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ЕЖЕМЕСЯЧНЫЙ НАУЧНЫЙ ЖУРНАЛ

Медицинские новости Грузии

საქართველოს სამედიცინო სიახლენი

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თანამშრომლობითა და მისი პატრონაჟით

ЕЖЕМЕСЯЧНЫЙ НАУЧНЫЙ ЖУРНАЛ

ТБИЛИСИ - НЬЮ-ЙОРК

GMN: Georgian Medical News is peer-reviewed, published monthly journal committed to promoting the science and art of medicine and the betterment of public health, published by the GMN Editorial Board and The International Academy of Sciences, Education, Industry and Arts (U.S.A.) since 1994. **GMN** carries original scientific articles on medicine, biology and pharmacy, which are of experimental, theoretical and practical character; publishes original research, reviews, commentaries, editorials, essays, medical news, and correspondence in English and Russian.

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GMN: Медицинские новости Грузии - ежемесячный рецензируемый научный журнал, издаётся Редакционной коллегией и Международной академией наук, образования, искусств и естествознания (IASEIA) США с 1994 года на русском и английском языках в целях поддержки медицинской науки и улучшения здравоохранения. В журнале публикуются оригинальные научные статьи в области медицины, биологии и фармации, статьи обзорного характера, научные сообщения, новости медицины и здравоохранения.

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GMN: Georgian Medical News – საქართველოს სამედიცინო სიახლენი – არის ყოველთვიური სამეცნიერო სამედიცინო რეცენზირებადი ჟურნალი, გამოიცემა 1994 წლიდან, წარმოადგენს სარედაქციო კოლეგიისა და აშშ-ის მეცნიერების, განათლების, ინდუსტრიის, ხელოვნებისა და ბუნებისმეტყველების საერთაშორისო აკადემიის ერთობლივ გამოცემას. GMN-ში რუსულ და ინგლისურ ენებზე ქვეყნდება ექსპერიმენტული, თეორიული და პრაქტიკული ხასიათის ორიგინალური სამეცნიერო სტატიები მედიცინის, ბიოლოგიისა და ფარმაციის სფეროში, მიმოხილვითი ხასიათის სტატიები.

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При представлении в печать научных экспериментальных работ авторы должны указывать вид и количество экспериментальных животных, применявшиеся методы обезболивания и усыпления (в ходе острых опытов).

4. К статье должны быть приложены краткое (на полстраницы) резюме на английском, русском и грузинском языках (включающее следующие разделы: цель исследования, материал и методы, результаты и заключение) и список ключевых слов (key words).

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2. Size of the article, including index and resume in English, Russian and Georgian languages must be at least 10 pages and not exceed the limit of 20 pages of typed or computer-printed text.

3. Submitted material must include a coverage of a topical subject, research methods, results, and review.

Authors of the scientific-research works must indicate the number of experimental biological species drawn in, list the employed methods of anesthetization and soporific means used during acute tests.

4. Articles must have a short (half page) abstract in English, Russian and Georgian (including the following sections: aim of study, material and methods, results and conclusions) and a list of key words.

5. Tables must be presented in an original typed or computer-printed form, instead of a photocopied version. **Numbers, totals, percentile data on the tables must coincide with those in the texts of the articles.** Tables and graphs must be headed.

6. Photographs are required to be contrasted and must be submitted with doubles. Please number each photograph with a pencil on its back, indicate author's name, title of the article (short version), and mark out its top and bottom parts. Drawings must be accurate, drafts and diagrams drawn in Indian ink (or black ink). Photocopies of the X-ray photographs must be presented in a positive image in **tiff format**.

Accurately numbered subtitles for each illustration must be listed on a separate sheet of paper. In the subtitles for the microphotographs please indicate the ocular and objective lens magnification power, method of coloring or impregnation of the microscopic sections (preparations).

7. Please indicate last names, first and middle initials of the native authors, present names and initials of the foreign authors in the transcription of the original language, enclose in parenthesis corresponding number under which the author is listed in the reference materials.

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In GMN style for each work cited in the text, a bibliographic reference is given, and this is located at the end of the article under the title "References". All references cited in the text must be listed. The list of references should be arranged alphabetically and then numbered. References are numbered in the text [numbers in square brackets] and in the reference list and numbers are repeated throughout the text as needed. The bibliographic description is given in the language of publication (citations in Georgian script are followed by Cyrillic and Latin).

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ავტორთა საყურადღებოდ!

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1. სტატია უნდა წარმოადგინოთ 2 ცალად, რუსულ ან ინგლისურ ენებზე,დაბეჭდილი სტანდარტული ფურცლის 1 გვერდზე, 3 სმ სიგანის მარცხენა ველისა და სტრიქონებს შორის 1,5 ინტერვალის დაცვით. გამოყენებული კომპიუტერული შრიფტი რუსულ და ინგლისურენოვან ტექსტებში - **Times New Roman (Кириллица)**, ხოლო ქართულენოვან ტექსტში საჭიროა გამოვიყენოთ **AcadNusx.** შრიფტის ზომა – 12. სტატიას თან უნდა ახლდეს CD სტატიით.

2. სტატიის მოცულობა არ უნდა შეადგენდეს 10 გვერდზე ნაკლებს და 20 გვერდზე მეტს ლიტერატურის სიის და რეზიუმეების (ინგლისურ, რუსულ და ქართულ ენებზე) ჩათვლით.

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6. ფოტოსურათები უნდა იყოს კონტრასტული; სურათები, ნახაზები, დიაგრამები - დასათაურებული, დანომრილი და სათანადო ადგილას ჩასმული. რენტგენოგრამების ფოტოასლები წარმოადგინეთ პოზიტიური გამოსახულებით **tiff** ფორმატში. მიკროფოტოსურათების წარწერებში საჭიროა მიუთითოთ ოკულარის ან ობიექტივის საშუალებით გადიდების ხარისხი, ანათალების შეღებვის ან იმპრეგნაციის მეთოდი და აღნიშნოთ სურათის ზედა და ქვედა ნაწილები.

7. სამამულო ავტორების გვარები სტატიაში აღინიშნება ინიციალების თანდართვით, უცხოურისა – უცხოური ტრანსკრიპციით.

8. სტატიას თან უნდა ახლდეს ავტორის მიერ გამოყენებული სამამულო და უცხოური შრომების ბიბლიოგრაფიული სია (ბოლო 5-8 წლის სიღრმით). ანბანური წყობით წარმოდგენილ ბიბლიოგრაფიულ სიაში მიუთითეთ ჯერ სამამულო, შემდეგ უცხოელი ავტორები (გვარი, ინიციალები, სტატიის სათაური, ჟურნალის დასახელება, გამოცემის ადგილი, წელი, ჟურნალის №, პირველი და ბოლო გვერდები). მონოგრაფიის შემთხვევაში მიუთითეთ გამოცემის წელი, ადგილი და გვერდების საერთო რაოდენობა. ტექსტში კვადრატულ ფჩხილებში უნდა მიუთითოთ ავტორის შესაბამისი N ლიტერატურის სიის მიხედვით. მიზანშეწონილია, რომ ციტირებული წყაროების უმეტესი ნაწილი იყოს 5-6 წლის სიღრმის.

9. სტატიას თან უნდა ახლდეს: ა) დაწესებულების ან სამეცნიერო ხელმძღვანელის წარდგინება, დამოწმებული ხელმოწერითა და ბეჭდით; ბ) დარგის სპეციალისტის დამოწმებული რეცენზია, რომელშიც მითითებული იქნება საკითხის აქტუალობა, მასალის საკმაობა, მეთოდის სანდოობა, შედეგების სამეცნიერო-პრაქტიკული მნიშვნელობა.

10. სტატიის ბოლოს საჭიროა ყველა ავტორის ხელმოწერა, რომელთა რაოდენობა არ უნდა აღემატებოდეს 5-ს.

11. რედაქცია იტოვებს უფლებას შეასწოროს სტატია. ტექსტზე მუშაობა და შეჯერება ხდება საავტორო ორიგინალის მიხედვით.

12. დაუშვებელია რედაქციაში ისეთი სტატიის წარდგენა, რომელიც დასაბეჭდად წარდგენილი იყო სხვა რედაქციაში ან გამოქვეყნებული იყო სხვა გამოცემებში.

აღნიშნული წესების დარღვევის შემთხვევაში სტატიები არ განიხილება.

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THE ROLE OF LACTOBACILLI IN THE HUMAN MICROBIOME AND METHODS OF THEIR CULTIVATION AND PRESERVATION

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Abstract. The extremely important role of the microbiome for human life and health has long been known. Many studies around the world are devoted to studying the mechanisms of action and functions of various bacteria that are permanent residents of our body. Connections between the bacteria of our microbiome and all organs and systems of the human body (intestine, brain, nervous and cardiovascular systems) have been identified. However, the effect of bacteria can be positive or negative, which affects the emergence and development of diseases or promotes healing.

Genus *Lactobacillus* is one of the most numerous populations of bacteria in the human body. Moreover, they have a significant positive effect on health. Scientists are actively researching methods of cultivating and using bacteria of this genus in the pharmaceutical and industrial fields. Most probiotics contain lactobacilli strains. Therefore, the study of methods of cultivation and storage of lactobacilli in order to find ways to improve their viability and functionality and, at the same time, the invention of options to protect cell culture from various harmful factors is extremely important.

In our review, we considered the importance of the microbiome for human health and the role of bacteria of the genus *Lactobacillus* as its component. Scientific works on studying the mechanisms of influence of lactobacilli on the functional capacity of human organs and systems have been studied. Much of the review is devoted to the study of lactobacilli cultivation methods, the diversity of culture media, and the importance of their components to improve the viability of lactobacilli culture because they are quite demanding and vulnerable. Attention is also paid to the development of methods of storage of grown cultures of bacterial cells and their improvement in order to obtain functional and suitable for further use in the pharmacological and industrial areas of bacterial strains.

Keywords. Lactobacilli, microbiome, probiotic, bacterial culture, cultivation medium.

Introduction.

The human microbiome, its importance, and role. The human gut microbiome is one of the most actively researched microbial communities. This is due to the incredible complexity of its composition and the range of its interactions with the human body. All hypotheses about the involvement of intestinal microbiota in the pathogenesis of various diseases, the number of which increases every year. The formation of the human intestinal tract microbiome is a multi-stage process. This process begins due to bacteria penetrating from the intestines, oral cavity, and maternal vaginal microbiome [1]. The child receives microorganisms when passing through the birth canal, as well as from the breast milk, which is non-sterile and contains significant concentrations of *Streptococcus, Staphylococcus, Propionibacterium, and Bifidobacterium* [2]. Shortly after birth, a typical child's type of intestinal microbiota is characterized by high concentrations of the *Bifidobacterium* genus representatives [3], which is largely determined by the content of oligosaccharides found in human milk. After two years, the relative abundance of *Bifidobacterium* gradually decreases and develops the final variant of the intestinal microbiota. In children born by cesarean section, during the first months of life, there are differences in the representation of individual certain groups of bacteria in the microbiota, which can be associated with the lack of contact with the maternal vaginal microbiota, taking antibiotics, and later start of the breastfeeding [3,4]. The intestinal microbiome of adults can include representatives of more than 600 different species of bacteria [5]. About 90% of the total microbiota are *Firmicutes* and *Bacteroidetes*, predominantly represented by hard-to-cultivate obligate anaerobes. In the European population the most often meeting types are phylum *Firmicutes, Faecalibacterium prausnitzii*, and bacteria genus *Blautia, Dorea, Roseburia, and Coprococcus*, the main representatives of intestinal *Bacteroidetes* include bacteria of the genera *Bacteroides, Parabacteroides, Prevotella, Odoribacter, Barnesiella* and *Alistipes* [6]. A certain percentage of the adult's gut microbiota are bacteria *Actinobacteria* and *Proteobacteria* types [7], a smaller part - *Fusobacteria, Verrucombicrobia*, and also methanogenic archaea of the *Euryarchaeota* type [6].

The concentrations of individual representatives in the intestinal microbiota are not independent - they are connected by a complex network of symbiotic and antagonistic static interactions. As a result, the space of all possible compositions of intestinal microbiocenosis is not filled evenly - you can distinguish stable combinations of ratios of microorganisms (enterotype), which are more common than transitional forms between these combinations. Researchers identify three main enterotypes, characterized by high concentrations of *Bacteroides, Prevotella,* and *Ruminococcaceae* correspondingly [8]. However, this division does not describe all the variety of qualitative and quantitative characteristics of intestinal microbiocenosis. It was shown that such factors as consumption food intake, smoking, age, body mass index, the concentration of hemoglobin and erythrocytes in the blood, and taking antibiotics influence the composition of the microbiota [5]. Inter-population differences, presumably related with the nature of nutrition are also expressed [7]. Thus, despite the active use of the term "dysbiosis" in the literature as a link in the pathogenesis of various diseases, the concept of the "normal" composition of the human gut microbiota has not yet been clearly defined.

The human gut microbiome produces many various substances that can enter the bloodstream and act on distant organs and systems. The microbiome is even called the "virtual endocrine organ" [9]. For example, intestinal bacteria biota can secrete

into the blood such signaling substances as serotonin, gamma-aminobutyric acid, histamine, acetylcholine, dopamine, and norepinephrine [10]. An important role in regulating the immune system activity plays receptor ligands synthesized by microorganisms of innate and adaptive immunity: flagellin, formylmethionine-containing peptides, lipopolysaccharide, as well as capsular polysaccharides such as polysaccharides *A Bacteroides fragilis* [11].

Lactobacilli may be called one of the most important bacteria for the human microbiome. The habitat of lactobacilli are various parts of the gastrointestinal tract - from the oral cavity to the large intestine. In the process of normal metabolism, they are able to form lactic acid, hydrogen peroxide, produce lysozyme and other substances with bactericidal activity (reuterin, plantaricin, lactocidin, lactolin). In the stomach and small intestine, lactobacilli are the main microbiological link in the formation of colonization resistance. For this phenomenon, there is the term "quorum sensing", which denotes the coordinated collective behavior of microbial populations with the aim of optimal adaptation and interaction/competition with other microbes in a specific ecological niche [12].

A significant amount of data has been accumulated confirming the critical role of lactobacilli in the pathogenesis of various metabolic, immunological, and neurological diseases. In this regard, the possibility of influencing human health with the help of personalized nutritional and therapeutic strategies aimed at modifying the intestinal microbiota, which includes the use of probiotics, is attracting increasing attention. The expediency of using live symbionts/commensals to modulate human immune responses is undeniable. The appeal of many developers of probiotic products and preparations of lactobacilli is quite justified due to the steadily expanding evidence base regarding their safety and immunocorrective effects on human health. The established mechanisms of molecular action, detailed structural and genetic characterization, data from randomized trials and meta-analyses, as well as vast experience in the effective practical use of lactobacilli make them the means of choice for the prevention and reduction of the severity of many human diseases mediated or accompanied by immune imbalance. Unfortunately, the fact of a high level of vulnerability of lactobacilli to various damaging factors is known - the effect of antibacterial preparations, oxidative stress, changes in acidity in the stomach and intestines, etc. Taking into account the above facts, it seems quite logical that the development of methods for increasing the stress resistance of these bacteria is one of the priority areas in science. This review will discuss methods to reduce the level of damage to lactobacilli during their cultivation and storage of bacterial cultures for further use in the industrial and medical fields.

What are Lactobacilli, their types, and importance to human health.

Lactobacillus is a genus of lactic acid bacteria with more than 260 different species [13]. They are non-pathogenic Gram-positive obligate or facultative anaerobes with high enzymatic activity. The genus *Lactobacillus* belongs to the family *Lactobacillaceae*, order *Lactobacillales*, class *Bacilli*, phylum *Firmicutes*, *Terrabacteria* group, kingdom Bacteria. According to modern taxonomy, the genus *Lactobacilli* includes: the *Lactobacillus casei* group, which includes the following species: *L. casei, L. paracasei, L. zeae*; and a large amount of other species: *L. acetotolerans, L. acidifarinae, L. acidipiscis, L. acidophilus, L. agilis, L. algidus, L. alimentarius, L. alvei, L. alvi, L. amylolyticus, L. amylophilus, L, amylotrophicus, L. amylovorus, L. animalis, L. animata, L. antri, L. apinorum, L. apis, L. apodermi, L. aquaticus, L. aviarius, L. backii, L. bifermentans, L. bombi, L. bombicola, L. brantae, L. brevis, L. brevisimilis, L. buchneri, L. cacaonum, L. camelliae, L. capillatus, L. catenefornis, L. caviae, L. cerevisiae, L. ceti, L. coleohominis, L. colini, L. collinoides, L. composti, L. concavus, L. coryniformis, L. crispatus, L. crustorum, L. curieae, L. curvatus, L. delbrueckii, L. dextrinicus, L. diolivorans, L. equi, L. equicursoris, L. equigenerosi, L. fabifermentans, L. faecis, L. faeni, L. farciminis, L. farraginis, L. fermentum, L. floricola, L. florum, L. formosensis, L. fornicalis, L. fructivorans, L. frumenti, L. fuchuensis, L. furfuricola, L. futsaii, L. gallinarum, L. gasseri, L. gastricus, L. ghanensis, L. gigeriorum, L. ginsenosidimutans, L. gorillae, L. graminis, L. guizhouensis, L. halophilus, L. hammesii, L. hamsteri, L. harbinensis, L. hayakitensis, L. heilongjiangensis, L. helsingborgensis, L. helveticus, L. herbarum, L. heterohiochii, L. hilgardii, L. hokkaidonensis, L. hominis, L. homohiochii, L. hordei, L. iatae, L. iners, L. ingluviei, L. insectis, L. insicii, L. intermedius, L. intestinalis, L. iwatensis, L. ixorae, L. japonicus, L. jensenii, L. johnsonii, L. kalixensis, L. kefiranofaciens, L. kefiri, L. kimbladii, L. kimchicus, L. kimchiensis, L. kisonensis, L. kitasatonis, L. koreensis, L. kullabergensis, L. kunkeei, L. larvae, L. leichmannii, L. letivazi, L. lindneri, L. malefermentans, L. mali, L. manihotivorans, L. mellifer, L. mellis, L. melliventris, L. micheneri, L. mindensis, L. mixtipabuli, L. mobilis, L. modestisalitolerans, L. mucosae, L. mudanjiangensis, L. murinus, L. nagelii, L. namurensis, L. nantensis, L. nasuensis, L. nenjiangensis, L. nodensis, L. odoratitofui, L. oeni, L. oligofermentans, L. oris, L. oryzae, L. otakiensis, L. ozensis, L. panis, L. pantheris, L. parabrevis, L. parabuchneri, L. paracollinoides, L. parafarraginis, L. parakefiri, L. paralimentarius, L. paraplantarum, L. pasteurii, L. paucivorans, L. pentosiphilus, L. pentosus, L. perolens, L. plajomi, L. plantarum, L. pobuzihii, L. pontis, L. porcinae, L. psittaci, L. rapi, L. rennanquilfy, L. rennini, L. reuteri, L. rhamnosus, L. rodentium, L. rogosae, L. rossiae, L. ruminis, L. saerimneri, L. sakei, L. salivarius, L. sanfranciscensis, L. saniviri, L. satsumensis, L. secaliphilus, L. selangorensis, L. senioris, L. senmaizukei, L. sharpeae, L. shenzhenensis, L. sicerae, L. silagei, L. silagincola, L. siliginis, L. similis, L. songhuajiangensis, L. spicheri, L. sucicola, L. suebicus, L. sunkii, L. taiwanensis, L. thailandensis, L. timonensis, L. tucceti, L. ultunensis, L. uvarum, L. vaccinostercus, L. vaginalis, L. vermiforme, L. versmoldensis, L. vespulae, L. vini, L. wasatchensis, L. xiangfangensis, L. yonginensis, L. zymae.*

In the composition of some species of *Lactobacilli*, subspecies are distinguished:

- *L. aviarius: Lactobacillus aviarius subsp. araffinosus, Lactobacillus aviarius subsp. Aviarius*;
- *L. brevis: Lactobacillus brevis subsp. coagulans, Lactobacillus brevis subsp. Gravesensis*;

- *L. coryniformis: Lactobacillus coryniformis subsp. coryniformis, Lactobacillus coryniformis subsp. Torquens*;
- *L. delbrueckii: Lactobacillus delbrueckii subsp. bulgaricus, Lactobacillus delbrueckii subsp. delbrueckii, Lactobacillus delbrueckii subsp. indicus, Lactobacillus delbrueckii subsp. jakobsenii, Lactobacillus delbrueckii subsp. lactis, Lactobacillus delbrueckii subsp. sunkii;*
- *L. helveticus: Lactobacillus helveticus subsp. jugurti;*
- *L. kefiranofaciens: Lactobacillus kefiranofaciens subsp. kefiranofaciens, Lactobacillus kefiranofaciens subsp. Kefirgranum*;
- *L. plantarum: Lactobacillus plantarum subsp. argentoratensis, Lactobacillus plantarum subsp. plantarum*;
- *L. sakei: Lactobacillus sakei subsp. carnosus, Lactobacillus sakei subsp. sakei.*

Many types of lactobacilli are part of the normal microflora of the gastrointestinal tract (GIT). The largest number of lactobacilli is found in the large intestine (10^6–10^7 (colony-forming units) per 1 g of feces). They are mainly represented by *L. acidophilus, L. casei, L. bulgaricus, L. plantarum, L. salivarius, L. reuteri*, and *L. rhamnosus*. The amount of lactobacilli in the stool largely depends on the nature of the diet. The average number of lactobacilli in the feces is examined in the analysis for dysbacteriosis is from 10^6 to 10^7 CFU/g lactobacilli for children under one year old, from 10^7 to 10^8 CFU/g lactobacilli for patients from one to 60 years old, and from 10^6 to 10^7 CFU/g for patients over 60 years old.

The role of lactobacilli in human health.

Lactobacilli represent a smaller part of the intestinal microflora; however, they perform no less important metabolic functions than the main representative of the normal microflora of the large intestine - *bifidobacteria*. Lactobacilli inhibit the growth of putrefactive and opportunistic microorganisms (OPM) in the intestine due to the ability to form substances such as lactic acid, lysozyme, and bacteriocins (lactocins B, F, J, M, lactobrevin, plantaricin, etc.). These waste products of lactobacilli have a pronounced antibacterial effect, and also affect epithelial cell membranes, DNA synthesis, and protein synthesis in the intestinal mucosa. In the course of clinical and experimental studies, it was found that lactobacilli inhibit the reproduction of pathogenic microflora and UPM - *Klebsiella pneumoniae, Proteus vulgaris, Pseudomonas aeruginosa, Salmonella typhosa, S. schottmuelleri, Sarcina lutea, Shigella dysenteriae, Sh. paradysenteriae, Serratia marcescens, Staphylococcus aureus, Streptococcus faecalis, S. lactis, Vibrio comma*, etc. [14,15].

Lactobacilli have the ability to activate cellular immunity and suppress the production of class E immunoglobulins (Ig). The immunomodulatory effect of lactobacilli is associated with the presence of peptidoglycans and teichoic acids, known polyclonal inducers, and immunomodulators, in their cell wall. It was also shown that the introduction of lactobacilli into mice was accompanied by an increase in the number of plasma cells, an increase in the synthesis of antibodies to the influenza virus and rotavirus, as well as an increase in the synthesis of IgA and IgM by bronchial mucosal cells. The protective properties of secretory IgA (sIgA) are associated with their ability to prevent adhesion and invasion of pathogenic bacteria, as well as to penetrate intestinal enterocytes and affect the replication cycle of intracellular viruses. Unlike other immunoglobulins, sIgA act as a specific immunological barrier, binding antigens on the surface of the epithelium and preventing their penetration into the body, thereby reducing the likelihood of both inflammatory and allergic processes [16,17].

In the course of clinical studies, it was found that different types of lactobacilli have an immunomodulatory effect of different severity. For example, *L. casei* is a powerful stimulator of the production of interleukins (IL) 6 and 12, tumor necrosis factor-alpha (TNF-alpha), and the expression of maturation markers by various cells. At the same time, *L. reuteri* is a weak inducer of IL-12 and suppresses the secretion of cytokines and the expression of maturation markers caused by other species of lactobacilli. Oral bacteriotherapy of *L. rhamnosus* GG (ATCC 53103) in children with atopic dermatitis and intolerance to cow's milk led to an increase in the production of the anti-inflammatory cytokine IL-10 and a weakening of the clinical manifestations of the disease. At the same time, the high level of the pro-inflammatory cytokine, TNF-alpha, characteristic of such patients, also decreased [18]. It has been established that the inhibition of peripheral blood lymphocytes by these bacteria leads to a decrease in the release of pro-inflammatory cytokines (TNF-alpha, IL-2, etc.) and an increase in the level of regulatory cytokines - TGF-beta (transforming growth factor-beta) and IL-10 [19,20].

One of the most studied and tested probiotic strains is *Lactobacillus rhamnosus* ATCC 53103 (*Lactobacillus rhamnosus* GG or LGG). It has been established that LGG produces antimicrobial factors that inhibit the activity of microorganisms such as *Clostridium spp., Pseudomonas spp., Salmonella spp., Escherichia coli, Staphylococcus and Streptococcus spp*. Although LGG actively suppresses the growth of intestinal pathogens, this strain demonstrates pacifism concerning other symbionts/commensals, not competing with them and not displacing other lactobacilli, and also has a positive effect on the adhesion of bifidobacteria [21].

Lactobacilli actively participate in the processes of digestion, turning protein into easily digestible components. Preclinical and clinical studies have shown that *L. acidophilus* can break down cholesterol in serum lipids. They also help reduce cholesterolemia by blocking the enzyme hydroxymethyl-glutarate-CoA reductase, which limits the rate of cholesterol synthesis. Lactobacilli are actively involved in the metabolism of lactose [22,23], producing enzymes: B-galactosidases, glycolases, and milk dehydrogenases. This is of great clinical importance in children with congenital lactase deficiency, as well as developing after intestinal infections or courses of antibiotic therapy. The inclusion of biological preparations or therapeutic and prophylactic infant formulas enriched with lactobacilli into the complex therapy of viral diarrhea is considered pathogenetically justified since the main "trigger" mechanism of diarrhea and the infectious process in viral diarrhea (rotavirus infection, etc.) is disaccharidase deficiency.

Based on the foregoing, the relevance of manufacturing a variety of mono- and multi-probiotic lactobacilli-based

preparations for the treatment of a wide range of diseases, both in humans and animals, is absolutely clear. Therefore, the improvement of methods for growing cultures of lactobacilli to maximize their accumulation of biomass, and maintain their stress resistance, stability, and activity in preparations are being actively studied.

Results and discussion

The importance of probiotics. According to scientists, the term "probiotics" refers to microorganisms that benefit hosts when administered in adequate amounts [24,25,26]. The real probiotic should preferably be of human origin, safe, and free from vectors capable of transmitting resistance to antibiotics and pathogenicity or toxicity. In addition, the probiotic must have a high ability to survive in intestinal conditions (acidic pH, enzymes, bile salts, etc.). Moreover, the probiotic should antagonize pathogens and stimulate the immune system and, ultimately, should have a clear beneficial effect on the host. Finally, the preservation of the activity, viability, and growth efficiency of the probiotic during technological processing should be demonstrated [27,28].

Cultures of lactic acid bacteria are the basis of many probiotic drugs for the therapy of different diseases. They are widely used for the production of drugs, in ferments of mono- and complex composition for the preparation of dessert and dietary fermented milk products, and in ensiling - for canning and increasing the nutritional value of feed. Issues of isolation, selection, and improvement of new strains of lactic acid bacteria remain relevant today [29]. Despite a large number of publications on lactic acid bacteria and their positive properties for the body, the search for new strains remains promising.

Extremely important are also the antagonistic properties of lactic acid bacteria, which are described in the literature quite fully. The mechanisms of antagonistic action are different. In some cases, the antimicrobial effect of these microorganisms is due to the action of the main product of metabolism - lactic acid, which lowers the pH of the environment and plays the role of a bactericidal factor in enzymatic fermentation in food and feed [30]. In other cases, the antagonistic effect is caused by neutral products, sometimes pigments, which are released by the cell into the environment.

Some substances that produce lactic acid bacteria are characterized by high antagonistic activity even at low concentrations in the environment. This category includes antibiotics (lactocil, lactobrevin, nisin, lactobacillin, etc.) [29,31]. According to the literature, lactic acid bacteria are able as antagonists to produce hydrogen peroxide in the presence of oxygen, which inhibits *Staphylococcus aureus, Pseudomonas* and others.

The antimicrobial effect of hydrogen peroxide is due to the denaturation of some enzymes, increased membrane permeability, and destruction of DNA by free radicals [31]. In addition, hydrogen peroxide activates the lactoperoxidase system, which forms oxidation products - inhibitors for a wide range of gram-positive and gram-negative bacteria [32].

Another mechanism of antibacterial activity of lactic acid bacteria has been identified - the ability to produce lysozyme, which destroys the wall of bacterial cells, creating a non-specific antibacterial barrier [33].

The antagonistic action of some lactic acid bacteria is characterized by bactericidal properties of substances of protein nature (bacteriocins), which have high specificity in relation to closely related or different strains of the same species [34].

The ability of lactic acid bacteria to inhibit the growth of a wide range of microorganisms (especially gram-negative) plays an important role in the formation of the microbiocenosis of the gastrointestinal tract of humans and animals. According to investigations, the highest antibiotic activity against gram-negative and gram-positive bacteria is characterized by cultures of *Lactobacillus acidophilus* and *L. bulgaricus*, which inhibited the growth of opportunistic pathogenic microflora even in high dilutions [35].

The lactobacilli of the intestinal microflora play an important role in maintaining the immunological reactivity and tolerance of the organism. It activates the induction of immunoglobulins, γ-interferon, and some other cytokines and increases the phagocytic activity of macrophages, neutrophils, monocytes, and other leukocytes [36]. Antagonistic activity and interaction with the immune system determine the most important property of probiotic microorganisms - providing the so-called colonization resistance which is understood as the protection of the intestinal wall from penetration into the internal environment of the body of bacteria, toxins, and toxic products of various origins.

The protective function of probiotics is also expressed in their antimutagenic activity. This property of probiotics explains their antitumor and antiallergic effects. Probiotics are involved in the hydrolysis of carcinogenic metabolic products of proteins, lipids, carbohydrates, inactivation of histamine, xenobiotics and pro-carcinogenic substances, deconjugation of bile, and hydroxylation of fatty acids [37]. Probiotics enhance the digestive and motor functions of the gastrointestinal tract. By participating in the enzymatic processes of the breakdown of food components, for example, fermentation, the normal microflora performs a digestive function in the body, which is especially important for lactase deficiency in children. As a result of the activity of probiotic microorganisms in the intestines, favorable conditions are created for the absorption of iron, calcium, and vitamin D. However, the participation of intestinal microflora in the metabolism of vitamins is not limited to enhancing their absorption. Probiotics are involved in the synthesis of vitamins B1, B2, B3, PP, K, and E, as well as folic and ascorbic acids. Normal microflora fully meets the human needs for vitamins B and H (biotin); Vitamin B12 is synthesized in nature only by microorganisms. In addition to vitamins, microorganisms of the human digestive tract synthesize biologically active compounds such as histidine, histamine, cholesterol, serotonin, γ-aminobutyric acid, aminoglycosides, antibiotics, and some toxins, participate in the production of aromatic compounds (indole and skatole) and amino acids (arginine, tryptophan, tyrosine). By participating in the production of carbon dioxide, hydrogen, hydrogen sulfide, ammonia, and methane, the microflora regulates the gas composition in the intestine [38].

This way experimental and clinical evidence supports the effectiveness of lactobacilli for the treatment of several pathological conditions. Long-term consumption of lactobacilli induces qualitative and quantitative modifications in the human gastrointestinal microbial ecosystem. That's why lactobacilli

are widely investigated as one of the most effective components of the probiotics, but the pharmacological profile of lactobacilli needs to be further characterized in order to avoid translocation-related risks. [14].

Methods of Lactobacillus cultivation.

The study of the biological properties of lactobacilli, as well as other microorganisms, requires the ability to long-term preservation and cultivation culture. This is necessary both to maintain the collections of lactic acid bacteria in a highly active state and for the manufacture and storage of probiotics [39].

In the biotechnological process of creating therapeutic and prophylactic probiotics, much attention is paid to achieving the maximum level of biomass yield of viable bacterial cells and, accordingly, the biologically active substances synthesized by them. It is important when selecting strains to take into account their manufacturability in production conditions and stability in cultivation, taking into account the preservation of probiotic properties. These indicators determine the productivity, competitiveness, and profitability of technological processes [40,41,42].

Lactobacilli have certain cultural properties [43]. On dense nutrient media, lactobacilli form colonies spherical, often lenticular, smooth, opaque, sometimes shiny, convex, with even clear contours. Usually, the colonies are small, but in some species, their size can exceed 4 mm in diameter. Colonies are generally unpigmented, white or slightly cream-colored, sometimes yellowish or reddish. Some species form rough colonies. On media with proteins or lipids, zones of clearing around the colonies usually do not form. However, most lactobacilli have weak proteolytic activity (due to secreted and associated with the cell wall proteases and peptidases) and weak lipolytic activity (due to intracellular lipases). Amylolytic activity on agar media with starch is found only in some species: *L. amylolyticus, L. amylophilus, L. amylovorus*, and *L. fermentum*. Certain types of lactobacilli (*L. plantarum, L. delbrueckii, L. casei*) are able to form extracellular nucleases when grown on agar containing DNA or RNA [30].

With deep sowing on a solid nutrient medium, dense colonies are formed in the form of regular lenses (lenticular), triangular and irregular in shape, or tender, resembling a snowflake or a ball of cotton wool [43]. If chalk was added to the medium, then a zone of chalk dissolution is formed around the colonies due to the accumulation of lactic acid. Good growth is observed in a semi-liquid nutrient medium containing 0.15-0.75% agar. Small concentrations of agar provide a low redox potential in the medium and create favorable microaerophilic conditions. According to the nature of growth in a semi-liquid medium, five variants are distinguished:

- growth in balls,
- in the form of longitudinal stripes,
- near-bottom,
- surface,
- uniform turbidity of the medium.

When growing on liquid nutrient media, lactobacilli most often cause uniform turbidity, soon after the cessation of growth, settling in the form of an even, homogeneous, less often flaky precipitate, never forming films on the surface of the medium [43].

Lactobacilli are chemoorganoheterotrophs. Very demanding on food sources, need rich complex environments. From carbohydrates, they mainly ferment hexoses (glucose, fructose, mannose, galactose) and disaccharides (lactose, maltose, sucrose), and only heterofermentative species, for example, some strains of *L.plantarum*, ferment pentoses (ribose, xylose, arabinose). Lactose is a disaccharide, therefore, before entering the path of catabolism, it must be cleaved by the enzyme galactosidase to glucose and galactose. Galactose is then phosphorylated to form glucose-6-phosphate [44]. In addition to carbohydrates, lactobacilli need various growth factors for their development: amino acids, vitamins, and nucleotides. Riboflavin, pantothenic and nicotinic acids are the most necessary for the life of most species, thiamine is needed mainly by heterofermentative lactobacilli, biotin, and vitamin B12 - only by some strains. Requirements for folic acid, riboflavin, pyridoxal phosphate, and para-aminobenzoic acid vary between species.

Sometimes lactobacilli are called "metabolic invalids" because they have lost the ability to synthesize several metabolites, probably as a result of their specialization (growth in milk and other media rich in nutrients and growth substances). So, they are not able to form porphyrins, in particular, heme. However, some lactobacilli are able to use environmental porphyrins (for example, when growing on media with blood) and, due to this, demonstrate catalase and nitrite reductase activity and even form cytochromes [39].

Almost all lactobacilli are mesophilic. The temperature optimum for development lies in the range of 30-40°C. The upper-temperature limit (maximum) for them is 40°C, however, there are thermophilic species that grow well and have an active metabolism at a temperature of about 45°C [44]. Lactobacilli are facultative anaerobes, sometimes microaerophiles. Although most strains are aerotolerant, anaerobic and microaerophilic conditions are optimal for growth. Lactobacilli usually grow weakly in air, better - with reduced oxygen content. Increased carbon dioxide concentration (≈ 5%) can stimulate growth; under strictly aerobic conditions, as a rule, growth slows down. Some species are strict anaerobes.

To date, a large number of methods for growing lactobacilli have been developed. Below we have listed the most commonly used culture media with a brief description of their components (Table 1).

Sources for the isolation of lactobacilli are silage and grasses, food (especially sour milk) products, and feces. To determine the presence and account for the number of lactobacilli, a sample of the test material is diluted in physiological saline and sown in liquid storage media or immediately on solid media. Since lactobacilli do not always occupy a predominant position in natural substrates, elective nutrient media are used to isolate them, which promote their growth and inhibit the growth of the accompanying microflora. Thus, the species identification of lactobacilli is preceded first by obtaining an enrichment culture of lactic acid bacteria, and then by isolating a pure culture of microorganisms on dense nutrient media.

In the study of Zavgorodny A.I. [45], it is shown that the growth of microorganisms depends on the pH and the temperature of the

Table 1. The most used culture media for cultivating lactobacilli.

The name of the cultivation medium	Information about medium	The composition of the cultivation medium	Notes
MRS culture medium (DeMan-Rogosa-Sharpe)	Designed for isolation and cultivation of lactobacilli. Designed by De Man, Rogosa, and Sharpe (1960).	Per 1000 ml of distilled water (g): - yeast extract - 4.0 - meat extract - 10.0 - casein hydrolyzate - 10.0 - glucose - 20.0 - ammonium citrate (disubstituted) - 2.0 - sodium acetate - 5.0 - twin 80 - 1.0 - K2HPO4 - 2.0 - MgSO4•7 H2O - 0.2 - MnSO4•4H2O- 0.05 Sometimes also: - sorbic acid - 0.4 - cysteine HCl - 0.4	To obtain a solid medium, it is recommended to add 20 g of agar. Dissolve the components in water, adjust pH to 6.2–6.5, and autoclave at 0.5 atm. within 20-30 min.
Cattail medium	Selective medium for the isolation and counting of lactobacilli in the control of meat, milk, and other products. Developed by Rogosa, Mitchell, and Wisemann (1951).	For 1000 ml of tap water (g): - peptone - 10.0 - yeast extract - 10.0 - meat extract - 10.0 -casein hydrolyzate - 5.0 - glucose - 20.0 - sodium acetic acid - 15.0 - ammonium citrate - 2.0 - sodium citrate - 2.8 - KH2PO4 - 6.0 - MgSO4 - 0.575 - MnSO4 - 0.12 - FeSO4 - 0.034 - twin 80 - 1.0	To obtain a dense medium, add 15-20 g of agar. Dissolve the components in water, adjust pH 5.5 with glacial acetic acid, and autoclave at 3/4 atm. within 20 min.
Nutrient medium for the cultivation of lactobacilli with ethanol		Prepare and sterilize separately, then mix (ml): - skimmed milk - 45 - 5% yeast extract – 5 - 2,5% agar - 50	The components of the medium are sterilized by autoclaving at 0.5-1 atm. within 20-30 min. Add 4-8% ethyl alcohol to the obtained 100 ml of the medium. Store no more than two days, after which the alcohol evaporates.
Dairy culture medium for the cultivation of lactobacilli	Skimmed milk powder contains fats - 1%, proteins - 36%, lactose - 52%, minerals - 6%.	Per 1000 ml of distilled water (g): - skimmed milk powder - 100 - sodium citrate - 1.5 - glucose - 10 - agar - 20	Milk casein forms a buffer system, binding a large number of acid metabolites into caseinates. To prevent coagulation of casein, a solution of sodium citrate is introduced into the milk base, which has strong stabilizing and buffering properties. The growth properties of the nutrient medium are enhanced by the content of lactose and glucose in it.
Cabbage agar	To obtain cabbage broth, pour 200 g of chopped fresh cabbage into 1 liter of distilled water and boil for 30 minutes. Filter the broth through a cotton-gauze filter, then bring the volume of the filtrate with tap water to 1 liter.	For 1000 ml of cabbage broth (g): - peptone - 10.0 - glucose - 20.0 - CaCO3 - 30.0 - agar - 20.0	Sterilize the medium by autoclaving at 0.5 atm. within 20-30 min. When growing on this medium, lactic acid bacteria form clearing zones around the colonies due to the conversion of insoluble calcium carbonate to soluble calcium lactate.
Medium for isolation of lactic acid bacteria (according to Netrusov A.I.)	To obtain a vegetable decoction, pour 100 g of chopped fresh cabbage or carrots into 1 liter of distilled water and boil for 30 minutes. Filter the broth through a cotton-gauze filter, then bring the volume of the filtrate with tap water to 1 liter.	For 1000 ml of herbal decoction (g): - yeast autolysate - 10.0 - peptone - 10.0 - glucose - 20.0	Sterilize the medium by autoclaving at 0.5 atm. within 20-30 min. Add 8-16% ethyl alcohol to the liquid medium inoculated with the substrate after 18-24 hours of cultivation. To prepare a solid medium of the same composition, add 2% agar and 4% crushed chalk and sterilize by autoclaving at 0.5 atm. within 20-30 min. Do not add alcohol. When growing on this medium, lactic acid bacteria form clearing zones around the colonies due to the conversion of insoluble calcium carbonate to soluble calcium lactate.
Reverse (skimmed milk)	Milk contains all the nutrients necessary for the development of heterotrophic microorganisms: lactose - about 4.5%, proteins - 5%, mineral compounds - 1%, and vitamins. Milk without additives contains approximately 0.01% free amino acids, which is less than 20% of the amino acids found in optimal bacterial growth media. To achieve normal growth on a medium with milk casein as the main source of nitrogen, organisms must have a certain ability to proteolyze.		Because the fat in milk can adversely affect the growth of certain microorganisms, the milk is defatted. To do this, whole milk is centrifuged for 15 min at 700-1500 g, then boiled and settled in the refrigerator for 2 days, after which it is sterilized by autoclaving at 0.5 atm. 30 minutes. Before sterilization, the acidity of the milk should not exceed 22°T, otherwise, the milk will curdle. When sterilized in an autoclave, browning of milk is sometimes observed due to caramelization of lactose and peptonization of casein. With prolonged sterilization, casein precipitates to the bottom, which can be partially peptonized. Overheated brown milk should not be used as a medium. For the growth of lactic acid microorganisms, the reverse can be diluted with water in a ratio of 2:1.
Glucose-peptone medium (GPS)		For 1000 ml of tap water (g): - peptone - 5.0 - glucose - 10.0 - NaCl - 5.0	To obtain a dense medium - glucose-peptone agar, add 20 g of agar. Sterilize the medium by autoclaving at 0.5 atm. 30 minutes.
Luria-Bertani medium (LB)		Per 1000 ml of distilled water (g): - trypton - 10.0 - yeast extract - 5.0 - NaCl - 5.0	To obtain a dense medium - LA - add 20 g of agar. Dissolve the components in water, bring the pH to 8.5, and autoclave at 1 atm. within 20-30 min.

nutrient medium. The higher the pH of the medium, the fewer lactic acid cells develop in the nutrient medium. The results of studies of the test medium pH were from 5.5 to 8.5, the most intensive growth was observed at pH 6.5–7.5. It should be noted that the maximum accumulation of cultures was observed at pH - 7.0 (control 24.3 ± 1.30; experiment 25.0 ± 1.14 cells). The lowest concentration of microorganisms in the nutrient medium was observed at pH - 8.5. Having obtained positive results in determining the optimal pH conditions, the optimal temperature regimes were determined by growing lactic acid bacteria in the proposed environment. In experiments, the incubation of sown culture was carried out at temperatures 30°C - minimum and 50°C – maximum. During statistical processing, it was found that the best growth and development of lactobacilli takes place at temperatures of 37–40°C. Thus, studies have shown that at different temperatures and pH, the best of them is what has pH - 7, and the incubation of seed takes place at 37°C. Scientists established that at different temperatures and pH of the medium the best of them for lactobacilli is one with a pH of 7.0, and the incubation of the seed takes place at a temperature of 37°C.

As far as *Lactobacillus spp*. are characterized by high nutritional requirements, due to their weak ability to synthesize amino acids and vitamins from B-group, their cultivation needs to be conducted in a rich medium, which includes fermentable carbohydrates, nucleic and amino acids, B-complex vitamins, as well as different minerals [39]. Moreover, Lactobacillus spp. bacteria primarily use peptides to fulfill their demand for nitrogen. For LAB cultivation purposes, on a laboratory scale, meat or yeast extract is commonly used as a nitrogen source; however, these components contribute to the high cost of ordinarily used de Man, Rogosa, and Sharpe medium (MRS). Low-cost medium alternatives are looked for mainly among food and agriculture by-products and wastes [46]. Cereals such as wheat, barley, maize, or rye, which are commonly used for animal feed, are proven to be a good source of nutrients for many LAB species [39].

To date, the optimal environment for the cultivation of lactobacilli is MRS [47]. But at the stage of the main technological process of obtaining probiotics - industrial cultivation, use of the complex and expensive nutrient medium is economically impractical, especially in the case of food impurities and functional products. Therefore, there is an urgent need to reduce the cost and improve existing technologies for growing probiotic microorganisms through the introduction of new physiologically effective and cheap nutrient media that can simultaneously increase the viability of the culture.

Methods of increasing the stress resistance of lactobacilli during cultivation and storage.

For industrial and medical use, probiotic strains are selected for a number of biological properties and the manifestation of their functional activity, in particular *in vitro* experiments. Their milk coagulation activity, the limit of acid formation, the patterns of growth of pure cultures, the number of microorganisms by the number of colony-forming units (CFU) on agar medium, the ratio of cultures to bacteriophages, resistance to bile, antagonistic activity of lactic acid bacteria against test cultures of pathogenic and opportunistic microorganisms, hydrophobicity of cells, electrokinetic studies of cell surface structures, the activity of lactobacilli, resistance to oxidative and other types of stress [48,49].

Lactobacilli do not contain porphyrins, in particular heme, therefore they are deprived of hemoproteins such as cytochromes and catalase [50]. Despite this, they are characterized by quite diverse mechanisms of protection against the toxic effects of reactive oxygen species (ROS):

- the enzyme superoxide dismutase, which catalyzes the dismutation reaction of superoxide radicals with the formation of hydrogen peroxide and oxygen).
- high intracellular concentrations of Mn2+ ions (up to 30 mM), which can effectively eliminate superoxide ions.
- pseudocatalase (in *L. mali*).
- the mechanism of accelerating the glycolytic decomposition of glucose under aerobic conditions (under aerobic conditions, hydrogen from NAD-H2 is directly transferred to O2, freeing part of pyruvate from its acceptor function in lactic acid fermentation. Pyruvate is oxidized to acetyl-CoA, the subsequent metabolization of which to acetate leads to the synthesis of the ATP molecule).

The physiological feature of lactobacilli is their acid resistance [51]. For the growth of lactobacilli, slightly acidic media with an initial pH of 5.4-6.4 are most favorable, and the growth of the culture slows down when pH 3.6-4.0 is reached, depending on the species and strain. *L. suebicus, L. casei*, and *L. plantarum* maintain growth even at pH 2.8. In alkaline and neutral environments, the growth of lactobacilli usually slows down.

Another distinctive feature of this group of microorganisms is their alcohol resistance [52]. They are able to develop in nutrient substrates at high concentrations of ethyl alcohol (18-24% vol).

Nitrate reduction is not typical for lactobacilli, except when the final pH is maintained at 6.0 and/or heme is contained in the medium [53]. Gelatin is not liquefied. Casein is not broken down, but most strains form small amounts of soluble nitrogen. Indole and hydrogen sulfide does not form. The content of guanine and cytosine in DNA is 32-55 mol %.

One of the main factors determining the possibility of production of microbiological remedies is the selection of nutrient medium taking into account the physiological needs of selected bacterial strains, their biosynthetic activity, the cost of environmental components, and the stability of the finished product [44].

In some studies, techniques for increasing stress resistance and survival of lactic acid bacteria are used, such as access restriction air, combined growth of lactic bacteria [54], and the use of stabilizing additives and protective media [55,56]. For example, to protect the lactic acid organism from damage during drying, methods of growing them in a highly concentrated wort are used [57].

Another method of protecting lactobacilli is cultivating them as lactic acid organisms (LAO), the combination of lactic acid bacteria and fungi, based on the use of stabilizing additives [58], which were used as antioxidants (orcin and hexylresorcinol), carbohydrates, and protein polymers (starch, carboxymethylcellulose and gelatin), as well as milk fat. A significant effect of stabilizing additives was found mainly for the 12-month-old variants storage: for hexylresorcinol and

gelatin - increase in CFU 2 times, for orcin and starch - 5–6 times. The addition of milk fat definitely had a negative effect: the number of CFU decreased by 1–2 orders of magnitude, which, apparently, is associated with the ability of fats to be oxidized to form long-lived peroxides that damage cell membranes [59].

The results indicate that storage in conditions of limited air access radically changes the rate of decrease in the number of CFU, which is an indicator of the death of microorganism cells. So, when stored for 6 months under conditions of free air access, the titer (the number of CFU) of LAO decreased to a level of 102 CFU / ml, while when stored for 15 months under conditions of limited air access, the number of colony-forming cells stabilized at the level of 1.2–1.4 × 107 CFU/ml, recommended for LAO titer in products nutrition [60].

As a result of the studies, aimed at increasing the viability of lactic acid organisms, new algorithms for the consistent application of microbiological and biochemical techniques were invented, the use of which provides a significant reduction in the rate of death of LAO cells:

- use of natural associations of lactic acid organisms to isolate LAO strains naturally adapted to co-cultivation.
- selection of natural associations of LAOs according to a given technological property, in this study - based on the duration of preservation of cell viability.
- restriction of air access during the storage of culture/products based on LAO.
- introduction of stabilizing additives (antioxidants, polymers of carbohydrate and protein nature) in binary (multicomponent) LAO cultures stored in the absence of oxygen.

In a recent investigation [61] using modern methodological approaches, the strains of lactic acid bacteria such as *Lactobacillus casei 302* and *Lactobacillus acidophilus 35* have a high level of biological activity were selected. The high biological potential of selected cultures of lactic acid bacteria, which could provide stability for the technological process of production and essential characteristics of bacterial preparations and fermented products, was set. *In vitro*, the experiments demonstrated that selected strains had valuable production properties: the ability to reduce the level of cholesterol and lactose during development in milk, were resistant to virulent bacteriophages and aggressive compounds of the gastrointestinal tract, and high adhesive and antagonistic activities as well.

When developing probiotics, the improvement of the technology and composition of probiotic preparations is also an urgent issue, which should be aimed at providing favorable conditions for bacteria during their storage and passage through the gastric barrier. This issue can be solved, among other things, by using adsorption and spatial immobilization of bacteria under mild conditions, which will allow them to maintain their viability [62,63,64].

One way to increase the resistance of bacterial drugs is immobilization. Cell immobilization and its methods found in literary sources can be divided into three types: adsorption immobilization using solid carriers; spatial immobilization by including the carrier itself in the structure and the method of membrane immobilization. Adsorption immobilization can occur using various reagents and carried out through covalent bonds. Spatial immobilization is actually microencapsulation, inclusion in the gel structure itself, and it is also possible to use membrane technologies [65]. Adsorption immobilization is the most preferred method for fixing living cells since the process occurs in the mildest conditions compared to spatial immobilization of various organic polymers. The most popular is the use of carbon carriers in the creation of structures for the adsorption immobilization of living microorganisms [66,67].

For adsorption immobilization, it is also possible to use other materials as carriers - graphite, ceramics, polyurethane, clay, cellulose, glass, chitosan, peat, and others. As shown, the use of such materials provides strong immobilization with maintaining the stable activity of living cells [68].

Various methods of spatial immobilization of the microorganisms living cells are also known, allowing to significantly increase the performance of biotechnological processes. These methods are based on the inclusion of biological agents in the structures of various gels and allow you to create high concentrations of microorganism cells, as well as provide protection against the aggressive effects of various factors environment, such as exposure to foreign microorganisms, the influence of toxic substances (including organic solvents), changes in temperature, pH level, etc. [69,70,67]. For spatial immobilization of cells and microorganisms as carriers, it is possible to use various carriers of organic or inorganic nature in the form of gels, films, and fibers. Most often, the basis includes such materials as gelatin, alginate, collagen, carrageenan, agar, chitosan, polyurethane, cellulose polyvinyl alcohol, etc. [71]. Materials that form the basis of the matrix for cell immobilization microorganisms, can be used both independently and in combinations with each other.

For immobilization of lactobacilli used in production cheeses, yogurts, kefir, and other lactic acid products, metabolites, including lactic acid, as well as for obtaining probiotic preparations and fortified food, such method as incorporation into the gel structure is quite often used. When choosing a carrier for the needs of the food industry, the following requirements are taken into account: lack of toxicity, availability, and low cost [72,73]. Based on these requirements, polymers are used: carrageenan, agar, gelatin, chitosan, and alginate. The advantage of these polymers is also that they can serve as an additional food component. It was shown that cell immobilization in carrageenan and alginate provides effective protection of lactobacilli from possible death in the freezing-thawing process - a technological operation for obtaining probiotics, contributing to the preservation of their stability in the process of storage and fermentation. In addition, immobilized cells differ better enzymatic characteristics in comparison with native [74,75]. The advantages of immobilization of lactobacilli are not only increasing stability in the technological cycle but also increasing the time storage of drugs and food. Currently, immobilization of microbial cells by inclusion in the alginate gel is recognized as the most effective and common.

Another interesting material for microorganism immobilization is agar, which is successfully used in various areas, especially in the cultivation of microorganisms. The constituent components of agar have an affinity for protective substances shells of

microorganism cells, which allows to provide protection of the microbial cells from the adverse effects of the external environment and carry out long-term storage of microbial cultures [76].

Microencapsulation can also be attributed to membrane technologies. The system is biomass enclosed in a semipermeable membrane, while the cells of microorganisms themselves remain spatially limited, and solutes freely pass through the membrane [77]. Microencapsulation also allows increased stability of the probiotics to aggressive factors of the gastrointestinal tract - low pH-environment of the stomach, the action of enzymes, and bile [78]. Research on microencapsulation into xanthan-chitosan capsules has shown the ability to increase the survival of probiotics *L. acidilactici* in the gastrointestinal tract [79]. The main substances used for probiotics microencapsulation are alginate and its combinations. For microencapsulation, calcium alginate is used at a concentration of 0.5-4%. They easily form a shell around the bacterium, are non-toxic to the body, uncomplicated in technology, dissolve in the gastrointestinal tract and release bacteria. However, they also have a number of disadvantages. For example, they are sensitive to an acidic environment and are prone to destruction in it. Moreover, if the environment contains phosphate, lactate, and citrate ions that react with calcium, the stability of microcapsules is broken. This is apparently due to the fact that these substances interact with calcium ions, due to which there is an "extraction" of the crosslinker and the destruction of the carrier matrix. For the use of such capsules, for example, in dairy products, it is necessary to develop additional operations leading to their strengthening [80].

New insights into oxidative stress influence on lactic bacteria physiological and biochemical features.

It is known that with a change (deterioration) cultivation condition, for example, when nutrients and energy sources are under the influence of adverse factors, such as reactive oxygen species (ROS), etc., microorganisms activate several protective mechanisms to ensure adaptation within reaction norms of a species or for its survival as a species resting forms under non-growth conditions [81]. For a long time, it was thought that stress adversely affects microorganisms, reducing their physiological activity and, consequently, the efficiency of biosynthesis. However, the current stress impact is widely used to improve cultivation processes efficiency, since certain conditions under the influence of stress factors improve individual indicators of biosynthesis [82]. Besides, microorganisms that are resistant to some exposed stress factors, can tolerate the influence of others (cross adaptation), without reducing the physiological activity.

Stress factors initiate the occurrence of DNA damage that is repaired through various reparation systems, one of which is the photoreparation system [83]. Photoreparation usually occurs against the background illumination of cells near UV and visible light. This process is associated with the action of photolyase - photoreactivating enzyme, which is a flavoprotein. A study of the effect of oxidative stress on physiological and biochemical characteristics of culture lactic acid bacteria looks very promising for the selection of conditions intensification of lactic acid biosynthesis.

Development of methods for safe storage of lactobacilli cultures.

In addition to improving methods for obtaining a viable and effective culture of lactobacilli, the development of methods for storing grown cultures is also an important issue. One of the basic principles of maintaining bacteria in the collection centers is to minimize the number of strain generations from the moment its entry into the collection before issuance to the applicant. For this, methods have been developed for storing bacteria for a long time without reseeding. Lyophilization (synonyms - freeze-drying, drying from a frozen state) is one of the most recommended methods for long-term storage of bacteria in biological resource centers and collections cultures [84]. Many known bacterial species remain viable after 50 years of storage in the dried state [85]. Even single living cells preserved in dry preparations, after confirmation of their authenticity can be used to get the next generation strain.

But, in the process of lyophilization cells are exposed to damaging stress factors. Low temperatures, water crystallization, osmotic process, pH alterations, and dehydration affect cell cultures and molecules. Oxidative reactions, running in dry cell preparations, change the composition and structure of lipids, proteins, and nucleic acids, thereby reducing the number of living cells during the storage [86]. One of the key factors that influence bacterial viability after lyophilization and storage is the composition of the shielding medium, with which the cells are mixed up before conservation. Utilization of protective media, containing carbohydrates, amino acids, restored milk, gelatin, and other components, decreases the probability of cell elements damaging and extends the assured storage life [87,88,89].

Conclusions.

The intestinal microbiota is a separate organ of a macroorganism that has a decisive influence on the health of the host organism and the development of diseases. Disorders of qualitative, quantitative composition and functional activity of intestinal microbiota are reduced microbial diversity, depletion of the pool of beneficial bacteria, expansion of pathogens (*Enterobacteriaceae, Pseudomonadaceae, Staphylococcus*, etc.), and changes in microbial metabolism. Microecological disorders are accompanied by dysfunction of the mechanisms of innate and adaptive immune defense, development of inflammation in the intestinal mucosa with activation of oxidation processes, increased permeability of the intestinal barrier to inflammatory products and microbial toxins, and increased risk of microbial translocation and metabolic changes in inflammation.

Representatives of the genus *Lactobacillus* are natural inhabitants of the intestine, who were among the first to colonize this habitat, play a key role in maintaining and restoring intestinal homeostasis, regulating the microflora of other habitats, determining the development and functioning of the immune and other vital systems. The powerful probiotic potential of lactobacilli underlies their use for therapeutic purposes. Therefore, the study of the mechanisms of functioning of lactobacilli, increasing their stress resistance, studying the possibilities of their effective cultivation, safe storage, and

creation of probiotic preparations based on them is an extremely important area.

The nutrient medium is of great importance in the cultivation of bacteria. Today there are a large number of modifications depending on the strains of lactobacilli and the purpose of future use of the culture. In addition, methods of increasing the survival of lactobacilli during cultivation and increasing their stress resistance are being actively studied. At present, the choice of nutrient medium largely depends on the strains that are planned to be grown on it, but the MPS environment with certain modifications remains one of the most commonly used.

Lyophilization or sublimation from the frozen state is the basic method of bacteria preservation in culture collections and biological resource centers. In the process of lyophilization, cells are exposed to damaging stress factors. One of the key factors that influence bacterial viability after lyophilization and storage is the composition of the shielding medium, with which the cells are mixed up before conservation. It was shown that utilization of protective media, containing carbohydrates, amino acids, restored milk, gelatin, and other components, decreases the probability of cell elements damaging and extends the assured storage life.

In a process of increasing stress resistance, an important role has different types of bacteria immobilizations (spatial and adsorption). Scientists' opinion regarding immobilization methods differs, but the method of microencapsulation is claimed to be very perspective.

But, despite significant advances in the study of the genus *Lactobacillus*, the mechanisms of their functioning, and use as probiotics, there are still many unexplored issues and the invention of new strains of lactobacilli and methods to increase their resistance to damaging factors remains relevant which will be explored in our future work.

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THE ROLE OF LACTOBACILLI IN THE HUMAN MICROBIOME AND METHODS OF THEIR CULTIVATION AND PRESERVATION

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Abstract. The extremely important role of the microbiome for human life and health has long been known. Many studies around the world are devoted to studying the mechanisms of action and functions of various bacteria that are permanent residents of our body. Connections between the bacteria of our microbiome and all organs and systems of the human body (intestine, brain, nervous and cardiovascular systems) have been identified. However, the effect of bacteria can be positive or negative, which affects the emergence and development of diseases or promotes healing.

Genus *Lactobacillus* is one of the most numerous populations of bacteria in the human body. Moreover, they have a significant positive effect on health. Scientists are actively researching methods of cultivating and using bacteria of this genus in the pharmaceutical and industrial fields. Most probiotics contain lactobacilli strains. Therefore, the study of methods of cultivation and storage of lactobacilli in order to find ways to improve their viability and functionality and, at the same time, the invention of options to protect cell culture from various harmful factors is extremely important.

In our review, we considered the importance of the microbiome for human health and the role of bacteria of the genus *Lactobacillus* as its component. Scientific works on studying the mechanisms of influence of lactobacilli on the functional capacity of human organs and systems have been studied. Much of the review is devoted to the study of lactobacilli cultivation methods, the diversity of culture media, and the importance of their components to improve the viability of lactobacilli culture because they are quite demanding and vulnerable. Attention is also paid to the development of methods of storage of grown cultures of bacterial cells and their improvement in order to obtain functional and suitable for further use in the pharmacological and industrial areas of bacterial strains.

Keywords. Lactobacilli, microbiome, probiotic, bacterial culture, cultivation medium.