and bifidobacteria.

The results are well-correlated with the literature [15–18]. According to the papers [20, 21], a consortium of bifidobacteria and lactobacilli was resistant to norfloxacin but somewhat sensitive to azithromycin. The high resistance to the tested antibiotic groups was explained by LAB consortia as probiotics as the more stable alternative to probiotic monocultures.

The previous results [25, 26] for the TM "VIVO" starter for api-products-enriched fermented milk drinks also proved the possibility of obtaining concentrated fermented milk starter for bread making, particularly, for yeast-free bread products based on the fermentation microflora of the starter enriched with pure LAB cultures.

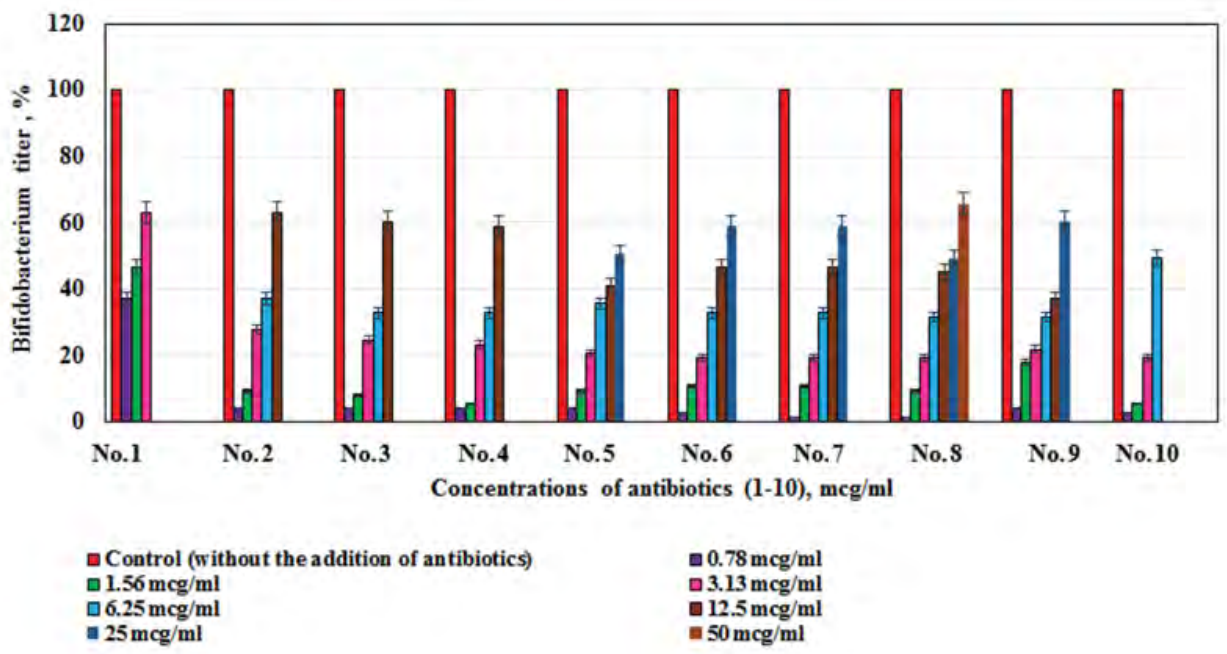


*Fig. 5.* **Bifidobacteria titer (%) of the control sample (without antibiotics) and of the antibiotic-augmented samples:**
1 — benzylpenicillin; 2 — azithromycin; 3 — lincomycin; 4 — gentamicin sulfate; 5 — ceftriaxone; 6 — norfloxacin; 7 — amoxil; 8 — streptomycin; 9 — tetracycline; 10 — erythromycin

## Conclusions

Today, popular functional products of everyday consumption include fermented milk drinks with live probiotics beneficial to humans and antagonistic to the opportunistic and pathogenic microbes, which restore the gut microbiome during antibiotic therapy and protect the normal microbial flora.

One of the main components of probiotic cultures of the LAB is the resistance to various antibiotic compounds.

We employed the double dilution test. The bacteria were cultured on selective media, Lactobacagar and Bifidoagar; the tested antibiotics were common — benzylpenicillin, azithromycin, lincomycin, gentamicin sulfate, ceftriaxone, norfloxacin, amoxil, streptomycin, tetracycline, and erythromycin.

According to the results, the range of the minimal inhibiting concentrations of these substances for the probiotic cultures of the "VIVO probioyogurt" starter is 3.13–100 μg/mL.

The experiment showed that the bifidobacteria are the most susceptible to benzylpenicillin (MIC 6.25 μg/mL). The most resistant the lactobacilli and bifidobacteria were to streptomycin, erythromycin, amoxil, and ceftriaxone (MIC 50–100 μg/mL). The lactobacilli were the most susceptible to such antibiotics as lincomycin, gentamicin sulfate, and benzylpenicillin (MIC 3.13–12.5 μg/mL).

According to the antibiotic resistance results for the LAB of the "VIVO probioyogurt" starter advertised for preparation of fermented milk products, it can be recommended for milk fermentation to obtain high-quality fermented dairy products to support gut microbiota, in particular during the antibiotic therapy, as these LAB exhibit sufficient resistance to the range of antibiotic substances.

The results of the bioactivity assay of the probiotic cultures of this starter regarding the titrated acidity parameters, fermentation time, and the organoleptic evaluation of the obtained drink are evidence that the preparation can be recommended as a base for fermented dairy products enriched by plant-derived functional components or for other new products' fermentation.

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## АНТИБІОТИКОРЕЗИСТЕНТНІСТЬ МОЛОЧНОКИСЛИХ БАКТЕРІЙ ЗАКВАСКИ «VIVO ПРОБІОЙОГУРТ»

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Молочнокислі бактерії відіграють ключову роль в мікроекології людини та біотехнологіях — формують органолептичні показники продуктів; підвищують харчову і біологічну цінність функціональних продуктів харчування. Природна стійкість до антибіотиків — один

## АНТИБИОТИКОРЕЗИСТЕНТНОСТЬ МОЛОЧНОКИСЛЫХ БАКТЕРИЙ ЗАКВАСКИ «VIVO ПРОБИОЙОГУРТ»

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Молочнокислые бактерии играют ключевую роль в микроэкологии и биотехнологиях — формируют органолептические показатели продуктов; повышают пищевую и биологическую ценность функциональных продуктов питания. Естественная устойчивость к анти-

з важливих чинників, що визначають пробіотичні властивості лакто- та біфідобактерій.

*Мета.* З'ясувати антибіотикорезистентність функціонально-активних пробіотичних культур закваски «VIVO пробіойогурт» задля встановлення доцільності вживання кисломолочного продукту, виготовленого на її основі, під час антибіотикотерапії для підтримки та відновлення нормальної мікрофлори кишковика.

*Методи.* Для дослідження було обрано чисті культури молочнокислих бактерій (МКБ): (*Lactobacillus delbrueckii* ssp., *L. acidophilus, L. casei, L. rhamnosus, L. paracasei, Streptococcus thermophiles, Bifidobacterium lactis, B. infantis*), які входять до складу закваски «VIVO пробіойогурт», якість якої підтверджено сертифікатами Міжнародної організації зі стандартизації ISO 9001:2008, а також ISO 22000:2005. Методика експерименту складалась із таких етапів: приготування живильних середовищ («Лактобакагар», «Біфідоагар», глюкозо-пептонне середовище), робочих розчинів антибіотиків, робочої суспензії МКБ, суспензії культур (лакто- та біфідобактерій), культивування МКБ на елективних живильних середовищах із додаванням антибіотиків та оцінювання результатів досліджень. Визначення антибіотикорезистентності МКБ проводили методом подвійних розведень.

*Результати.* Використання такої методики дало змогу встановити мінімальну інгібувальну концентрацію (МІК) антибіотиків різних груп стосовно МКБ. Результати досліджень оброблено за допомогою ліцензованої комп'ютерної програми Microsoft Excel.

*Висновки.* За результатами досліджень з визначення МІК антибіотиків — бензилпеніцилін, азитроміцин, лінкоміцин, гентаміцину сульфат, цефтріаксон, норфлоксацин, амоксил, стрептоміцин, тетрациклін, еритроміцин щодо МКБ було встановлено, що лакто- та біфідобактерії закваски «VIVO пробіойогурт» можна віднести до умовно резистентних пробіотичних культур; кисломолочний продукт, виготовлений на основі цієї закваски, доцільно використовувати під час анибіотикотерапії задля відновлення мікрофлори кишковика.

***Ключові слова:*** антибіотикорезистентність, молочнокислі бактерії, мінімальна інгібувальна концентрація, закваска, пробіотики.

биотикам — один из важных факторов, определяющих пробиотические свойства лакто- и бифидобактерий.

*Цель.* Выяснить антибиотикорезистентность функционально-активных пробиотических культур закваски «VIVO» пробиойогурт для установления целесообразности употребления кисломолочного продукта, изготовленного на ее основе, во время антибиотикотерапии для поддержания и восстановления нормальной микрофлоры кишечника.

*Методы.* Для исследования были выбраны чистые культуры молочнокислых бактерий (МКБ): (*Lactobacillus delbrueckii* ssp., *L. acidophilus*, *L. casei, L. rhamnosus, L. paracasei, Streptococcus thermophiles, Bifidobacterium lactis, B. infantis*), которые входят в состав закваски «VIVO пробиойогурт», качество которой подтверждено сертификатами Международной организации по стандартизации ISO 9001:2008, а также ISO 22000:2005. Методика эксперимента состояла из следующих этапов: приготовление питательных сред («Лактобакагар», «Бифидоагар», глюкозо-пептонная среда), рабочих растворов антибиотиков, рабочей суспензии МКБ, суспензии культур (лакто- и бифидобактерий), культивировании антибиотиков и оценке результатов исследований. Определение антибиотикорезистентности МКБ проводили методом двойных разбавлений.

*Результаты.* Использование такой методики позволило установить минимальную ингибирующую концентрацию (МИК) антибиотиков разных групп в отношении МКБ. Результаты исследований обработаны с помощью лицензированной компьютерной программы Microsoft Excel.

*Выводы.* По результатам исследований на предмет определения МИК антибиотиков – бензилпенициллин, азитромицин, линкомицин, гентамицина сульфат, цефтриаксон, норфлоксацин, амоксил, стрептомицин, тетрациклин, эритромицин относительно МКБ установлено, что лакто- и бифидобактерии закваски «VIVO пробиойогурт» можно отнести к условно резистентным пробиотичным культурам. Кисломолочный продукт, изготовленный на основе этой закваски, целесообразно использовать при анибиотикотерапии для восстановления микрофлоры кишечника.

***Ключевые слова:*** антибиотикорезистентность, молочнокислые бактерии, минимальная ингибирующая концентрация, закваска, пробиотики.