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ANALYSIS OF THE CURRENT STATUS OF PROBIOTIC DRUG DEVELOPMENT

Olga Bliznjuk, Igor Ryshchenko, Nataliia Masalitina, Daria Pylypenko, Yuriy Krasnopolsky

The aim *of the work is to analyze the current state of the creation of probiotic preparations.*
Materials and methods. *The work analyzes strains of probiotic bacteria Lactobacillus, Bifidobacterium, Streptococcus, Bacillus, Saccharomyces and other genera. Methods for obtaining probiotics are considered, including the selection of producer strains, the scheme and parameters of culturing producers, collection and concentration, lyophilization, formulation of the product composition, selection of the dosage form. Following technological methods for obtaining dosage forms of probiotics are used: capsules, emulsions, hydrogels, suppositories, tablets, etc.*
Results. *The main functions of probiotics in various parts of the human body are considered. Bacterial strains that are part of prophylactic and medicinal preparations are analyzed. The creation of new probiotic preparations is carried out in several directions: the creation of recombinant microorganisms by genetic engineering methods and the development of new generation therapeutic preparations to improve human health, as well as the development of probiotic delivery systems into the human body. Engineered probiotics are a type of new microorganisms obtained by modifying the original bacteria and yeast. The possibility of using a new generation of strains (Akkermansia muciniphila, Ruminococcus bromii, etc.) that demonstrate high therapeutic potential in the treatment of metabolic diseases is discussed. New data and a deep understanding of the microbiome have helped to identify useful commensals and their therapeutic potential. The prospects for the use of probiotics, prebiotics and postbiotics in preparations, including a new generation of probiotic strains, are shown. The effectiveness of probiotic products for restoring the microflora of the oral cavity, intestines and vagina in various dosage forms was assessed: hydrogels, capsules and tablets.*
Conclusions. *Functional products of probiotic and postbiotic origin have antiviral, antibacterial and antitumor activity. Probiotics are effective and safe, have high therapeutic potential for the prevention and treatment of diseases of various etiologies. Various dosage forms of probiotics are highly effective for restoring the microbiome of the human body. The prospects for the use of various probiotic strains, including a new generation of microorganisms, are discussed*
Keywords: *probiotics, prebiotics, postbiotics, dosage forms, new generation of probiotics, recombinant strains, cultivation, lyophilization, prevention and treatment*

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1. Introduction

One of the important areas of pharmaceutical science is the creation of prophylactic and medicinal products based on probiotic (PR) microorganisms to restore the microbiota (MB) of the intestine [1] and the human vagina [2]. Recent studies, including clinical trials, have convincingly demonstrated the active role of PR in the prevention and treatment of infectious diseases. PR have a unique ability to create a biofilm that acts as a protective barrier and replaces any pathogen in the composition of the MB [3].

PR are living microorganisms that provide the normal composition of the human MB, the presence of which in various parts of the body (oral cavity, upper respiratory tract, gastrointestinal tract (GI – esophagus, stomach, small and large intestine), vagina, skin) gives reason to evaluate PR as an important factor for human life [4, 5].

Research on probiotics is constantly revealing new benefits of using these microorganisms. A wide range of beneficial effects of probiotics for humans has been confirmed. During the first half of the 20th century, the main role of probiotics was considered to be in maintaining the intestinal microbiota by preventing or treating infections, modulating the host immune response, and enhancing vitamin secretion.

Clinicians are faced with a violation of the spectrum of probiotics in the human body, which leads to a pathological condition. The composition of probiotics, both quantitative and qualitative, changes significantly under various factors associated with diseases. To maintain the composition of probiotics necessary for the human body for normal functioning, work is being carried out to create drugs containing a different spectrum of probiotics [6]. The probiotic is a factor that affects the physiological processes that determine human health, including antibiotic therapy.

Main representatives of the genera of PR strains used in preparations:

– *Lactobacillus: L. casei, L. rhamnosus, L. helveticus, L. reuteri, L. delbruesckii (bulgaricus), L. plantarum, L* and others;

– *Bifidobacterium*: B. *breve, B. longum, B. bifidum-I, B. infantis* and others;

– *Streptococcus: S. thermophylus, S. faecalis; Bacillus: B. clausii, B. coagulans, B. subtilis, B., B. licheniformis, B. pumilus, B. megaterium* and others;

– *Saccharomyces*: *S. boulardii* and others; as well as other genera.

When developing PR drugs, it is necessary to consider the site of action of the PRs that are administered, which determines the creation of a particular dosage form. To increase the effectiveness of PRs, when developing and using them, it is necessary to take into account the area in the body that the PRs should affect, which determines their delivery system (DDS). The creation of effective PR drugs is based on the mechanisms of their action.

2. Research planning (methodology)

The aim of the work is to analyze the current state of the creation of PR preparations. To achieve this goal, the following research plan has been developed:

– to analyze the used strains of probiotic bacteria, such as *Lactobacillus, Bifidobacterium, Streptococcus, Bacillus, Saccharomyces*, etc., including the mechanisms of their probiotic activity (antagonistic spectrum, immunomodulatory and biosynthetic activity);

– to consider methods for obtaining PR preparations, including the selection of producer strains, the scheme and parameters of producer cultivation, collection and concentration, lyophilization, formulation of the product composition, selection of the dosage form;

– to consider technological methods for obtaining PR dosage forms: capsules, emulsions, hydrogels, suppositories, tablets, etc.

3. Materials and methods

To search for sources of information for the study, open access electronic resources of scientific periodicals were used: scientific databases Google Scholar, PubMed, Clarivate, Web of Science, Scopus and others; electronic repositories of higher education institutions and scientific institutions, where dissertation abstracts, scientific publications and other scientific works are stored, including the results of their own previous research. Biological experiments conducted with at least three repetitions were considered, and the data are presented as the average value with the standard deviation. Differences between groups were determined using one-way analysis of variance (ANOVA) followed by Dunnett's test for multiple comparisons. The value $p<0.05$ was considered statistically significant. To calculate statistical values, the authors mainly used the following software:

1. IBM SPSS (ver 21, USA).
2. GraphPad Prism.
3. Microsoft Excel.

Research methods: information search, theoretical analysis and systematization of data from scientific sources, logical analysis.

4. Research results

4. 1. Mechanisms of action of PR

The creation of effective drugs containing PR is based on the mechanisms of their action:

I. Effect on immunomodulatory activity, which leads to increased immunity in humans [7, 8].

II. Synthesis of metabolites with anti-inflammatory local and systemic activity. Metabolites are represented mainly by short-chain fatty acids (SCFA): butyric, acetic, propionic, lactic, etc. [9].

III. Production and release of neurotransmitters (g-aminobutyric acid – GABA), serotonin, catecholamines and histamine or their precursors, which send signals to the CNS through enterochromaffin cells and intestinal nerve receptors. GABA is the main inhibitory neurotransmitter of the CNS. The products of PR metabolism are bacteriocins that have antibiotic effects (acidol, lactolin, lactacin, acidophilin, etc.) [10].

IV. Ability to coaggregate, which forms a protective barrier of the epithelium against colonization by pathogens and inhibition of the synthesis of bacterial toxins [11].

V. Enhancement of the synthesis and absorption of vitamins (mainly group B, but also PP and K), mineral compounds, stimulation of the production of enzymes involved in the digestive processes in the enzymatic deconjugation of bile acids by hydrolysis of bile acid salts. PRs are able to bind cholesterol (Chol) in the small intestine [12, 13].

VI. Production of various substances with antimicrobial activity: peroxide, SCFA, antibiotic-like peptides, lysozyme, etc. It has now been established that SCFA perform a number of functions in the body: energy support, stimulation of the functions of non-pathogenic symbiotic flora, anti-inflammatory and bacteriostatic (relative to pathogenic microflora) activity, maintenance of the necessary pH values in the intestine. The antimicrobial properties of PR also include competition with pathogens for adhesion to the epithelium and nutrients [14, 15].

VII. Effectively reduce total Chol and low-density lipoprotein (LDL) levels. PFs are capable of converting Chol to coprostanol or reducing Chol esters in LDL particles [16, 17].

VIII. PFs may be responsible for the detection and degradation of potential carcinogens and could both reduce and increase the production of anti-inflammatory cytokines, which play an important role in preventing carcinogenesis. A correlation has been demonstrated between the consumption of dairy products with PFs and the risk of developing colon and rectal cancer. In addition, PFs can activate phagocytes to destroy cancer cells at an early stage. The effectiveness of PFs (*Lactobacillus, Bifidobacterium, Saccharomyces*, etc.) has been shown in some types of cancer: stomach, liver, lung, breast, oral cavity and other tissues [18–20].

The total number and composition of bacterial microflora vary significantly in individual parts of the gastrointestinal tract and depend on the pH value and oxygen concentration. Each department differs in the composition of MB. Several strains are present in different departments. In the oral cavity, the *St. salivarius* strain is found, which normally makes up 40 % of the total population. *St. salivarius* also colonizes the naso-

pharynx, large and small intestines, producing two bacteriocins (salivaricin A2 and salivaricin B), which have antagonistic activity in pathogenic microflora. The smallest number of bacteria is present in the stomach, in the lumen of which the low pH value serves as the main growth-limiting factor.

4. 2. Technological aspects of obtaining PR drugs

When creating PR preparations, it is necessary to consider a number of factors, such as production technology, determination of the dosage form and method of its administration. Obtaining PR preparations includes: selection of producer strains and their identification, determination of the optimal composition of the nutrient medium, the scheme and parameters of cultivation of producer strains, studying the possibility of co-cultivation (if necessary), collection and concentration of PR, formulation of the product composition, addition of stabilizers, prebiotics, cryoprotectants or other biologically active components, selection of the dosage form of the developed product, which can largely determine the need for drying biomass, control of the finished product [21, 22]. The above scheme for obtaining PR preparations is also followed by other researchers [23, 24]. In our opinion, the selection of producer strains is decisive in this scheme: criteria for safety assessment and identification, study of antagonistic activity against host pathogens. PR strains must be of human origin. In addition, it is necessary to evaluate the possibility of choosing a PR strain, considering its need and application: oral, vaginal or transdermal.

Several technological methods are used to dry the biomass of PR strains: spray drying (less often) or freeze drying. It should be noted that both types of drying lead to significant losses of PR activity, which are accompanied by changes in the structural features of the strain, a decrease in the ability to adhere and the number of living cells, changes in morphological and biochemical properties, a decrease in the synthesis of acids and other products of PR metabolism. It is also important to use DDS to deliver PR to the human body. For this purpose, it is necessary to develop systems for introducing PR into the human body with a minimal decrease in the effectiveness of PR upon contact with biological fluids of the human body, where microorganisms are subjected to stress caused by temperature, oxidation, pH, the presence of enzymes, bile acids and other factors. PR preparations are mainly represented by products for oral administration, which are supplied in the form of capsules or tablets, and PR directly contact in vivo in the gastrointestinal tract and vagina. To stabilize the strain during the manufacturing, storage and application process, a number of substances are used that ensure the viability of the PR when moving through the gastrointestinal tract and regulate the release rate depending on the part of the body for which they are intended. One of such substances is hydroxypropyl methylcellulose (HPMC), which is used in pharmacy for oral use (tablets, capsules) as a material for an intersoluble coating that regulates the release rate [25]. Hydrophilic polymers significantly improve solubility and bioavailability. Various cryoprotectants are used to stabilize the PR during drying: sucrose, lactose, trehalose, skim milk, inulin, maltodextrin, etc. In addition to cryoprotection, excipients can be PR stabilizers and prebiotics, which requires determining their composition for a specific PR strain [26].

4. 3. Dosage forms of PR drugs

Today, there are known following dosage forms of PR: capsules, powders, spore suspensions, emulsions, hydrogels, drops, suppositories, tablets, patches, etc. [27, 28]. Based on the characteristics of PR strains and their localization in the body, it is necessary to determine the method of product administration and the task set. PRs are administered orally, nasally, transdermally, externally (locally), vaginally, rectally. When studying the PR of a product, it is necessary to assess the stability of the PR properties when administered into the body, taking into account their contact with biological fluids in which the PR is affected by pH, enzymes (pepsin, trypsin and chemotrypsin), bile acids, which act as biological detergents that disrupt the PR membranes, damaging them.

Tablets containing PR.

The tablets are supplemented with excipients that allow them to pass through the stomach and release PR in a controlled manner in the required area of the gastrointestinal tract, including the oral cavity. Tablets containing *Lactobacillus* (*L. reuteri, L. casei, L. paracasei, L. rhamnosus*, etc.) and *Bifidobacterium* are used to treat oral diseases, such as caries or yeast infections [29]. The possibility of colonization of the oral cavity by PR has been shown, which leads to a decrease in the number of pathogenic MB. Chewable tablets containing PR have been successfully used for oral hygiene [30]. Substances introduced into the tablets allow the creation of pH-sensitive forms of drugs. Tablets coated with phthalyllinulin (PI) sensitive to alkaline pH have been proposed [31] to protect *L. reuteri* LRT18 from the pH of gastric juice. Tablets were prepared using different compression forces and the survival of the PR, tablet disintegration time and kinetics in a fluid simulating the GI tract were measured. It was shown that higher compression forces resulted in higher viability of the PR. The degree of tablet swelling in simulated intestinal fluid was higher than in simulated gastric fluid due to the insensitivity of PI to the acidic pH characteristic of gastric juice. PI-coated tablets improved the survival of the PR in the stomach and rapidly released the PR into the intestinal fluid.

Multilayer tablets containing *L. gasseri* PA 16/8, *B. longum* SP 07/3 and *B. bifidum* MF 20/5 have been proposed [32]. To study the survival of PR in a tablet, the authors used a dynamic computer model of the stomach and small intestine TIM-1 (TNO, Zeist, the Netherlands), which simulates the gastrointestinal system of an adult after a meal. The TIM-1 system allows for the study of PR of drugs in various forms (tablets, capsules, etc.) and is a multi-chamber, dynamic, computer-controlled model that simulates the upper human gastrointestinal tract. The main parameters of human digestion, such as pH, body temperature, peristaltic mixing and transport, gas-

tric, biliary and pancreatic secretion, as well as passive absorption of small molecules and water, are reproduced with the greatest possible accuracy. Experiments with multilayer tablets were conducted both in the gastric compartment model and in the complete system (stomach+small intestine). Initially, an uncoated tablet (core) was tested in TIM-1 under these conditions: gastric survival increased dramatically to 31.3 % (*Bifidobacterium*) and 24 % (*Lactobacillus*). As a result of the studies, an enteric-coated tablet was developed, which increased the delivery of viable cells reaching the small intestine to 72 % (gastric survival for *Bifidobacterium*) and 53 % (gastric survival for *L. gasseri*). Survival in the small intestine increased by about an order of magnitude. It should be noted that enteric coating of the tablet leads to a 20-40-fold increase in the delivery of viable PR to the small intestine. These tablets are presented in a three-layer structure, in which one layer contains vitamins, the second - minerals and trace elements, and the third – PR. Such a structure can ensure the viability of PR and guarantee their delivery to the site of action in the intestine.

Orodispersible PR tablets have been proposed using mucoadhesive polymers for buccal mucoadhesion [33]: Carbopol 971P NF (anionic), Metolose 655H50 (nonionic), cationic Chitosan (Chit). The polymers were incorporated into the tablet either by direct compression or after granulation with PR of different *Lactobacillus* strains. The disintegration of the tablet, mucoadhesion and stability of the PR during storage were studied. It was found that when a sufficient amount of polymer is included, high mucoadhesion of the PR can be achieved. The authors showed that tablets with Carbopol 971P NF retained the stability of the PR and mucoadhesion during long-term storage, unlike tablets based on Metolose 655H50 and Chit, when using which the mucoadhesive effect was noticeably reduced. The possibility of creating tablets that disperse in the mouth for the treatment of gingivitis, periodontitis and caries is shown.

Capsules containing PR.

When obtaining PR in the form of capsules, various fillers are studied and compared. For example, Neuslin U52 (magnesium aluminometasilicate) [34] – a widely used inert filler with unique properties, was used to obtain capsules from *L. plantarum* R2 Biocenol. A capsule form of lyophilized PR was proposed. Lyophilizates were obtained from silicates (Neuslin U52), cellulose derivatives (Avicel hY-101) and sugars (inulin, sucrose, modified starch 1500). Their physicochemical properties (pH, moisture, water absorption, wetting time, density and fluidity) were evaluated by pharmacopoeial methods. The viability of *L. plantarum* was confirmed for 6 months at 4–8 °C. PR with Neuslin U52 turned out to be the most viable [35].

The TIM-1 system was used to study the survival of the PR of the drug using the "capsule-in-capsule" technology (Duocap) [36]. *Bifidobacterium* and *Lactobacillus* strains contained within the inner capsule were studied. The second capsule contained Ahiflower oil. The survival of PR in powder (with or without oil) and capsules with PR present only in the inner capsule were studied. Survival of strains after passage through the stomach compartment in the Duocap capsule was approximately 1.5 times higher compared to other options. After passage through the entire gastrointestinal tract, the survival of PR inside the Duocap capsule was approximately 2 times higher than that of strains inside the inner capsule or powder. The developed "capsule in capsule" technology increased the number of viable PR in the upper gastrointestinal tract, mainly due to the presence of polyunsaturated fatty acids of the oil contained in the outer capsule, which protects the PR mixture in the small intestine.

B. breve was included in alginate (Alg) capsules and then subjected to layer-by-layer coating with Alg and Chit alternately. These multilayer coated structures showed better protection of PR in an acidic environment than uncoated Chit structures. The viability of PR was increased from <3 log (CFU/mL) to 8.84±0.17 log (CFU/mL) in the three-layer coated matrix [37].

Hydrogels containing PR.

Hydrogels are a network of cross-linked hydrophilic polymer chains in the form of a colloidal gel in which water is the dispersion medium. A three-dimensional solid is formed because hydrophilic polymer chains are held together by cross-links [38]. Hydrogels are widely used in clinical practice due to their adjustable physical properties, biocompatibility, ability to protect drugs from degradation and its controlled release. In addition, the mucoadhesive properties of some hydrogels allow them to be immobilized at the injection site. Hydrogels are suitable materials for the encapsulation of PR due to their biocompatibility, high moisture content, softness, flexibility and versatility of fabrication [39].

Hydrogels containing probiotics for oral administration.

Currently, polysaccharide (PolyS)-based hydrogel systems have become popular due to their ability to provide a physical barrier between encapsulated PRs and the "harmful" environment. These systems not only increase the viability of PRs in the gastrointestinal tract but also improve their stability under various storage conditions [40]. Since PolyS (Chit, pectin, Alg, etc.) are biocompatible, biodegradable and affordable, they are used to deliver PRs either individually, or as a combination of two PolyS, or a combination of PolyS and non-PolyS. PolyS-based hydrogel systems should have a sufficiently small size compared to the size of bacterial cells, thus retaining the entrapped PRs in the hydrogel matrix until the network is destroyed.

A pectin/starch hydrogel was obtained by extrusion, into which *L. plantarum* was included [41]. *L. plantarum* encapsulated in the hydrogel structure remained significantly more viable than free forms of PR in experiments with gastric juice or bile acid solution. It was confirmed that the developed hydrogels protect PR from adverse conditions of the gastrointestinal tract.

Alg-based hydrogels were used to protect PR [42]. Alg is a hydrocolloid, its interfacial polymerization occurs instantly. The hydrogel was obtained from encapsu-

lated *L. plantarum* in granules and soy protein isolate and Alg-calcium (ratio 1:8). The preparation was studied under different pH and temperature conditions. The survival rate of PR in encapsulated beads was assessed under pH conditions of 2.3 and 6.5 and in bile salt. At pH 2.3, the survival of encapsulated PR was significantly higher than that of free PR. The number of live PRs in the bile after incubation did not differ from the initial number. To increase the effectiveness of Alg hydrogels containing *B. breve*, they are coated with cationic polysaccharides, such as Chit, which stabilizes the controlled swelling, while increasing the strength of the hydrogel and reducing the leakage of PRs included in the gel [37].

Alg-calcium/fucoidan (sulfated heteropolysaccharide from seaweed) hydrogels containing PR (*L. rhamnosus*) have been used as bioactive patches to accelerate the healing of oral ulcers [43]. The composite hydrogel demonstrated high cyto/histocompatibility and antimicrobial efficacy, leading to healing of oral wounds in contrast to commercial oral patches.

Synthetic polymers, including polyvinyl alcohol (PVA), polymethyl methacrylate, poly-D,L-lactic-co-glycolic acid, polyethylene glycol (PEG), polyethylene oxide, and polyacrylamide, have also been used to prepare hydrogels. These synthetic polymers have low immunogenicity, and their mechanical and physicochemical properties can be easily controlled [44]. However, the application of synthetic polymers in PR is still limited because the use of organic solvents can cause damage to PR cells. Of interest is the work in which organic solvents were not used to prepare the hydrogel [45]. The authors prepared an enzymatically crosslinked hydrogel of gelatin and PVP loaded with *L. plantarum spp.* CM-CNRG TB9 bacteria for oral delivery. In this study, *L. plantarum* was encapsulated in a gelatin-PVP hydrogel prepared in a "green" manner using microbial transglutaminase as a cross-linking agent [46]. The hydrogel is suitable for oral administration of PR due to its physicochemical properties, lack of cytotoxicity, and protection in the stomach. The hydrogel was fully characterized and its ability to entrap and protect *L. plantarum* was investigated. The gel was found to protect PR both during lyophilization and under conditions simulating the gastrointestinal tract. The hydrogel showed high PR loading efficiency (over 90 %) and lyophilization survival (91 %) of the total number of bacteria included. In the gastric model, no degradation of the hydrogel was observed, which preserved *L. plantarum*, with a survival of over 94 %. In the intestinal model, the hydrogel completely dissolved, facilitating PR release.

Hydrogels with PR for external use.

The current direction of creating hydrogels with PR is the possibility of their use to combat pathogens of wound infections. Considering that wound infections are a significant problem in medicine, and traditional antibiotic treatment often leads to the development of resistant pathogens, the use of hydrogels is justified. The ProGel system has been developed, containing *L. plantarum* placed in a gelatin matrix obtained from adipic acid dihydrazide of gelatin cross-linked with benzaldehyde-PEG. Thanks to the system of two ProGel syringes, the gel can be easily mixed and applied to any shape of wound, forming a hydrogel *in situ*. The hydrogel demonstrates reliable mechanical and self-healing properties and has clear advantages compared to direct injection of the finished hydrogel into the wound. The cross-linked hydrogel mesh can provide controlled release of PR, ensuring their constant presence in the wound to increase therapeutic efficacy. ProGel exhibits a broad spectrum of antimicrobial efficacy against pathogens commonly associated with wound infections: *Ps. aeruginosa*, *S. aureus*, and *C. albicans* (40–70 % reduction) [47]. The efficacy of ProGel has been demonstrated in clinical trials on human skin wound models infected with *Ps. aeriginosa* and *S. aureus*. Hydrogels as universal biomaterials are well suited for wound healing due to their unique properties. They act as a protective barrier, managing exudate and protecting the wound from external contaminants and mechanical trauma. A preparation using a cell-free biomimetic hydrogel based on PR membrane vesicles (MVs) has been proposed for wound healing [48]. MVs – bacterial outer membrane vesicles – are lipid vesicles that bud from the outer membrane of gram-negative bacteria. With their help, bacteria "communicate" with bacteria of their own genus and other species, as well as the environment. These vesicles contain a variety of signalling molecules: DNA, RNA, proteins (enzymes), endotoxins and other virulence factors [49]. The authors proposed a bacteriomimetic hydrogel based on MVs produced by *L. plantarum* and *L. casei*, using synthetic microparticle surfaces [48]. The wound microenvironment changes during wound healing, including pH adaptation and changes in oxygen supply. The proteomic characterization of MVs was studied, and several unique proteins expressed and sorted by MVs for each culture condition were identified. The ability of bacteriomimetic hydrogels to improve wound healing in a full thickness wound model in mice in vivo was demonstrated.

Hydrogels containing PR for vaginal use.

Representatives of the vaginal normoflora are a component of the human MB and are represented by a diverse spectrum of bacteria [50]. Most often, *Lactobacillus* is found in the healthy human vagina, among which *L. crispatus*, *L. gasseri*, *L. iners*, *L. jensenii* predominate (95 % of the normal vaginal microflora). It has been established that the vaginal microflora can change during menstruation, pregnancy and sexual activity. *Lactobacillus* produce protective metabolites: lactic acid, peroxides, bacteriocin-like chemicals, biosurfactants, etc. Biosurfactants are a structurally diverse group of surface-active molecules that can reduce the attachment of pathogens to host cells. These compounds acidify the vaginal microenvironment, inhibit the proliferation of pathogenic microorganisms and thereby maintain a eubiotic MB. *Lactobacillus* can restore homeostasis of the vaginal flora through several mechanisms: co-aggregation with pathogens can occur when the *Lactobacillus* cell surface contains various mucin-, fibronectin- and collagen-binding proteins, and these surface proteins can enhance the ability to attach to pathogens. Hydrogels with *Lactobacillus* can be used to treat vaginal infec-

tions [51, 52]. There are about 170 species of *Lactobacillus*, but only a few of them are used to treat vaginal infections. The acidic pH of a healthy vaginal ecosystem is believed to be between 4 and 4.5, which determines the selection of microorganisms capable of colonization and is crucial for suppressing the proliferation of pathogens such as *Gardnerella*, *Megasphera* and *Prevotella* or *Candida ssp.* This characteristic is the result of the production of lactic acid by *Lactobacillus* from glycogen derived from vaginal epithelial cells. Furthermore, *Lactobacillus* compete with pathogens for adhesion sites and nutrients and produce bacteriostatic and bactericidal products that control the growth of pathogenic strains [53, 54]. In general, strains belonging to the phylogenetic group *L. acidophilus* are commonly used in vaginal probiotics. However, studies of human vaginal flora using molecular methods have shown that this is not the most common type of healthy vaginal microflora, which is usually dominated by *L. crispatus*, *L. gasseri*, *L. iners*, *L. jensenii*. Probiotics are widely used in the treatment of intestinal diseases, but the effect of probiotics on the health of the female reproductive system is still controversial [55]. As a type of vaginal probiotic, *Lactobacillus* can not only acidify the vaginal environment, stabilize the microflora and enhance the function of vaginal epithelial cells, but also kill cervical cancer cells. *Lactobacillus* adsorb and occupy the vaginal epithelium, preventing the adhesion of aggressive pathogenic bacteria that cause malignant neoplasms [56]. *Lactobacillus* can inhibit the proliferation of cancer cells by secreting peptidoglycan and PolyS. Hydrogels with PR, mainly with *Lactobacillus*, demonstrate positive results in the treatment of vaginal candidiasis [57]. PR enhance the body's immune process, promote the production of cytokines, and inhibit monocyte proliferation. PRs have been shown to act directly on cervical cancer cells [58]. Recent studies have shown that PRs, such as *L. casei* and *L. rhamnous*, play an anticancer role by activating the maturation of NK lymphocytes and dendritic cells [59, 60].

5. Discussion of research results

Probiotics are defined as living microorganisms that, when administered in adequate amounts, confer a health benefit on the host. These beneficial microorganisms were discovered in the late 19th century and are collectively referred to as the body's probiotics. The global market for probiotic products was estimated at $4.62 billion in 2019 and is expected to reach $7.59 billion in 2026. The development of probiotics is taking place in several directions: the search for unique strains; the creation of recombinant probiotics (rPs) through genetic engineering; the development of new-generation probiotics; the development of DDSs for delivering probiotics to specific areas of the human body; and the creation of adhesive materials to improve probiotic colonization *in vivo* [61].

Unique PR strains include *E. coli* Nissle 1917, which has been used for about 100 years. The Nissle strain does not exhibit pathogenic activity and can be used in the treatment of gastrointestinal diseases, which is since Nissle does not produce P- and S-fimbrial adhesins, which are important virulence factors in other E. coli strains [62]. Based on the Nissle strain, the PR drug "Mutaflor" was created with proven clinical efficacy, which is used in Europe for the treatment of gastrointestinal diseases: acute and prolonged diarrhea, ulcerative colitis and other diseases. With the help of fimbriae, the strain attaches to the mucosa of the colon and forms colonies in the form of a biofilm. Due to the presence of flagella, the Nissle strain is characterized by mobility, which gives it an advantage in colonizing the large intestine. Nissle flagella are responsible for the ability to induce the production of human b-defensin-2. The properties of the Nissle strain allow it to effectively compete with several bacterial pathogens for binding sites on host tissues.

New PR strains include the yeast *S. cerevisiae* CNCM 1-3856 (CNCM 1-3856). Yeast is currently a highly effective PR product, such as *S. cerevisiae var boulardii*. The main mechanisms of action of yeast PR are inhibition of intestinal pathogens, modification of host signaling pathways, especially those involved in inflammatory reactions, stimulation of the immune system, and effects on the intestinal mucosa. CNCM 1-3856 has antagonistic activity against pathogenic *E. coli*, and its use reduces mucosal inflammation, as shown in a mouse model of induced colitis caused by adhesive-invasive *E. coli*, which mimics Crohn's disease [63].

The production of rPs is actively developing [64, 65]. Constructed PR is a type of new microorganisms obtained by modifying the original PR. Based on rPs, the following have been created: *p-L. casei* ANCC 334, which synthesizes the adhesion protein of *L. monocytogenes*. Colonization of the intestine *p-L. casei* ANCC 334 protects the intestinal tract of mice from *L. monocytogenes* [66]. *p-B. subtilis* 2335(105) synthesizes human α-2-interferon and has antibacterial and antiviral efficacy when colonizing the human intestine (the drug "Subalin") [67]. *p-E. coli* SLIC synthesizes nanoantibodies, promotes T-cell activation, increases the number of memory T-cells and enhances the antitumor immune response, thereby suppressing tumor growth [68]. *p-E. coli* Nissle 1917 synthesizes human α-defensins, which provide antimicrobial host defence in the small intestine through their expression and release [69]. *P-L. lactis* strains that recognize the cholera autoinducer (CAI-1) produced by Vibrio cholerae are actively used to obtain rPs strains [70]. *p-L. lactis* modified with a plasmid delivering the protein P62, which can reduce the severity of colitis in mice. P62 is a multifunctional human adaptor protein involved in key cellular processes (tissue homeostasis, inflammation and cancer). The use of pPR synthesizing P62 protects the mucosa of mice in model experiments [71]. Oral administration of *p-L. paracasei* expressing angiotensins 1-7 to adult diabetic mice improved glucose tolerance by increasing insulin levels and reducing diabetes-induced kidney and retinal damage [72]. With the help of pPR, bioactive peptides with antimicrobial, antiviral and antidiabetic activities are synthesized [73].

As producer strains are used p-*L. lactis*, p-*L. casei* BL23, p-*L. plantarum* WCFS1, p-*E. coli* Nissle 1917, p-*B. longum*, p-*Streptococcus*, p-*S. boulardi*, p-*B. subtilis* and others [74, 75].

It has been proposed to use next-generation probiotics as therapeutic agents to improve human health [76]. Five new probiotic strains have been identified: *Akkermansia muciniphila, Ruminococcus bromii, Faecalibacterium prausnitzii, Anaerobutyricucum hallii*, and *Roseburia intestinalis*, which have shown efficacy in the treatment of metabolic diseases. New data and a deeper understanding of the microbiota have helped to identify beneficial commensals and their therapeutic potential. Certain commensal strains of human intestinal microbiota have been positively associated with human health and have recently been named next-generation probiotics [77, 78].

The bacterium A. *muciniphila* plays an important role in the condition of the intestinal mucosa and enhances the action of insulin and helps to fight obesity and regulate blood sugar levels. The action of this bacterium is due not so much to its properties as a PR, but to the metabolites it produces [79]. *R. bromii* is the main intestinal MB species found in humans and animals, which synthesizes a narrow spectrum of carbohydrate-active enzymes that play a crucial role in the degradation of dietary resistant starch. The strain encodes a gene responsible for the formation and germination of spores that survive exposure to oxygen [80]. *R. intestinalis* ensures the growth of proximal Bacteroides thetaiotaomicron by releasing glucose during starch degradation. *R. intestinalis* produces butyrate in the colon, which affects energy metabolism, the intestinal barrier and has anti-inflammatory effects. *R. intestinalis* prevents intestinal inflammation and maintains energy homeostasis by producing metabolites [81]. Patients with inflammatory bowel disease show significant changes in the abundance of *R. intestinalis*. *R. intestinalis* stimulates intestinal cells, secretes cytokines, promotes the differentiation of regulatory T cells, and activates innate lymphoid cells type 3 (ILC3). *R. intestinalis* has also been shown to affect the energy metabolism of the gut–brain axis. *R. intestinalis* is also capable of enhancing antitumor activity [82]. Butyrate produced by *R. intestinalis* enhances the efficacy of anti-PD-1 in colorectal cancer by activating cytotoxic CD8+ T cells. Results of a study of the number of *R. intestinalis* showed a significant depletion of bacteria in the feces of patients with colorectal cancer compared with healthy controls. Administration of *R. intestinalis* significantly inhibited tumor formation in mice, which restored the intestinal barrier function. *R. intestinalis* or butyrate inhibited tumor growth by inducing the cytotoxic metabolites interferon-γ and tumor necrosis factor TNF-α.

Currently, the release of PR in capsules containing GLP-1 is underway: A. muciniphila, *R. bromii*, *F. prausnitzii*, *A. hallii* and *R. intestinalis.*

In recent decades, researchers have become increasingly interested in functional products that include probiotics, prebiotics, and postbiotics. Probiotics are living microorganisms, and prebiotics are substrates that are selectively used by host microorganisms. A separate group is postbiotics, which are metabolites and cell wall components that are beneficial to the host and are released by living bacteria or appear after lysis due to the fermentation activity of probiotics [83]. According to a number of researchers, the use of bacterial components is safer than the use of whole bacteria. Postbiotics include SCFA and long-chain fatty acids (LCFA), proteins, peptides, bacteriocins, and other compounds that improve the functioning of various body systems and perform some of the functions of live probiotics [84]. LCFAs with antimicrobial activity include eicosapentaenoic, lauric, and myristic, which inhibit the growth of gram-positive pathogens by causing lysis and increased membrane permeability. The effect of fatty acids induced by *L. fermentum*, *L. acidophilus*, and *L. paracasei* on Klebsiella oxytoca has been demonstrated. Peptides kill microorganisms by inhibiting the synthesis of macromolecules and destroying microbial membranes. Postbiotics also include hydroxyl radicals – peroxides, which have powerful oxidizing properties. Many PRs produce hydrogen peroxide, which is the main metabolite of lactic acid bacteria and is usually found in catalase-negative bacteria during aerobic cultivation [85, 86].

Methods for encapsulation of PR are being developed. Various strains with PR properties and substances for encapsulation of strains are being considered [87–89]. Today, there are a number of technological methods for encapsulation of PR, namely: coacervation, extrusion, sublimation, spray drying, spray-freeze drying, emulsification, electrospinning, etc. [90, 91].

Currently, PRs attract the attention of both doctors and researchers for the prevention and treatment of many diseases. This interest is associated with the various mechanisms of action of PRs in humans. The connection between the intestinal microbiota and the liver, the so-called “microbiota-gut-liver axis”, between the intestinal microbiota and the CNS through the “microbiota-gut-brain axis” has been demonstrated; and the microbiota-gut-lung axis in bacterial and viral infections [92]. The use of PR in various pathologies is currently under discussion.

Practical significance. PRs attract the attention of clinicians as highly effective and safe drugs, which are an important tool in the treatment of many human diseases. The practical significance of PR drugs for the prevention and treatment of human diseases of various etiologies is determined by the various properties of probiotic microorganisms. Understanding the diversity of PR mechanisms of action is important in creating effective drug delivery systems.

Study limitations. The available literature on the topic of obtaining new-generation PRs and recombinant PRs contains limited information on the technological aspects of cultivating strains and manufacturing new dosage forms based on them.

Prospects for further research. Further research into PR drugs will be aimed at identifying new pharmacological properties of microorganisms and creating new dosage forms of bacteria and yeasts that will ensure their delivery to the human body in the most native form possible. More clinical studies are also needed to confirm the efficacy and safety of PR strains.

6. Conclusions

Functional products of probiotic and postbiotic origin have antiviral, antibacterial and antitumor activity. Probiotics are effective and safe, have high therapeutic potential for the prevention and treatment of diseases of various etiologies. Various dosage forms of probiotics probiotics are highly effective for restoring the microbiome of the human body. The prospects for the use of various probiotic strains, including a new generation of microorganisms, are discussed.

The scientific community considers it necessary to conduct more clinical studies on large samples of patient populations so that the assessment of their therapeutic potential provides specialists with solid evidence of their effectiveness and safety in clinical use.

Conflict of interest

The authors declare that they have no conflict of interest regarding this study, including financial, personal, authorship, or other, that could influence the study and its results presented in this article.

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The manuscript has no associated data

Use of artificial intelligence

The authors confirm that they did not use artificial intelligence technologies in creating the presented work.

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Olga Bliznjuk, Doctor of Technical Sciences, Professor, Head of Department, Department of Biotechnology, Biophysics and Analytical Chemistry, National Technical University «Kharkiv Polytechnic Institute», Kyrpychova str., 2, Kharkiv, Ukraine, 61002

Igor Ryshchenko, Doctor of Technical Sciences, Professor, Director, Educational and Scientific Institute of Chemical Technology and Engineering, National Technical University «Kharkiv Polytechnic Institute», Kyrpychova str., 2, Kharkiv, Ukraine, 61002

Nataliia Masalitina, PhD, Associate Professor, Department of Biotechnology, Biophysics and Analytical Chemistry, National Technical University «Kharkiv Polytechnic Institute», Kyrpychova str., 2, Kharkiv, Ukraine, 61002

Daria Pylypenko, PhD, Department of Biotechnology, Molecular Biology and Aquatic Bioresources, State Biotechnological University, Alchevskikh str., 44, Kharkiv, Ukraine, 61002

Yuriy Krasnopolsky*, Doctor of Pharmaceutical Sciences, Professor, Department of Biotechnology, Biophisic and Analytical Chemistry, National Technical University «Kharkiv Polytechnic Institute», Kyrpychova str., 2, Kharkiv, Ukraine, 61002

***Corresponding author:** *Yuriy Krasnopolsky, e-mail: yuriykrasnopolsky@gmail.com*