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Review

RECOMBINANT PROBIOTIC PREPARATIONS: CURRENT STATE, DEVELOPMENT AND APPLICATION PROSPECTS

A.D. Khablenko[1*], S.G. Danylenko[2], O.I. Yalovenko[1], O.M. Duhan[1], O.I. Potemskaia[2], D.S. Prykhodko[1]

[1]Igor Sikorsky Kyiv Polytechnic Institute, Kyiv, Ukraine
[2]Institute of Food Resources of the National Academy of Agrarian Sciences of Ukraine, Kyiv, Ukraine

*Corresponding author: khablenkoad@gmail.com

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The article is devoted to the latest achievements in the field of research, development, and implementation of various types of medicinal products based on recombinant probiotics. The benefits of probiotics, their modern use in medicine along with the most frequently used genera and species of probiotic microorganisms were highlighted. The medicinal and therapeutic activities of the studied probiotics were indicated. The review suggests various methods of creating recombinant probiotic microorganisms, including standard genetic engineering methods, as well as systems biology approaches and new methods of using the CRISPR-Cas system. The range of potential therapeutic applications of drugs based on recombinant probiotics was proposed. Special attention was paid to modern research on the creation of new, more effective recombinant probiotics that can be used for various therapeutic purposes. Considering the vast diversity of therapeutic applications of recombinant probiotics and ambiguous functions, their use for the potential treatment of various common human diseases (non-infectious and infectious diseases of the gastrointestinal tract, metabolic disorders, and allergic conditions) was investigated. The prospects for creating different types of vaccines based on recombinant probiotics together with the prospects for their implementation into medicine were considered. The possibilities of using recombinant probiotics in veterinary medicine, particularly for the prevention of domestic animal diseases, were reviewed. The prospects for the implementation of recombinant probiotics as vaccines and diagnostic tools for testing certain diseases as well as modeling the work of the human digestive system were highlighted. The risks of creation, application, including the issues related to the regulatory sphere regarding the use of new recombinant microorganisms, which can potentially enter the environment and cause unforeseen circumstances, were outlined.

Keywords: genetic engineering; biotherapy; living microorganisms; cell-factory; metabolic disorders; immune stimulants; probiotic-based vaccines.

Introduction

Human use of microorganisms for various purposes (initially – in a passive form, then after the famous discovery by Louis Pasteur of the role of microorganisms in the conversion of glucose into alcohol – in a conscious form) has been observed throughout the history of mankind [1]. It is important to note that the development of such biological disciplines as biochemistry, genetics, microbiology, cosmetology, pharmaceutics, medicine, etc. took place in parallel, and discoveries, e.g., in the field of genetics, had a significant impact on the development of microbiology and biochemistry.

Among the most important discoveries of the 20th century are as follows: 1) the decoding of the double helix of the DNA molecule as the carrier of the structural unit of heredity – the gene; 2) the development of recombinant DNA technology. These achievements gave impetus to the development of genetic engineering as well as the development of highly effective and genetically stable probiotic strains of microorganisms on its basis. Other numerous discoveries in the field of enzymology, such as restriction enzymes, gave molecular biologists an opportunity to develop methods for DNA sequencing and cloning, which also contributed to the production of highly effective probiotic strains of microorganisms [2]. Thanks to these discoveries, it became possible to obtain genetically modified organisms (GMOs), which could significantly increase the synthesis of biological active compounds (BAC), enzymes, vitamins, antibiotics, and other compounds useful for humans, as well as obtain cultivated plants that are resistant to climate change [3]. Modern research and the discovery of the CRISPR-Cas system made it possible to carry out genetic modifications of various biological objects based on simpler DNA recognition, which significantly expanded the range of scientific possibilities for creating GMOs [4].

Due to the development and implementation of various tools in molecular biology, numerous interdisciplinary fields of science appeared, among which are genomics, transcriptomics, proteomics, metabolomics, microbiomics, metagenomics, etc. [5].

Among these new "omics sciences", microbiomics (an interdisciplinary field that studies the human microbiome) is of considerable interest. The rapid development of microbiomics over the past decades became possible due to the substantial historical base of research on the human microbiome, numerous studies on the discovery of the "probioticity" phenomenon, massive use of genetic engineering technologies together with molecular biology tools [6,7].

There are several definitions of probiotics. The most comprehensive, in our opinion, is the following: probiotics (probiotic microorganisms) are living microorganisms that are considered as a normal and useful component of the human microflora, the waste products of which have therapeutic properties to some extent: they stimulate the secretion of gastric juice and natural enzymes during the digestion of food, reduce the number and severity of side effects of antibiotics, promote the breakdown of salts of bile acids and normalization of lipid metabolism, normalize the composition of normal intestinal microflora [9, 10, 12]. Generally, this group of microorganisms has been known for their beneficial properties since the epoch-making discoveries of Louis Pasteur in the mid-19th century (e.g., can be used in the production of fermented milk products or as food additives) [8–10]. The commonly accepted definition of "probiotics" was formed by the WHO and the FAO (Food and Agriculture Organization of the United Nations) and has the following wording: probiotics are live microorganisms that, when administered (or consumed) in sufficient quantities, provide health benefits for the host [11, 12]. Usually, probiotics include microorganisms of prokaryotic and eukaryotic origin, but most probiotic microorganisms are prokaryotes [13]. Among prokaryotes, which are generally recognized probiotics, the following genera are distinguished: *Lactobacillus*, *Bifidobacterium*, *Enterococcus*, *Streptococcus*, *Bacillus*. Representatives of the *Lactobacillaceae* family are especially beneficial, studied in detail and the following genera are most often used: *Lactobacillus*, *Lacticaseibacillus*, *Lactiplantibacillus*, *Leuconostoc*, *Limosilactobacillus*, etc. [13, 14]. Eukaryotic probiotics have not been studied enough. They include various types of yeast, but only one strain, *Saccharomyces boulardii*, has documented probiotic properties [14, 15].

It is now known that using (as a food supplement) certain groups of probiotics as a part of probiotic preparations can have a significant impact on the health of the holobiont (host) [16]. Besides, their administration helps in the prevention and control of foodborne diseases. In addition, they have several mechanisms of competitive exclusion (destruction) of pathogenic microbiota in the gastrointestinal tract (GIT) and take part in the modulation of the host's immune system. [6,11]. Even though many probiotics can inhibit intestinal pathogens, they still have some limitations in their antimicrobial activity and can induce a low level of immune response [11]. The individual mechanisms of antimicrobial and immunological action have not yet been fully investigated [17].

As the first studies of probiotic microorganisms date back to the 19th century, the emergence of studies on the creation of recombinant probiotics appeared at the end of the 20th century. Therefore, the development of "designer" or recombinant probiotic microorganisms [18] is an indisputable breakthrough towards the creation of qualitatively new probiotic preparations with specified properties.

The main prerequisites for the use of genetic engineering to create highly effective probiotic strains were some negative factors in the field of health care, among which are as follows: 1) unsystematic intake of antibiotics by patients, which led to the acquisition of resistance to pathogenic microorganisms [17]; 2) the spread of infectious diseases, which also requires the creation of fundamentally new vaccines [19, 20].

In addition to the ability of probiotic microorganisms to suppress the development of pathogenic microbiota in the human body, they can also enhance certain specific properties of existing strains with declared therapeutic properties of the products of their vital activity [11]. Today, thanks to modern methods of genetic engineering, new opportunities are opening up for the design and creation of genetically modified probiotic strains with specified characteristics or targeted at a specific pathogen. [21]. Modern research and scientific works in this direction cover numerous fields of medicine together with veterinary medicine, which is discussed in the article. The leading ones include the use of recombinant probiotics for the therapy or prevention of gastrointestinal diseases, the creation of various vaccines, and the prospects for using recombinant probiotics for the treatment of a significant number of metabolic disorders [21, 22].

The purpose of the article is to describe and analyze modern recombinant probiotics, contemporary methods of their creation as well as their use for therapeutic and preventive purposes.

1. General characteristics of modern probiotic microorganisms

The concept of probiotics appeared in 1908 with the discovery by Stamen Grigorov of the relationship between longevity and yogurt consumption along with the further development of this idea by Ilya Mechnikov. Furthermore, the development of knowledge about probiotics was influenced by the research of Pasteur's students. The term "probiotic" was proposed in 1965 [22, 23].

It is known that probiotic microorganisms have a number of beneficial properties for the human body. All of them are safe and have GRAS (Generally recognized as safe) status [24]. However, probiotics, whose by-products have certain therapeutic properties and can potentially be used for medical purposes, are subject to a certain number of requirements, shown in Fig. 1 [24, 25].

The most numerous groups of probiotic microorganisms include LAB (lactic acid bacteria), which usually meet the above-mentioned parameters. In fact, the distribution of known and therapeutically valuable species is rather uneven. The proportion for each species is shown in Fig. 2. Other genera with probiotic properties usually include *Bacillus*, *Bacteroides*, *Saccharomyces*, etc. The complete species list is given in Table 1.

According to the constructed diagram and Table 1, the largest share of probiotics falls on LAB, whereas the smallest falls on the yeast of *Saccharomyces* genus. In addition, the leaders in the number of probiotic species are the *Bifidobacterium* and *Lactobacillus* genera.

It is known that most of the above-mentioned genera and species of probiotics belong to the normal human microbiota, mainly the intestine [21], skin, and genitourinary system [26–28]. Regardless of the organ system, the normal microbiota plays an important role in maintaining the function of that system and providing protection against pathogens [15]. Some studies on the human microbiome indicate that the microbiome may function as a distinct organ [15, 29, 30]. There is also evidence for the effect of normal microbiota on other organ systems, e.g., the central nervous system (CNS) [31], improving the functioning of the gastrointestinal tract [32]. The normal microbiota of human organ systems plays a preventive role in bacterial infections [33]. Such effects are achieved by the peculiarities of the mechanism of action of both individual representatives of the normal human microbiota and when consuming probiotic preparations based on artificially created probiotic microorganisms [22, 34]. Quite a few mechanisms of action of various types of probiotics are known; among which are: production of antimicrobial compounds (organic acids, hydrogen peroxide, diacetyl, bacteriocins, biosurfactants) [35], immunomodulation (modulation of humoral and cellular immune responses, stimulation of nonspecific immunity) [22, 32], competitive exclusion of pathogens, improvement of barrier body functions [9, 11]. Other beneficial effects of probiotics include lowering the amount of cholesterol in the blood, reducing the risk of colon cancer, reducing the level of toxicogenic or mutagenic waste products of harmful microorganisms, and improving the digestion of lactose [9, 36].

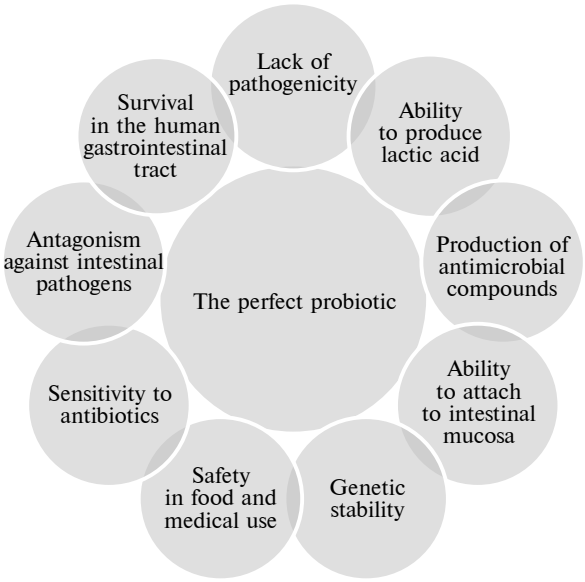

Figure 1: Requirements for an ideal probiotic [24, 25]

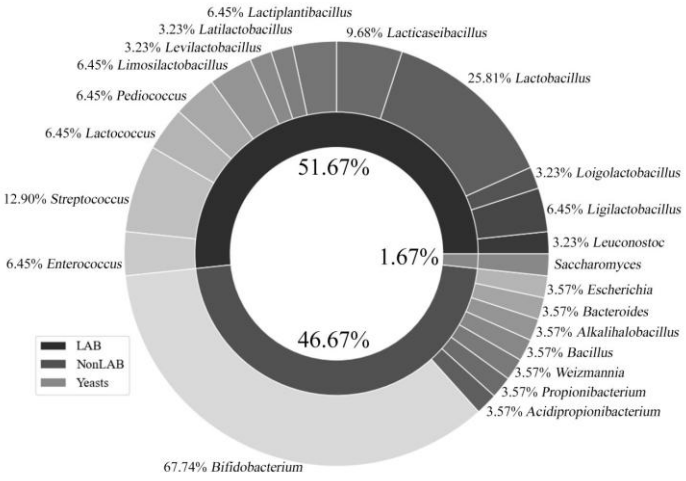

Figure 2: Diagram of the proportions of probiotics by genus and relation to LAB / non-LAB [13, 25]

Table 1: Species list of therapeutically valuable probiotics [13, 22, 25]

Family	Genus	Species
Lactobacillaceae	*Ligilactobacillus*	*Lg. aviarius*, *Lg. salivarius*
	Loigolactobacillus	*Lo. coryniformis*
	Lactobacillus	*Lb. delbrueckii*, *Lb. kefiranofaciens*, *Lb. acidophilus*, *Lb. crispatus*, *Lb. gasseri*, *Lb. johnsonii*, *Lb. helveticus*, *Lb. amylovorus*
	Lacticaseibacillus	*Lcb. paracasei*, *Lcb. casei*, *Lcb. rhamnosus*
	Lactiplantibacillus	*Lpb. plantarum*, *Lpb. pentosus*
	Latilactobacillus	*Llb. sakei*, *Llb. curvatus*
	Levilactobacillus	*Lvb. brevis*
	Limosilactobacillus	*Lmb. fermentum*, *Lmb. reuteri*
	Leuconostoc	*L. mesenteroides*
	Pediococcus	*Pc. acidilactici*, *Pc. pentosaceus*
Streptococcaceae	*Lactococcus*	*Lc. lactis*, *Lc. cremoris*
	Streptococcus	*Str. thermophilus*, *Str. oralis*, *Str. sanguinis*, *Str. mitis*
Enterococcaceae	*Enterococcus*	*Ec. faecalis*, *Ec. faecium*
Bifidobacteriaceae	*Bifidobacterium*	*Bif. adolescentis*, *Bif. angulatum*, *Bif. animalis*, *Bif. asteroides*, *Bif. bifidum*, *Bif. boum*, *Bif. breve*, *Bif. catenulatum*, *Bif. choerinum*, *Bif. coryneforme*, *Bif. cuniculi*, *Bif. dentium*, *Bif. longum*, *Bif. magnum*, *Bif. merycicum*, *Bif. minimum*, *Bif. pseudocatenulatum*, *Bif. pseudolongum*, *Bif. psychraerophilum*, *Bif. pullorum*, *Bif. ruminantium*, *Bif. saeculare*, *Bif. scardovii*, *Bif. subtile*, *Bif. thermacidophilum*, *Bif. thermophilum*
Propionibacteriaceae	*Acidipropionibacterium*	*A. jensenii*
	Propionibacterium	*P. freudenreichii*
Bacillaceae	*Weizmannia*	*W. coagulans*
	Bacillus	*B. subtilis*
	Alkalihalobacillus	*Ab. clausii*
Bacteroidaceae	*Bacteroides*	*Bt. uniformis*
Enterobacteriaceae	*Escherichia*	*E. coli*
Saccharomycetacee	*Saccharomyces*	*S. cerevisiae* var. *boulardii*

Due to the variety of beneficial properties for human health, probiotics are widely used for therapeutic purposes, which is shown in Table 2.

Even though many probiotic species and strains are studied nowadays, several more therapeutically valuable probiotic microorganisms were discovered over the last twenty years, which include unconventional strains of *Akkermansia muciniphila*, *Faecalibacterium prausnitzii*, *Bt. fragilis* together with the members of Clostridia clusters IV, XIVa and XVIII [74, 75].

Different approaches are used to artificially create therapeutic probiotics, or pharmabiotics. The most promising approaches are as follows: 1) the creation of multistrain compositions [74]; 2) a personalized approach when using probiotics considering characteristics of the patient's microbiome [75]; 3) the creation of "designer probiotics" [17].

The concept of creating recombinant probiotics appeared due to the need to find new antimicrobial agents, which, unlike antibiotics, will be safer, biodegradable, more effective against many microorganisms as well as resistant to certain groups of drugs [17, 18]. Due to their primary beneficial properties (listed above), the ability to influence the microbiome and human health, probiotics are ideal objects for improving their properties or acquiring new ones via genetic engineering [11, 17, 77]. It should be noted that traditional probiotics have certain disadvantages, such as: lack of specificity, lower efficiency [78], limited adhesion inhibition of certain pathogens, low level of anti-inflammatory action, low tolerance to stress factors during production or consumption [11]. Therefore, the use of various methods of genetic engineering can help to create probiotic microorganisms of different action spectra, more resistant pharmabiotics and producers of certain therapeutically important BACs [17, 21].

Table 2: Therapeutic use of probiotics

Nosological form	Probiotics	Mechanism of action and effect on microbiota	Source
Gastrointestinal disorders			
Antibiotic-associated diarrhea (AAD)	*S. cerevisiae* var. *boulardii*	Increase of secondary bile acids, decrease of Escherichia and Parabacteroides bacteria	[37, 38]
	Lcb. rhamnosus (LGG) and *S. boulardii*	The compatible mechanism is unknown	[39]
	Lactobacillus Rosell-52, *Bifidobacterium* Rosell-175 and *Lactobacillus* Rosell-11	Cleavage of toxins by proteases, inhibition of toxin synthesis, reduction of inflammation	[40, 41]
	Lb. acidophilus NCFM, *Lcb. paracasei* Lpc-37, *Bif. lactis* Bi-07 and *Bif. lactis* B1-04	Reduction of Bacteroides and Ruminococcus	[42]
Gastroenteritis	*Lactobacillus* spp., *Lmb. reuteri*, *Lcb. casei*	Increase of IgA levels and immunostimulation	[9]
	Str. thermophilus and *Bif. bifidum*	Competition with other microorganisms, immunomodulation	[43]
	Lb. acidophilus NCFM and *Bif. lactis* Bi-07	Not researched, increase of *Lb. acidophilus* and bifidobacterium (change in intestinal microbiota)	[44]
Lactose intolerance	*Bif. animalis*	A change in the metabolic activity of the microbiota, a change in intestinal pH, β-galactosidase expression and a positive effect on the colon microbiota	[45]
	Lb. acidophilus LA-1, *Lb. gasseri* OLL 2863 and strain OLL 2948	β-galactosidase activity, the ability to utilize lactose in the human intestine	[46]
Inflammatory bowel disease (IBD)	*S. boulardii* and *Lcb. rhamnosus* (LGG)	Increase of IgA level in the gastrointestinal tract	[47]
	E. coli Nissle 1917	Inhibition of pathogen adhesion and their effect on mucosal barrier function	[48]
Clostridium difficile infection	*S. boulardii* and *Lactobacillus* spp.	Strengthening the natural barrier of normal microbiota, immunomodulation, direct antimicrobial action and regulation of intestinal enzymes	[49]
Helicobacter pylori infection	*Lactobacillus* spp., *Bifidobacterium* spp.and *Lb. johnsonii*, *Lcb. rhamnosus* GG	Immunostimulation and direct effect on *H. Pylori* by bacteriocins, organic acids, inhibition of adhesion to epithelial cells	[50]
	Str. faecalis, *Lb. acidophilus*, *B. subtilis*	Bacteriostatic and bactericidal effects, indirect effect on enzyme activation	[51]
	Lmb. reuteri	Biosynthesis of reuterin that has an anti-*H. pylori* effect	[52]
Celiac disease	*Bifidobacterium* spp.	Immunomodulation, reduction and increase of TNF-γ levels, reduction of *Bt. Fragilis* and *Enterobacteriaceae*	[9]
Allergic conditions			
Atopic dermatitis	*Lactobacillus* spp., *Lactobacillus* compatible with *Bifidobacterium*	Contribute to the development of the mucous barrier and stimulate the immune system (activation of anti-inflammatory cytokines, increase the IgA level), reduce the concentration of IgE in serum	[47, 53]
Allergic rhinitis	*Lb. acidophilus* L-92, *Lcb. rhamnosus* GG, *Lb. gasseri*	Suppression of serum antigen-specific IgE, immunoregulatory effect	[54, 55]
Atopic eczema	*Lb. acidophilus* L-92, *Bifidobacterium* and *E. coli* Nissle 1917	Reduction of IgE level in serum, stimulation of Th1 cells	[56, 57]

Continuation of Table 2

Nosological form	Probiotics	Mechanism of action and effect on microbiota	Source
Respiratory diseases			
Asthma	*Ec. faecalis* FK-23	Not fully explored, reduction of Th17 cells and cytokines produced by lung cells	[58]
Cystic fibrosis	*Lcb. rhamnosus* GG	Not researched	[59]
Respiratory infections	LAB strains of all genera	Not enough researched, associated with the production of bacteriocins, hydrogen peroxide, the competition mechanism, and suppression of virulence factors	[59]
Neurological and psychiatric diseases			
Various neurological conditions	*Lcb. rhamnosus*, *Bifidobacterium* spp., *Lactobacillus* spp., *Str. thermophilus*	Formation of the gut microbiota-brain axis by increasing brain-derived neurotrophic factor, including IFN-dependent mechanism, i.e. by acting on neural, metabolic and immune pathways	[60]
Diseases of the autistic spectrum	*Lb. acidophilus* Rosell-11	Not studied, reduces the amount of D-arabinitol in urine	[61]
Autoimmune encephalomyelitis	*Lpb. plantarum*, *Lcb. paracasei*	Balance Mediation by induction of immunoregulatory reactions, anti-inflammatory effect, induction of IL-10 dependent cells differentiation	[62]
Liver diseases			
Cirrhosis and hepatic encephalopathy	Combination of LAB	Not researched, reduction of morbidity when consuming probiotics	[63]
Diseases of the genitourinary system			
Bacterial vaginosis and *Gardnerella vaginalis* infection	*Lactobacillus* spp.	Immunomodulation, normalization of vaginal microbiota and production of lactic acid, inhibition of pathogens growth and development	[47, 64]
Metabolic syndromes			
Diabetes and obesity	*Lactobacillus* spp., *Bifidobacterium* spp.	Production of short-chain fatty acids (SCFA), butyrate, GLP-1 secretion, appetite-suppressing mediator of the brain-gut axis	[65]
	Akkermansia muciniphila	Increases the thickness of the intestinal mucous layer, increases the content of biologically active lipids, has an insulinotropic effect	
Cardiovascular diseases			
Elevated cholesterol	*Lactobacillus* spp., *Bifidobacterium* spp.	Not fully explored	[66]
Diseases of the oral cavity			
Gingivitis Periodontitis	*Lg. salivarius*	Replacement of pathogenic microorganisms	[67]
Dental caries	*Lactobacillus* spp., *Lcb. rhamnosus*	Not fully investigated, but related to the detection of cariogenic pathogens and their inhibition	[68]
Oral candidiasis	*Lcb. rhamnosus* GG	Immunostimulation, IFN and cytokines production, *Candida* growth inhibition by producting antimicrobial compounds,	[69]

End of Table 2

Nosological form	Probiotics	Mechanism of action and effect on microbiota	Source
Autoimmune diseases			
Sjogren's syndrome	*Lactobacillus* spp., *Bifidobacterium* spp.	It is believed that there is a link between the pathogenesis of Sjogren's syndrome and dysbiosis	[70]
Rheumatoid arthritis		Reduction of the C-reactive protein index, stabilization of the insulin level, not fully explored	[71]
Systemic lupus erythematosus		Understudied, modulates the immune response, which may be related to pathogenesis, an imbalance is believed to contribute to disease development	[72]
Multiple sclerosis	*Lactobacillus* spp., *Bifidobacterium* spp.	Reduce the level of inflammatory cytokines and mucosal inflammation	[71]
Adjuvants for vaccines			
Flu vaccine	*Lcb. paracasei*, *Lcb. casei*	Increasing the vaccine immunogenicity by inducing phagocytes and NK-cells, promoting the IgA secretion to enhance the vaccine effect	[73]

2. The main approaches to the development of recombinant probiotics

The creation of genetically engineered probiotics is being developed in the following directions: 1) improving the primary characteristics of the probiotic, e.g., increasing stress tolerance or adhesive abilities [11, 79]; 2) using probiotics as a universal delivery system for certain active pharmaceutical ingredients [79], a diagnostic tool [80], an object to improve the specific properties and increase the synthesis of certain antimicrobial BACs [77].

2.1. Methods of introducing foreign DNA into probiotics

The introduction of foreign DNA into a microorganism is a mandatory stage for carrying out genetic manipulations to change the heredity of a biological object. Standard methods are generally used for probiotics [81]. Transformation can be achieved via natural and artificial methods. In relation to probiotics, natural methods are most often used, which include conjugation, phage transduction or natural competence. In turn, natural competence is the most common method for horizontal gene transfer in bacteria [81, 82]. For instance, natural competence allows us to transform *Str. Thermophilus* using classical vectors and linear fragments that can be directly integrated into the chromosome [81]. The natural competence method was recently discovered in *Lactococcus* species [83] Conjugation method is still used today to obtain new probiotic strains of LAB [84].

Artificial methods include electroporation (creating pores in the lipid bilayer of the cell membrane under the action of an electric field), chemical or thermal shock [82, 83]. Electroporation is the simplest method of artificial transformation. Although the electroporation method is quite effective, it is not always possible to use it for wild-type strains, especially those isolated from the human GI tract [81]. The possibility of using the protoplast fusion method was also reported [81, 83].

2.2. Methods of modifying the genome of probiotics

The use of genetic engineering to create probiotic microorganisms with specified properties is carried out by manipulating their own DNA and the DNA of other microorganisms (that also have GRAS status) via the following methods: induced mutagenesis (random mutagenesis); using vectors obtained from cryptic LAB plasmids or bifidobacteria; integration of target genes into the probiotic strain using site-specific recombination; using the CRISPR-Cas system [85].

2.2.1. Induced mutagenesis (random mutagenesis)

To obtain mutant LAB with given functions, it is possible to use induced mutagenesis by physical (irradiation) or chemical mutagenic agents. It is

noted that some types of LAB have greater UV resistance than *E. coli* and are unable to create mutant variants with increased synthesis of the substances needed by the researcher [86]. Chemical mutagenesis can increase the production level of many products, e.g., lactic acid or riboflavin [87, 88]. However, induced mutagenesis is not controlled and in many cases leads to the induction of unwanted mutations together with prolonged strain selection. Nowadays, these methods are considered outdated and almost not used [84].

2.2.2. Using cryptic plasmids to create recombinant probiotics

Food-grade vectors were developed to meet industrial needs for recombinant products. They (vectors) are constructed using the DNA of GRAS microorganisms. The main requirement for such vectors is the absence of antibiotic resistance markers. It can be said that LAB were the first microorganisms to be modified by such vectors. The name of these vectors is cryptic plasmid – an extrachromosomal DNA element in the form of a double-stranded DNA molecule closed in a ring [85, 86].

LAB have a large number of cryptic plasmids that are small in size and can show high resistance to *in vitro* manipulations. In addition, they contain genes necessary for plasmid replication and mobilization, and have no apparent effect on the host phenotype [85]. At this time, many critical LAB plasmids were isolated and used to improve LAB properties [86]. For instance, these are plasmids from the following LAB species: *Lc. lactis* (pWV01, pSH71, pD125, pLC2.1], *Str. thermophilus* (pA2, pA33, pSt04, pJ34, LeJ2, p4028, pRS1) [85, 86]. A large number of plasmids were also isolated from other probiotic genera, e.g., *Lactobacillus*, *Pediococcus*, and *Bifidobacterium* [85]. In general, the use of cryptic plasmids improves some properties of LAB, such as: resistance to bacteriophages, resistance to bacteriocins (e.g., nisin), acquisition of resistance to heavy metals (e.g., cadmium, copper), utilization of other carbon sources (e.g., lactose). [85, 86]. A striking example is the enhancement of bacteriophage resistance via conjugative transfer of the cryptic bacteriophage resistance plasmid pCI1750 from *Lc. lactis* UC653 to *Lc. lactis* CHCC1915 and CHCC1916 [86].

2.2.3. Using recombination to create recombinant probiotics

The main problem with the use of cryptic plasmids lies in the unsuitability of the created strains to be used in industrial conditions, mainly due to structural and mitotic instability [85]. Therefore, integrative "suicide" vectors and expression vectors containing regulatory regions, which also have "food-grade" status, were proposed to replace cryptic plasmids.

Integrative gene cloning leads to specific insertion of the gene into the bacterial chromosome without the need for antibiotics or other resistance markers [85, 86]. When integrating vector systems into the host chromosome, both homologous and site-specific recombinations are used. A common strategy for homologous recombination is to establish regions of homology between foreign DNA and a region of cognate DNA for insertion into the chromosome [81, 84]. For site-specific recombination, the most typical example is the integration of bacteriophages into the host chromosome, which occurs between the attB site in the bacterial chromosome and the bacteriophage attP attachment site [81, 85].

It is the use of different types of recombination that makes it possible to integrate certain specific genes, e.g., the human IL-10 gene into the *Lc. Lactis* chromosome.[89], or the aggregation promoting factor (apf) gene from *Lb. crispatus* to *Lcb. paracasei*, which provides the production of antibodies directed against rotavirus [90]. The use of LAB modified by homologous or site-specific recombination is quite promising for therapeutic use [86]. An additional advantage for using recombination on LAB is the availability of previous studies on many species: *Lc. lactis*, *Lmb. reuteri*, *Lb. gasseri*, *Lcb. casei* and *Lpb. plantarum* [83].

2.2.4. Using CRISPR-Cas9 to create recombinant probiotics

CRISPR-Cas systems are adaptive immune systems found in bacteria and archaea that are used as programmable tools for genome editing in eukaryotic and prokaryotic cells [84, 85]. It is the Cas9-DNA endonuclease of the type II CRISPR-Cas system that is the most widely used among the Cas family proteins [84]. Cas9 can create "blunt" double-stranded DNA breaks that cannot be repaired in prokaryotes, which is a powerful tool against selection in wild-type cells [85]. Today, the system is used in LAB for *Lmb. reuteri*, *Lpb. plantarum* and *Lc. lactis*. Cas9 was also used to remove large mobile genetic elements in *Str. thermophilus* and *Lc. lactis* [83, 85]. Another example of using the CRISPR system is the regulation of gene expression by CRISPRi interference with catalytically inactive variants of Cas9. For instance, transcriptional regulation using CRISPRi was reported to be used [80].

2.3. Systems biology approaches for the creation of recombinant probiotics

Nowadays, in addition to *in vivo* and *in vitro* studies, there are opportunities to combine computer methods for the development and introduction of new bioactive properties of probiotics and their subsequent use for a positive effect on the human microbiome or as "cell factories." There are numerous *in silico* methodological approaches that help to pre-model cellular metabolic pathways and improve certain biosynthetic properties of cells that can be used by different probiotics.

A rather important approach is "metabolic engineering" based on the analysis of metabolic pathways of a microbial cell and metabolic flux [85]. Given the well-studied genome of LAB and other types of probiotics, it is possible to modify their metabolic pathways for the purpose of synthesizing BACs useful for human (e.g., exopolysaccharides, alanine, sorbitol, lactic acid, antimicrobial compounds) [87]. For instance, such a comprehensively studied microorganism as *Lc. lactis* is considered as a "cell factory" for the synthesis of enzymes, bacteriocins and a model organism for *in silico* construction [91]. Other probiotics, which are metabolic models and can be used for *in silico* construction, belong to the *Lactobacillus*, *Enterococcus*, *Bacillus*, *Bifidobacterium* genera along with the well-studied *E. coli* microorganism. These models are important tools for the identification and further development of probiotic strains with increased properties for the synthesis of final products and their use as producers of various BACs [85].

3. Use of modern recombinant probiotics in therapeutic practice

Since probiotics have several mechanisms of positive influence on the health of the host, the creation of genetically engineered probiotics with increased properties of such influence will in the long run make it possible to use them directly for preventive and therapeutic purposes [92]. Improvements in genetic engineering methods will expand the range of microorganisms that can be used as effective probiotics [82, 93]. Although there is no direct use of recombinant probiotics in clinical practice today, modern research gives reason to believe that a qualitatively new generation of genetically modified probiotic strains is promising for their wide practical use in medical practice for the prevention and treatment of some infectious diseases. For example, the following medical areas of use are possible: treatment of GI disorders [79, 94], as vaccines [82, 92], as a means for immunomodulation [92], for disease diagnosis, as a "cell factory" to produce useful BACs, etc. [17, 18, 92].

It should be noted that the opinions of medical scientists regarding the so-called "therapeutic" use of recombinant probiotics are not unanimous.

It is believed that the use of recombinant probiotics for therapeutic purposes is usually considered simultaneously – both as the delivery of a certain therapeutically valuable BAC and the synthesis of this BAC (e.g., a certain antimicrobial peptide). Therefore, in some scientific works, the same recombinant probiotic is indicated both as a synthetic BAC and as a means of delivery. Either in the case of vaccines or antimicrobial peptides, such probiotic can simultaneously be considered as a synthetic BAC (it is a synthetic), as a means of delivery (the synthesis takes place *in situ* conditions), as a vaccine (in most cases some immunity is acquired), as antimicrobial drugs (in general).

Due to such variation of opinions regarding the lack of a unified classification, we will not further divide recombinant probiotics into the categories by means of delivery or synthetics *in situ* conditions but classify them by the use in certain organ systems or diseases of certain organ systems. This is what we had in mind when writing the paragraphs that recombinant probiotics can be considered as multifunctional therapeutic agents and the most accurate classification is based on therapeutic effects, which was used later.

For instance, it is stated that an antimicrobial peptide synthesized by a certain recombinant probiotic ensures the delivery of this BAC and its synthesis "on the spot." In the case of using recombinant probiotics as a vaccine preparation, they (probiotics) are considered as a delivery tool (sometimes an adjuvant) for a specific antigen.

In this review, we tried to link diseases of various human organ systems with the possible promising use of recombinant probiotics for the prevention and treatment of such systems, the separation of diseases of certain organ systems, and to elucidate other possibilities for the use of recombinant probiotics.

3.1. Recombinant probiotics as a means for the treatment of gastrointestinal diseases

Diseases of the digestive system are quite common and have a significant impact on people's lives. Conventional probiotics are successfully used to treat or prevent gastrointestinal diseases, espe-

cially those related to the gastrointestinal microbiota disorders [21].

Among probiotics, *Lc. lactis* is a well-known microorganism that is often used for modifications. *Lc. lactis* recombinant strains have the ability to biosynthesize a variety of anti-inflammatory proteins, and certain strains have been tested in preclinical and clinical experiments for the treatment of certain inflammatory bowel diseases [21, 95]. In addition to genetically modified strains of *Lc. lactis*, there are other representatives of the LAB, whose waste products have enhanced anti-inflammatory properties, e.g., *Lcb. casei*, *Lpb. plantarum* and *Str. thermophilus* recombinant strains [95].

3.1.1. Recombinant probiotics for the treatment of inflammatory bowel disease and related diseases

Thus, due to genetic engineering, wild-type strains of *Lc. lactis* acquired the ability to biosynthesize the KatE enzyme, or heme catalase, which is a natural producer of *B. subtilis*. The enzyme made the survival of *Lc. lactis* possible under the conditions of oxidative stress, i.e., an antioxidant strain that can synthesize catalase (an anti-inflammatory enzyme) was created [96]. Another similar study is the creation of synthetic strains of manganese-dependent catalase (MnKat) based on *Lcb. casei*. Hence, adding *Lcb. casei* BL23 recombinant strain to the mice diet with DSS-induced colitis (DSS – sodium dextran sulfate) had an anti-inflammatory effect, which indicated a protective effect of this modified strain [97]. Overall, studies suggest that increased synthesis of antioxidant enzymes by various LAB (superoxide dismutase (SOD) and various catalases) reduces reactive oxygen species (ROS), inflammation in colitis models and inflammatory bowel disease (IBD) [94, 97], increases the survival levels of modified LAB [98], which enables the creation of both resistant probiotic strains and probiotics as a "delivery tool" of anti-inflammatory enzymes. It is believed that *Lc. lactis* transformed with the *pprI* gene significantly improved the resistance to high salt concentrations and oxidative stress due to the regulation of genes responsible for resistance (e.g., the SOD encoding gene). It is believed that the use of *Llb. sakei* transfected with the *pprI* gene has the potential to be used for the prevention and treatment of radiation-induced enteritis [99].

Other studies include the creation of recombinant strains based on *Lc. lactis*, which can produce pancreatin-associated protein (PAP). This protein is a C-type lectin that usually has an anti-inflammatory effect and can reduce the severity of colitis and IBD-type diseases. The use of *Lc. lactis* strains with the ability to produce PAP (LL-PAP for short) showed a protective role through microbiota modulation in mice with DSS-induced colitis. In addition, a decrease in pro-inflammatory cytokines, which causes IBD progression, was found [100]. A convergent study using the PAP-secreting strain *Lc. lactis* also shows the possibility of preventing dysbiosis caused by 5-fluorouracil, which is used as a drug for chemotherapy. Mice that consumed LL-PAP were observed to have some changes in the composition of the microbiota: the number of microorganisms of *the Anaerotruncus*, *Corynebacterium and Enterobacteriaceae* genera in animals with mucositis decreased, while the number of bacteria of the *Akkermansia*, *Bilophila*, *Dehalobacterium*, *Desulfovibrio*, *Desulfovibrionaceae*, *Parabacteroides*, *Peptococceae* genera simultaneously increased [101].

Another method of reducing the manifestations and treatment of IBD, colitis and other inflammatory diseases – the creation of recombinant strains of LAB that can synthesize different interleukins (IL). An important IL is IL-10, which controls excessive immune response and reduces immunopathologies. This interleukin is synthesized by *Lcb. casei* recombinant strains. During *in vivo* mouse tests with an increase in IL synthesis, a decrease in the symptoms of DSS-colitis was observed. A possible mechanism of this phenomenon is the blocking of the NF-κB pathway [102]. A synthetic strain-producer of human IL-10 (hIL10) based on *Lc. lactis* was also created. Interestingly, the hIL10 gene was inserted in a place of the thymidalate synthase (*thyA*) gene. Thus, the accumulation of the gene-modified strain in the environment was prevented by excision of the *thyA* gene and insertion of hIL10, since it is known that bacteria deficient in this gene cannot accumulate in the environment [89]. There are also studies on the production of human IL-10 using genetically engineered *Bif. bifidum*. Compared to other probiotics ("food-grade bacteria"), the created strain produced the highest amount of the target product, which can be used for the treatment of various diseases [103]. It was shown that recombinant cathelicidin-related antimicrobial peptide (CRAMP), which is produced by *Lc. Lactis* recombinant strains, regulates the cytokine profile, reduces the production of IL-6, IL-1β, TNF-α, increases the production of IL-10. During *in vivo* mouse tests, it was established that two *Lc. lactis* NZ9000 recombinant strains, which produced CRAMP, protected mice from DSS-colitis by suppressing p-p38/NF-κB p-p65 signals,

due to which the detected regulation of the cytokine profile was achieved [104].

In addition to LAB, the non-pathogenic *E. coli* Nissle 1917 strain is actively used in the creation of recombinant probiotics for the treatment of IBD. *E. coli* Nissle 1917 recombinant strains can synthesize IL-10, contribute to the IL-10 accumulation in blood plasma in mouse studies, and reduce inflammation processes in DSS-colitis [105].

3.1.2. Recombinant probiotics for the treatment of infectious diseases of the gastrointestinal tract

Another concept of using recombinant probiotics for the treatment of gastrointestinal diseases is the prevention of infectious diseases of the gastrointestinal tract, which can be caused by such pathogenic microorganisms as *Vibrio cholera*, *Staphylococcus aureus*, *Clostridioides difficile*, etc., or other eukaryotic pathogens.

For the treatment of *V. cholera*, a protocol based on the consumption of recombinant probiotics with mimicry ganglioside GM_1 receptors on the cell surface was developed. It is the GM_1 receptors that have the ability to bind cholera toxin in large quantities. A recombinant probiotic based on *E. coli*, which can bind cholera toxin *in vitro*, was created. *In vivo* mouse studies demonstrate the ability of *E. coli* recombinant strain to reduce the mortality of infected mice, which was achieved by introducing *Campylobacter jejuni* glycosyltransferase genes and the *E coli* GalNAc-4-epimerase gene into the strain. In turn, such manipulations ensured the modification of the lipopolysaccharide of the recombinant strain, which had an imitation of the GM_1 receptor [106]. Another approach was the transformation of the probiotic strain *E. coli* Nissle 1917 to express the SAI-1 cholera autoinducer. In this way, a reduction in the expression of cholera toxin and toxin-conjugated pili in Caco-2 human epithelial cells was achieved [107] together with using an engineered strain in mice [108].

St. aureus is a microorganism with which a large number of the above-mentioned probiotics, such as LAB, compete. Preventing infections caused by this pathogen involves the strategy of the insertion of genes encoding various bacteriocins, such as lysostaphin (a glycine-zinc endopeptidase that can cleave staphylococcal cell walls). For example, enzyme synthesis by *E. coli* recombinant strains [109] and *Pichia pastoris* yeast [110] is possible. However, *in vivo* studies were not conducted. It is also known that *Lcb. rhamnosus* GG recombinant strains produce the SpaC protein (responsible for mucus binding), inhibit staphylococcal adhesion to keratinocytes, improve survival after pathogen infection [111].

Cl. difficile causes fairly persistent infections, whereas most antibiotic treatments are ineffective. The introduction of such probiotics as *Bif. breve*, *Lcb. rhamnosus*, *Lcb. casei*, *and S. boulardii*, either alone or together with antibiotic therapy, showed some treatment success in living models [112]. Today, there are studies on the creation of recombinant strains based on *Lb. acidophilus* and *Lcb. casei*, which contains a chimeric toxin *Cl. difficile* – surface layer protein SlpA. Such recombinant probiotics provide protection against infection and reduce the mortality of hamsters in *in vivo* studies [113].

Recombinant probiotics can be used to treat and prevent enterotoxinogenic diarrhea caused by *E. coli*. The study [114] used a non-pathogenic *E. coli* strain, on the basis of which a recombinant strain was created by inserting glycosyltransferase genes from *Neisseria meningitidis* and *Campylobacter jejuni*. As a result, a microorganism that produces a chimeric polysaccharide that has the ability to bind the heat-labile enterotoxin of pathogenic strains of *E. coli* was obtained.

Certain studies indicate that unlike wild strains, *Lcb. paracasei* are recombinant strains with the ability to synthesize the Listeria adhesion protein (LAP) and successfully prevent adhesion, invasion, and transepithelial localization of *Listeria monocytogenes* (bacteria that cause infections in immunocompromised people). Such data indicate the possibility of using recombinant strains to prevent listeriosis [115].

Among the Ukrainian scientific developments is the medical drug Subalin [116] containing the *B. subtilis* 2335/105 recombinant strain that can express human α2-interferon [117]. Today, the stability of the drug is being studied and its antagonistic activity against such opportunistic and pathogenic microorganisms as *Shigella sonnei*, *Salmonella typhimurium*, *Staphylococcus aureus*, *Candida albicans*, etc. was proven [118].

3.1.3. Recombinant probiotics for the treatment of other pathological conditions of the gastrointestinal tract

It was established that *E. coli* Nissle 1917 recombinant strains, capable of epidermal growth factor (EGF) secretion in combination with the lipase domain of ABC transporter recognition, can enhance the migration of human enterocytes *in vitro* on HST-18 cultures, thus ensuring the recovery of intestinal cells. However, further studies are needed for the safe use of such strategy [119]. A potential

use of recombinant probiotics is the treatment of liver fibrosis. For such purpose, a *Lc. lactis* recombinant strain that can produce the extracellular domain of TGFβR2 (binds to the liver fibrosis promoter TGFβ1) was created. In mouse models with liver fibrosis, it was found that the supernatant of recombinant bacteria could inhibit TGFβ1-induced collagen synthesis in hepatic stellate cells. In addition, the consumption of recombinant probiotics reduced the degree of liver fibrosis, and the use of bacteria had no side effects [120].

3.2. Vaccines based on recombinant probiotics

The development of therapeutic agents based on vaccines for the treatment of infectious diseases is a rather difficult task. However, thanks to advances in the biological sciences, vaccines have significantly reduced the threat of disease transmission and improved the ability to treat complex diseases. Recombinant DNA technology has also contributed to the development of vaccines that are based on amino acids and DNA [121]. Recombinant probiotics of various species and strains are one of the tools for creating vaccines in modern conditions. They usually play the role of various synthetic antigens in the development of vaccines, so the terms "vaccine carrier" or "vaccine delivery vector" are often used, but the general term that unites these concepts is "recombinant probiotic-based vaccine" [122]. In particular, the use of recombinant probiotics holds promise for both animal and human immunization not only against certain viral pathogens, but also for immunization and protection against certain eukaryotic pathogens. It should be noted that almost the entire spectrum of probiotics is used in the study of possible immunogenic properties or creating vaccines based on recombinant probiotics.

3.2.1. Antiviral vaccines based on recombinant probiotics

At present, there are certain developments regarding the use of the *E. faecium* L3 probiotic as a bacterial vector for an oral vaccine for immunization against the H1N1 influenza virus. Hence, full-length hemagglutinin protein 2 (HA2) (L3-HA2) is mounted into the genome of the *Ec. faecium* L3 bacterial cell, or its long alpha-helix (LAH) domain together with four tandem repeats of matrix protein 2 (M2e) (L3-LAH+M2e). When tested in mice, the created prototypes of oral vaccines induced antibodies either directed at the NA protein or M2e-specific antibodies. It was the L3-LAH+M2e vaccine that provided 100% survival in experimental animals, which is promising for further development and testing a candidate vaccine for influenza prevention [123]. The possibility of creating a vaccine against the H1N1 influenza virus by integrating the neuraminidase (NA) genes into *Ec. faecium* L3 DNA is also being considered. Oral administration of such a vaccine to mice produced an increase in virus-specific serum IgG and IgA antibodies, whereas immunization provided 34% survival [124].

There are approaches to the development of vaccines against the avian influenza virus based on recombinant probiotics. These approaches are due to the inability of conventional (traditional) vaccines to protect mucous membranes against the virus. A vaccine based on *Lpb. plantarum* recombinant strains expressing the HA1 antigen of the influenza virus with an adjuvant peptide targeting DCs was constructed. Oral immunization of mice with the recombinant vaccine strain demonstrated activation of DCs in Peyer's plaques, increased amount of CD4+IFN-γ and CD8+IFN-γ cells in the liver and mesenteric lymph nodes. An increase in B220+IgA+ cells in the PP and other parts of the intestine was also observed. In addition, there was an increase in specific IgG, IgG1, IgG2a and IgA antibodies. Thus, it can be stated that recombinant strains of *Lpb. plantarum* could potentially be candidates for further development of avian influenza vaccines [125].

Various LABs are considered as rotavirus (RV) vaccine candidates. There is some information on the creation of prototypes based on *Lc. lactis* (expression of RV outer layer proteins, such as VP6, VP4, VP7), *E. coli*, *Lcb. casei* (VP4 expression) [126], and *Lpb. plantarum* (VP7 expression) [127]. In most cases, an increase was observed in mucosal IgA and serum IgG or a combination of these immunoglobulins with the use of such prototype vaccines [126, 127].

The creation of a prototype vaccine against the rabies virus (RABV) based on recombinant probiotics is known to be created. A recombinant strain based on *Lpb. plantarum* NC8 was constructed by inserting two copies of the G gene, which targets DCs. Immunogenicity and protective functions were evaluated by oral administration in mice. After three cycles of immunization, induction of specific IgG antibodies and mixed responses of Th1/Th2 cells were observed. Even though the antibody titers were normal, the developed prototype vaccine protected 60% of mice against lethal RABV infection [128].

In the studies of coronavirus infection and the initial attempts to create vaccines, it was found that the mucosa plays a major role in the transmission of the disease. Accordingly, vaccination of the mucosa may be the best approach to detect and enhance the systemic immune responses of the mucosa itself [129]. Although there are certain theoretical grounds for the use of recombinant probiotics to create a vaccine against SARS-CoV-2, there is no probiotic-based drug today, and the developments are at the stage of preclinical or clinical trials [130]. For example, the creation of a prototype vaccine against COVID-19 by inserting the S protein into *Lb. acidophilus* was reported [129]. Studies with yeast have demonstrated an increase in specific IgG and IgA in experimental mice, as well as certain modulation of the gut microbiota [131] In addition, Phase I clinical trials of the *Bif. Longum*-based vaccine expressing adhesion protein (ClinicalTrials.gov Identifier: NCT04334980) are underway [129, 130].

3.2.2. Vaccines based on recombinant probiotics against bacterial pathogens

In addition to virus-based vaccine preparations, it is possible to develop vaccines against certain bacterial pathogens, both foodborne and certain zoonotic infections [132]. There are studies on the creation of a prototype vaccine against enterohemorrhagic *E. coli* O157:H7 (EHEC). Using *Lb. acidophilus*, certain procedures of virulent EHEC proteins insertion (toxins of type III secretion system T3SS) were carried out. When tested on the LoVo cell line, the safety of such a vaccine and the inhibition of the induction of cell lesions were observed. In an *in vivo* test, oral immunization induced high levels of antibody synthesis in mice, increased synthesis of IFN-γ, IL-4, IL-10, and the response of T-helper cells, suggesting induction of humoral and cellular immunity [133]. A vaccine to combat Group B *Streptococcus* (GBS) infection is being developed. When inserting the surface immune protein (SIP) into *Lc. Lactis*, the induction of humoral (increased levels of IgA, IgG) and cellular (activation of SIP-specific T cells) immune response is observed, which was demonstrated in an experimental model of GBS vaginal colonization in mice [134]. There are approaches to create a vaccine against brucellosis based on recombinant probiotics – studies based on using recombinant *Lcb. Casei* with the Omp19 antigen insertion [135] and *Lc. lactis* strains with an integrated omp31 gene fused to the usp45 signal peptide [136]. Both studies revealed powerful immunogenic properties of the created vaccines and stimulation of humoral and cellular immune responses [135, 136]. For some time, the possibility of creating an oral vaccine against Salmonella enterica serovar Enteritidis was considered. Thus, prototypes using recombinant *Lcb. casei* strains, which express FliC (flagellin), SipC (type III secretion protein), and OmpC (Salmonella outer membrane porin) antigens were proposed. Antigen-specific immune responses and enhancement of protective immunity were observed after immunization of mice [137]. Certain strategies for the treatment of cholera using recombinant probiotics have been discussed above, so the next step to prevent *V. cholera* infection is the creation of oral vaccines. Thus, the probiotic *S. boulardii* yeast was used to express the TcpA protein toxin precursor. The resulting recombinant strain was capable of protein synthesis after 5 hours. Extra studies are needed for making further conclusions regarding the immunogenicity of the resulting strain [138]. The creation of a vaccine against *Clostridium perfringens* infection (the causative agent of necrotizing enterocolitis) and other gastrointestinal tract diseases is being considered. The need to create a vaccine against this pathogen is due to its resistance to standard antibiotics and the belief that vaccination is more effective [112]. *Lcb. casei* recombinant strains were used in the development of the vaccine, which acquired the ability to synthesize *Cl. Perfringens* α-toxin. When tested on mice, the acquisition of humoral and cellular immunity was established [139]. In addition, using *Lcb. casei*, a strain expressing α-, ε-, β1- and β2-toxins was created. An immunogenicity study showed that the vaccine triggers both humoral (synthesis of sIgA, a number of ILs) and cellular (increase in % CD4+ and CD8+ T cells) immune responses [140].

3.2.3. Development of vaccines against trichinellosis

Trichinellosis is a rather dangerous disease of food origin that is difficult to treat. Modern treatment has many side effects from the drugs used. Consequently, there is a need to create a vaccine against this disease [141, 142]. Based on *Lpb. plantarum* NC8, recombinant strains expressing two *Trichinella spiralis* antigens (protein encoding gp43 hydrolase and proteins expressed at the muscle larva stage) were constructed. Oral immunization induced high levels of specific serum IgG and mucosal sIgA in mice. In addition, an increased number of cytokines, interleukins and CD4+ type cells were indicated. After infecting immunized mice

with pathogenic larvae, there was a 75.67% decrease in the number of adult worms 7 days after infection along with encysted larvae on the tongue and masticatory muscles [142]. Another study also used *Lpb. plantarum* recombinant strains expressing fibrinonectin-binding protein (FnBPA), adult *T. spiralis* serine protease (Ts-ADpsp), and murine IL-4. Experimental mice were orally immunized 3 times with 10-day intervals, after which they were infected with *T. spiralis* larvae. Due to immunization of mice, the number of specific IgG, cytokines and T-helpers increased [141].

3.2.4. Vaccines based on recombinant probiotics for veterinary use

The development of vaccines based on recombinant probiotics is promising not only for human use but can also be applied in veterinary practice. For example, a *B. subtilis*-based vaccine is under development to prevent porcine epidemic diarrhea virus (PEDV). Thus, a recombinant strain was constructed by inserting the adhesion protein of PEDV and L-lectin-β-GF, which can improve the attachment of *B. subtilis* to M-cells of the intestine. Based on the results of mouse studies, an increase in the percentage of T cells was observed, an increase in sIgA in the intestinal mucosa of mice was detected, and an increase in anti-PEDV IgG was discovered, indicating the effectiveness of oral immunization with the developed vaccine [143]. In addition to PEDV, a vaccine targeting porcine parvovirus (or porcine parvovirus infection PPV) has been developed. The PPV structural polypeptide VP2 was embedded into *Lcb. casei* ATCC 393. A strong specific immune response against VP2 was observed when administered intragastrically to mice, and the induced antibodies demonstrated a neutralizing effect on PPV [144]. A vaccine against the transmissible gastroenteritis coronavirus (TGEV), which is the biggest threat to pig farming, was also developed on the basis of recombinant probiotics. A set of *Lpb. plantarum* recombinant strains was created with a surface bearing a spike (S) protein derived from TGEV and fused to DC. As a result, immunized animals (pigs) had increased sIgA titers in feces and IgG titers in serum, indicating the induction of an immune response. This approach can be used to further develop vaccines against TGEV [145]. The use of recombinant probiotics allows the development of a vaccine against the African swine fever virus (ASFV). To date, there is no traditional vaccine against this virus, but research in this direction continues. Hence, in *Lpb. plantarum* a protein p14.5 was incorporated, which was chosen as an antigen. After oral immunization of mice with the recombinant strain, the level of differentiation and maturity of T-lymphocytes, B-lymphocytes and dendritic cells in mice was determined by flow cytometry and ELISA. Compared to the control group, these indicators were higher. In addition, the formation of specific antibodies and sIgA was observed, which made it possible to conclude about the effectiveness of the vaccine and the improvement of humoral, mucosal, and cellular immunity, which is the basis for further development of vaccines against ASFV [146]. Efforts to develop a vaccine to prevent post-weaning diarrhea (PWD) in piglets caused by enterotoxigenic *E. coli* (ETEC) are underway. For this purpose, the *E. coli* modified strain using the CRISPR-Cas9 technology was used. After modifications, the strain had the ability to express F4 and F18 fimbriae. Immunization of mice and piglets with the recombinant strain promoted the development of an immune response against both fimbriae. Furthermore, serum antibodies of immunized animals inhibited the adhesion of ETES strains [147].

Some experimental studies on the use of recombinant probiotics as immunostimulants are conducted in fish. For instance, salmon during the breeding season can be exposed to viral diseases. Today there are not enough methods to prevent fish diseases. In this regard, *in vivo* and *in vitro* studies using recombinant *Lc. L actis* that can express type I interferon to determine the possibility of its use against infectious pancreatic necrosis virus (IPNV), which commonly affects salmon (*Salmo salar*), were carried out. Thus, it was found that a recombinant synthetic interferon strain reduces the viral load *in vitro*, whereas oral administration of the strain stimulates the antiviral immune response in adult males [148].

3.3. Recombinant probiotics for the treatment of metabolic disorders

Nowadays there are studies on the possibility of using recombinant probiotics to reduce the symptoms or complications of such diseases as diabetes of the first and second type, and obesity. Other studies indicate the possibility of treatment of human metabolic diseases, e.g., phenylketonuria.

One of the promising strategies for the treatment of obesity with the help of recombinant probiotics is the synthesis of intestinal glucagon-like peptide (GLP-1) by various types of LAB. GLP-1 and its analogues are drugs of a new generation that can modulate the metabolism of human lipids. However, such drugs can only be administered

intramuscularly due to the intestinal degradation of peptides. Therefore, recombinant strains based on *Lcb. paracasei* NFBC 338, which could synthesize a long-acting GLP-1 analog, were created. A study in obese rats showed that short-term consumption of the recombinant microorganism reduced serum lipoproteins and cholesterol, whereas long-term consumption improved insulin secretion, glucose and cholesterol metabolism [149]. The creation of *E. coli* Nissle 1917 recombinant strain capable of synthesizing the N-acylphosphatidylethanolamines (NAPE) bioactive lipids by inserting NAPE synthase genes was reported. After short-term administration of the recombinant strains, mice became resistant to diet-induced obesity [150]. *Lc. lactis* was modified to obtain *in vivo* fibroblast growth factor 21 (FGF21), which is a regulator of metabolism. After administration of the strain to obese mice, the body weight of the animals decreased compared to the control group, while the activity of brown adipose tissue increased. A study indicates the possibility of synthesis and oral administration of FGF21 by recombinant probiotics to combat obesity [151]. The *Lmb. reuteri* recombinant strain, which can express IL-22 (ameliorates the course of diseases associated with metabolic syndrome), was developed. In murine models of nonalcoholic fatty liver disease (NAFLD), the recombinant strain was found to reduce liver weight and the manifestations of NAFLD compared to the control group of animals [152].

Certain recombinant probiotics that synthesize GLP-1 are also used to treat type 2 diabetes in mouse models. These are strains of *the Bifidobacterium* and *Lactococcus* genera [153]. The *Lcb. paracasei* BL23 recombinant strain was used to produce the GLP-1 analogue for further investigation of the antidiabetic effect in rats. As a result, it was found that the recombinant probiotic stimulated the production of insulin. In addition to this, the introduction of non-modified strains also contributed to the insulinotropic effect [154]. A recombinant strain based on *Lc. lactis* NZ9000 was created to treat type 1 diabetes without obesity, which synthesizes a specific protein HSP65-6P277 (reduces the incidence of diabetes and inhibits insulitis in mice). Mouse studies showed that the oral consumption of the recombinant strain prevented hyperglycemia, improved glucose tolerance, and reduced insulitis [155].

Recombinant probiotics are also being developed to reduce the symptoms of diabetes and prevent complications of this disease. The *Lcb. Paracasei*-based strains that can synthesize human angiotensin-converting enzyme (ACE) 2 (reduces inflammation and oxidative stress, protects against cardiovascular diseases and other metabolic diseases, including diabetes) were developed. Mouse studies with two models of diabetic retinopathy indicate a positive effect of the created probiotics: a reduction in the expression of inflammatory cytokines and a loss blocking of retinal ganglion cells were observed [156]. Another study also indicates the possibility of using recombinant *Lcb. paracasei* expressing angiotensin-(1-7). Oral administration of the strain reduced the loss of retinal vascular capillaries, ganglion cells, and decreased the expression of inflammatory cytokines [157].

Recombinant probiotics capable of in situ synthesis of phenylalanine hydroxylase (PAH) are currently being developed, which can become a strategy for the treatment of phenylketonuria. In this way, the defective enzyme is replaced, and the accumulation of phenylalanine is eliminated. Hence, based on *Lpb. plantarum*, a PAH-synthetic recombinant strain was created, and co-cultivation of bacteria with Caco-2 cells showed the efficiency of enzyme production and the possibility of its transport through a monolayer of cells. Test for PAH enzymatic activity secreted by recombinant *Lpb. plantarum* indicated enzyme activity and a decrease in L-phenylalanine levels [158]. Using *Lmb. reuteri* 100-23C, a recombinant synthetic strain of phenylalanine lyase, *Anabaena variabilis*, was created. Oral administration of lyophilized bacteria was used for *in vivo* studies. In experimental mice, L-phenylalanine levels were reduced after three to four days of probiotic administration and remained significantly reduced compared to the control group [159].

Recombinant probiotics may also be used as hypotensive (or antihypertensive) agents. For example, there are known studies on the synthesis of peptides inhibiting ACE, which regulates blood pressure. *E. coli* recombinant strains were typically used for the biosynthesis of such peptides. To study the effect of probiotics *in vivo*, a recombinant strain based on *Lpb. plantarum* NC8 was created, which had the ability to produce inhibitory peptides. A study in rats showed a reduction in systolic blood pressure compared to the control group [160]. The protective effect of the *Lpb. plantarum* recombinant strain was shown on human umbilical vein endothelial cells (HUVEC). The strain had the ability to express ACE-inhibiting peptide (NC8-pSIP409-alr-ACEIR). The experimental results indicate the possibility of using the strain to protect HUVECs from the damage induced by hydrogen

peroxide. Additionally, an increase in ACE2 expression was observed. The possibility of using recombinant probiotics to reduce the rate of apoptosis of vascular endothelial cells and hypertension was demonstrated [161].

3.5. Recombinant probiotics for the treatment of allergies

It is known that probiotics are used to treat some types of allergies, such as food or allergic dermatitis. Recombinant probiotics provide much broader opportunities for overcoming these manifestations, treating allergic conditions, and creating allergy vaccines [162].

There are studies on the creation of recombinant strains to reduce immune responses in house dust mite allergy. In *Lb. acidophilus* the Der p 5 gene was inserted, which is an allergen of *Dermatophagoides pteronyssinus* (house dust mite). The resulting strain was orally administered to mice, then 21 days after sensitization, they were subjected to an inhalation challenge. The consumption of the recombinant strain was found to significantly reduce the synthesis of Der p 5-specific IgE and allergen-induced hyperreactivity [163]. With the insertion of another Der p 1 allergen in *Lpb. plantarum* NCIMB8826, the immunomodulatory properties of this strain were determined and the possibility of its use as a live vaccine carrier was revealed. In mouse studies and prophylactic intranasal administration of the strain, the prevention of a typical allergic reaction was observed due to a decrease in specific IgE and allergen-specific IgG2a. In addition, both wild-type and modified probiotics reduced airway eosinophilia after allergen aerosol exposure [164]. The use of the *E. coli* Nissle 1917 probiotic strain, which expresses Der p 1, is also considered an effective way of combating allergy caused by dust mites. Vaccination of mice with this strain prevented an allergic reaction after treatment of the respiratory tracts of experimental animals with aerosol extracts of mites. Induction of allergen-specific IgG2a response as well as decrease in IL-5 secretion were additionally observed. It was the recombinant strains that inhibited the development of eosinophilia and neutrophilia in the airways of mice [165]. Using *Lc. lactis*, prototypes of house dust mite allergy vaccines were created, which had the ability to produce the Der p 2 allergen. Oral administration of preparations of the obtained strains prevented the development of respiratory tract depression in mice sensitized to Der p 2, which was determined by weakening the infiltration of inflammatory cells in the lung tissues and reducing the level of such cytokines as IL-4 and IL-5. In addition, a decrease in serum levels of allergen-specific IgE and IL-4 was observed [166].

In addition to house dust mite allergens, pollen allergens of various plants are clinically important. In this direction, there are also attempts to use recombinant probiotics to create effective means for allergen-specific sublingual therapy [162, 167]. Sublingual administration of *Bif. bifidum* recombinant strain expressing Bet v 1 (an allergenic component of birch) enhanced the tolerance induction in mice sensitized to this allergen and suppressed airway hyperreactivity. In addition, the induction of human dendritic cells maturation *in vitro* was observed. The studied immune responses make the *Bif. bifidum* recombinant strain as an adjuvant candidate for specific immunotherapy of certain types of allergies [167]. When using *Lcb. paracasei* expressing Bet v 1 in pregnant and lactating mice, prevention of airway inflammation in the offspring was observed [168]. *Lc. lactis* and *Lpb. Plantarum* recombinants, expressing Bet v 1 *in vitro*, caused a shift of the immunological reaction towards Th1. Intranasal immunization of mice, which was followed by sensitization, demonstrated reduced airway inflammation, eosinophilia, and IL-5 levels compared to the control groups [169].

A strain of *Lc. lactis* was constructed, which expresses profilin (Che a 2) – the main allergen of *Chenopodium album*. Immunological studies of the strain indicate the possibility of binding human IgE. Nevertheless, *in vivo* studies have not been performed [170]. In addition, *Lc. lactis* was used for the expression of the Ama r 2 (*Amaranthus retroflexus*) allergen. To evaluate the effectiveness of the strain, a probiotic "ice cream" was prepared. It was established that consumption of the product significantly reduced the level of IgE, IL-4 after sensitization, compared to the control group [171]. *Lc. lactis* recombinant strain was also tested on mice, expressing the Sal k 1 allergen (*Salsola kali* or prickly saltwor). After oral sensitization with the obtained strain and subsequent treatment with the allergen, a decrease in the levels of IgE, IL-4, IgG specific cytokines was found. A study showed that Th2 immune responses in mice were reduced after immunotherapy with the recombinant strain used [172].

There are studies using not only one allergen, but also several at the same time. Thus, a recombinant strain based on *E. coli* Nissle 1917, which simultaneously expresses allergens of birch pollen and grass (a polyallergenic construct), was created. Normal mice and gnotobionts received the strain intranasally during pregnancy and lactation. The

offspring were also exposed to intranasal administration of the strain. As a result, a decrease in allergic inflammation in the offspring was observed, and prevention with the use of gnotobionts was more effective [173].

Information on the possibility of treatment or prevention of food allergies using recombinant probiotics is available. *Lc. lactis*, expressing *Helicobacter pylori* protein subunit, activates neutrophils (NapA) and has a certain therapeutic potential in food allergic diseases. Mouse studies indicate a reduction in allergy symptoms (diarrhea, intestinal inflammation) and stimulation of the secretion of a number of cytokines IgG2a, IFN-γ [174]. A fairly common type of food allergy is a peanut allergy. In order to create allergen-specific immunotherapy, various recombinant strains of *Lc. lactis* were created, producing Ara h 2 (peanut allergen) in different forms: cytoplasmic, fixed and secretory. It is the last two forms that have proven to be more effective in redirecting the Th2 immune response to non-allergenic Th1. In addition, induction of sIgA and regulatory T cells was observed upon oral administration of the strain [175]. *Lc. lactis* recombinant strain, which expresses the major allergenic buckwheat protein (Fagag1) with and without GFP tag, which allowed measuring the expression of the target protein, was also created. Mouse splenocytes treatment with the isolated recombinant protein increased the expression of allergic cytokines (IL-4, IL-13, IL-17). Such results indicate the possibility of increasing the specificity of immunological responses, however, *in vivo* studies are needed for further conclusions [176].

Another type of food allergy is milk allergy, which is why recombinant probiotics are being developed to reduce the dangerous manifestations of the disease. For example, a bovine lactoglobulin (BLG) recombinant synthetic strain based on *Lc. lactis* was created. BLH is one of the main allergens of cow's milk. After intranasal administration of recombinant strains to mice, there was a decrease in IgG1 production in serum and bronchoalveolar lavage fluids as well as a switch from Th2 to Th1 immune response [177]. A synthetic strain of β-galactosidase *Lc. lactis* MG1363/FGZW was developed to reduce the symptoms of hypolactasia. In an *in vivo* study, a reduction in allergy symptoms was observed, which correlated with an increase in colonization of the gut with bifidobacteria [178].

Among other applications of probiotic strains may be the treatment of atopic eczema and asthma. *Lcb. rhamnosus* GG and *Lcb. rhamnosus* HN001 recombinant strains were reported to have positive effects [179].

3.6. Recombinant probiotics as biosensors and "diagnostics tools"

In addition to direct therapeutic applications of recombinant probiotics, there is the possibility of their use as a diagnostic tool or for disease detection. A fairly common system of using recombinant probiotics to detect signaling molecules in the body is the synthesis of *Lc. lactis* β-lactamase, showing colorimetric shifts that indicate the presence of the causative agent of cholera. Another example is using probiotics to find formyl peptides generated by pathogens [180]. Using the method of finding the "quorum", a biosensor based on *Lmb. reuteri* was constructed, which can determine autoinducing peptide-I (AIP-I), that is, molecules produced by *Staphylococcus* sp. during the disease [181]. Biosensors were also designed to detect thiosulfate and tetrathionate, which can be formed in colitis, *Shewanella halifaxensis* and *Salmonella typhimurium* infections. Typically, such sensors use *E. coli* Nissle 1917 [93]. *Lc. lactis* recombinant strain has also been developed to detect *Ec. faecalis*. When this pathogen is detected, recombinant strains synthesize anti-enterococcal peptides that inhibit the growth and reduce the viability of bacteria. The signal for detection is the cCF10 enterococcal sex pheromone, whereas antimicrobial activity is provided by three bacteriocins: enterocin A, hyracin JM79, and enterocin P [182].

Also, biosensors can be used not only to detect certain infectious disorders or disorders of the intestinal microbiota, but also to diagnose cancer. Based on *E. coli* Nissle 1917, a diagnostic drug that can detect liver tumors *in vivo* was created. The resulting diagnostic platform contained bacteria transformed with a lacZ vector and luxCDABE integrated cassette that provided luminescent visualization of metastases [183].

Bioluminescent recombinant probiotics are being developed to monitor the intestinal microbiota. The creation of bioluminescent probiotics is achieved by inserting the green fluorescent protein gene, which was tested on various microorganisms: *Lc. lactis* subsp. *cremoris* [184, 185], *Lpb. Plantarum* [184], *Bifidobacterium* sp. [185]. Luciferase genes (luc+) are often used in relation to *E. coli*, *Lc. lactis* [186], *Lcb. casei* [187], *Lpb. plantarum* [188], etc. The method is completely safe and allows real-time monitoring of bacteria but is still only a tool for certain studies [185].

3.7. Recombinant probiotics as "cell factories" or for the synthesis of biological active compounds

Various microorganisms are quite often considered by biotechnologists as "cellular factories". Hence, with the help of recombinant DNA methods or other genetic tools the tasks set for the synthesis of the necessary BAC can be easily achieved. Probiotics were not an exception for such use. In addition, probiotics have advantages when used as a "cell factory", the main part of which is the study of the genome of most species [50, 77, 83]. In addition to the usual *E. coli* and *S. cerevisiae* biotechnology model microorganisms, more and more attention is paid to other probiotic microorganisms, such as *Lc. Lactis* and *B. subtilis* [91, 189].

It is known that genetic manipulations on LAB make it possible to use them for the biosynthesis of organic acids (lactic acid [88]), sweeteners (sorbitol, mannitol), exopolysaccharides, and vitamins (riboflavin) [87]. Various genetically engineered probiotics can be used to synthesize certain bioactive antimicrobial or antiviral peptides [190]. For instance, the synthesis of bacteriocins that inhibit the growth of *Ec. faecalis* [182] by *Lc. Lactis* recombinant strain and the synthesis of the antilisteral peptide leucocin C by genetically modified *S. boulardii* [191]. Enterocin A-producing strains, which is a bacteriocin that suppresses the growth of listeria, were also created. High antimicrobial activity is demonstrated by *Lcb. casei* mutants, which synthesize enterocin A [192].

The studied recombinant proteins of bifidobacteria have prospects for the use in pharmaceutical development or for the creation of pharmacologically active food products. These proteins are usually various enzymes: endo- and exoglycosidases, pyrophosphorylases, and glycosyltransferases. The use of genetically modified strains of various *Bifidobacterium* species to produce such enzymes as β-galactosidase, α-L-fucosidase, amylase, etc. are being considered [193]. There are studies on the creation of a phytase producer from *Bif. longum* strains. Thus, intestinal phytate degradation may be increased and phosphorus excretion will be reduced [194, 195].

The achievements in genetic engineering allow the creation of producers of various chemical substances, e.g., S-butanediol, acetone, *Lc. Lactis*-based diacetyl, 3-hydroxypropionic acid, 1,3-propanediol using *Lmb. reuteri* [195].

It is reported about the possibility of biosynthesis of conjugated linolenic acid by numerous LAB, such as: *Lc. Lactis* mutant strains and *Lcb. paracasei* [196]. The possibility of creating *E. coli* Nissle 1917 probiotic strain with the ability to biosynthesize omega-3 fatty acids, particularly eicosapentaenoic and docosahexaenoic, is being investigated as well [197].

Additionally, the possibility of γ-aminobutyric acid (GABA) synthesis by mutant probiotics is considered. There are studies on using *Bif. adolescentis* and *E. coli* Nissle 1917. The amount of GABA was 415 mM with a glutamate conversion of 92–100% for the first microorganism [198] and 17.9 g/L in the case of the second [199]. Another GABA biosynthetic is *Lc. lactis*, which synthesized up to 33.52 g/L after numerous genetic modifications [200].

Studies on the synthesis of mannolytic and chitinolytic enzymes on the surface of *Lpb. plantarum* are quite interesting, which provides a new approach for the synthesis of potentially prebiotic oligosaccharides [201]. It is also possible to produce isomaltulose using an immobilized *E. coli* recombinant strain capable of expressing *Pantoea dispersa* sucrose isomerase using the same strategy. The obtained disaccharide increased the proliferation of various probiotic strains, whereas the maximum productivity was 240 g/L [202]. According to the same strategy, there is a possibility to obtain valuable neoagarooligosaccharides prebiotics (e.g., neoagarotetrose). For this reason, the insertion of *Bt. plebeius* β-agarase genes into *S. boulardii* was used [203]. Other probiotics were modified to biosynthesize valuable polysaccharides, such as heparosan – a precursor to heparin that can be used to deliver certain drugs. To increase the yield of valuable polysaccharide, it is possible to use genetic engineering on *E. coli* Nissle 1917 [204].

Also, genetic engineering makes it possible to create strains of probiotics that can synthesize anticancer compounds. Based on *Lc. lactis*, a recombinant IL-17A synthetic strain with antitumor properties was constructed. Conducted *in vivo* studies indicate efficient synthesis of the cytokine and its biological activity [205]. A producer of 5-aminolevulinic acid based on *E. coli* Nissle 1917 was also created, which can induce specific cytotoxicity against cancer cells [3].

4. Recombinant probiotics safety and regulatory principles

Despite the large number of beneficial properties of both recombinant and conventional (natural, unmodified) probiotics, the scientific commu-

nity still does not have a consensus on the safety of any probiotics and their use as medicinal products.

For example, a joint report by WHO and FAO raises some doubts concerning the safety of some probiotics [206]. It is known that probiotics can have negative metabolic activity, cause systemic infections and inadequate immune responses, and sometimes contain antibiotic resistance genes, which can cause horizontal gene transfer [207, 208]. Considering these reasons, the new probiotic strains are recommended to be additionally tested for antibiotic resistance, toxin-forming capacity, hemolysis, and potential harmful metabolic activity [206, 207].

Genetically engineered probiotics neither currently have specific regulatory approvals from the FDA nor specific status as non-modified [84]. Using recombinant probiotics has more risks than conventional probiotics, including:

1) the problem of over-efficiency of the gastrointestinal tract colonization by a recombinant microorganism, which can lead to dysbacteriosis and displacement of natural microbiota;

2) impossibility of achieving absolute specificity for pathogens;

3) resistance to pathogens and the acquisition of new forms or resistance due to the formation of biofilms or changes in signaling molecules;

4) emergence of the problem of biocontamination with new "artificial" microorganisms [82, 112].

In addition, recombinant probiotics in most cases are tested in animal models and full clinical use requires further improvements and studies [11, 112]. Although the use of genetically modified probiotics as biotherapeutic agents is not approved today, such microorganisms still have a practical value, e.g., as a "cell factory" for the synthesis of food components or enzymes. Due to the lack of legal regulation and prohibition, the majority of probiotic microorganisms used in industry are obtained by classical strain improvement (e.g., the use of "food-grade vectors"), not by genetic engineering methods [83]. The development of genetic engineering methods and the growing number of studies on the various properties of recombinant probiotics provide prospects for the creation of legal regulation of the use of genetically engineered microorganisms both for standard biotechnological processes ("cell factories", BAC synthesis) and modern biotherapeutic drugs as well.

Conclusions

Probiotics are therapeutically valuable microorganisms that include various families of bacteria, the largest and best known of which are as follows: *Lactobacillaceae*, *Bifidobacteriaceae*, and *Streptococcaceae*. The analysis of the genera of probiotics showed that the largest share is made up of lactic acid bacteria, especially the *Lactobacillus*, *Lacticaseibacillus*, and *Streptococcus* genera. Besides this, a large group makes up the genus of non-lactic acid bacteria – *Bifidobacterium*. Due to the large number of species and strains, different probiotics have different therapeutic effects on certain human diseases and, accordingly, different mechanisms of action, among which the most famous can be as follows: immunostimulation, synthesis of biologically active compounds (bacteriocins, organic acids), and displacement of pathogenic microorganisms.

Considering the significant beneficial properties that natural probiotics have, the use of genetic engineering and other genome modifications are quite promising and aimed at achieving various beneficial effects for humans, the main of which are related to improving the properties of natural probiotics and adding special properties, e.g., the delivery of specific biologically active compounds, or the use of probiotics as *in vivo* "cell factories."

Nowadays, there is a large amount of research and scientific works on the creation of recombinant probiotics for various therapeutic applications. The largest areas of research and testing are as follows: treatment and prevention of non-infectious diseases of the gastrointestinal tract, creation of various vaccines based on recombinant probiotics, treatment of human metabolic diseases and allergic disorders. The well-studied probiotics, such as: *E. coli* Nissle 1917, *Lc. lactis*, *Lcb. casei*, *Lcb. rhamnosus* GG, *Bif. Bifidum* are most often used in these directions. In all *in vivo* studies, using mouse models, positive effects that can improve the course of a certain disease or reduce symptoms and put the disease into remission were reported. Recombinant probiotics-based vaccines research on mice models was reported to have positive effects that include increased survival in the group, triggering humoral and cellular immune responses. In addition, the use of recombinant probiotics for the treatment of the gastrointestinal tract diseases, metabolic disorders and allergies allows a local effect on the causative agent or the use of a microorganism as an *in vivo* cell factory, which will lead to a faster relief of the symptoms of the disease along with its treatment.

Recent decades have seen the interest of the scientific community and a significant amount of research on recombinant probiotics. Thus, it can be said that this is a new field that requires a more thorough, systematic approach and cooperation

with medicine. In addition, when identifying the positive effects of a certain modified probiotic, not only the positive effects, but also the side effects of its long-term consumption should be studied. Moreover, the risks of its direct therapeutic use should be considered, which would allow us to expand and accumulate knowledge about recombinant probiotics.

Interests disclosure

The authors have no conflicts of interest to declare.

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А.Д. Хабленко[1], С.Г. Даниленко[2], О.І. Яловенко[1], О.М. Дуган[1], О.І. Потемська[2], Д.С. Приходько[1]

[1]КПІ ім. Ігоря Сікорського, Київ, Україна
[2]Інститут продовольчих ресурсів НААН України, Київ, Україна

РЕКОМБІНАНТНІ ПРОБІОТИЧНІ ПРЕПАРАТИ: СУЧАСНИЙ СТАН, ПЕРСПЕКТИВИ РОЗВИТКУ ТА ЗАСТОСУВАННЯ

Стаття присвячена сучасному стану досягнень у сфері досліджень, розробки та впровадження різних видів медичних препаратів на основі рекомбінантних пробіотиків. Висвітлено переваги пробіотиків, їх сучасне використання у медицині, проаналізовано найчастіше використовувані роди та види пробіотичних мікроорганізмів. Наведено лікувально-терапевтичні активності досліджених пробіотиків. Огляд ознайомлює з різними методами створення рекомбінантних пробіотичних мікроорганізмів, серед яких як стандартні методи генної інженерії, так і підходи системної біології та нові методи з використанням системи CRISPR-Cas. Наведено спектр потенційних терапевтичних застосувань препаратів на основі рекомбінантних пробіотиків, особливу увагу приділено сучасним дослідженням зі створення нових більш ефективних рекомбінантних пробіотиків, що можуть бути використані з різними терапевтичними цілями. Зважаючи на різноманітні можливості терапевтичного застосування рекомбінантних пробіотиків та їх неоднозначну функціональність, розглянуто їх використання для потенційного лікування різних поширених захворювань людини: не- та інфекційних хвороб шлунково-кишкового тракту, метаболічних розладів, алергічних станів. Розглянуто перспективи створення різних типів вакцин на основі рекомбінантних пробіотиків та перспективи їх впровадження у медицину. Оцінено перспективи застосування рекомбінантних пробіотиків у ветеринарії, особливо для профілактики захворювань свійських тварин. Висвітлюється перспектива впровадження рекомбінантних пробіотиків як вакцин і діагностичних засобів для тестування певних хвороб та моделювання роботи травної системи людини. Окремо розглянуто ризики створення та застосування й проблематику нормативної сфери щодо використання нових рекомбінантних мікроорганізмів, які потенційно можуть потрапити в навколишнє середовище і викликати непередбачені ситуації.

Ключові слова: генна інженерія; біотерапія; живі мікроорганізми; cell-factory; метаболічні розлади; імуностимулятори; пробіотичні вакцини.