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Therapeutic potential of modern probiotics

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This discussion article adduces a short review of clinical studies where the use of probiotics has shown the best results. The issues of whether probiotics are capable of having a protective effect on the intestinal barrier, an antagonist effect – on opportunistic microbes and a stimulatory effect – on the immune system, are discussed. The best studied production species and strains forming probiotic drugs are observed. The differences between monostrain and multispecies probiotics are given. The reasonability of using combined drugs with combinational additive or synergetic strain-specific effects is explained.

Keywords: *probiotics, intestinal microbiocenosis, bifidus bacteria, lactobacilli, monostrain, multistrain, multispecies drugs.*

As reported, key mechanisms involved in microflora disorders tend to be selective targets for different biological methods of exposure.

The use of the microgerm known as probiotic is considered to be one of the practical approaches to regulatory system recovery [1−9].

Probiotic (from Greek προ и βίοτοç “for life”). Experts from the World Health Organization give the following definition: a probiotic is a live microorganism. Probiotics are live microorganisms which, when used in the necessary amount, have beneficial effect on the health of the host organism [10]. According to the data of completed research, probiotic effect can be provided not only by viable or diminished cell (for instance radiated) but also by structural components of the non-viable bacteria (short DNA sequences, peptidoglycane, lipoteichoic acid) [11−16]. It is obvious that there is reason to expand the modern definition of “probiotic”.

Mechnikov I.I., the founder or probiotic concept, has been awarded Nobel Prize in Medicine in 1908 for his series of works [17]. Since then there has been made a sufficient number of studies of micro-organisms (tb. 1), that could be used in everyday medical practice for

pharmaceutical products and functional foods [15, 18]; however, only a few are officially recognized as such. The main criteria for this are the phenotypic, genetic characteristics and availability of probiotic effect, established in double-blind placebo-controlled studies. More accurate evidence has been obtained for *Bifidobacterium lactis, Bifidobacterium longum, Bifidobacterium infantis, Lactobacillus acidophilus, Lactobacillus* GG*, Lactobacillus reuteri, Lactobacillus casei, Strepto (Entero-) coccus faecium* SF68*, Streptococcus thermophilus, Saccharomyces boulardii.*

It is rational to highlight that the World Health Organization, the US Federation for quality control of food and drugs (FDA), the UN Food and Agriculture Organization (FAO) and other large public and expert groups conclude that probiotics are generally considered absolutely safe and, having a GRAS status (generally recognized as safe), can be put to use without limitations in food and pharmaceutical industry [10, 19].

The abovementioned microorganisms are a part of the pharmaceutical market of medical drugs with different product description: tablets, liquid suspensions, capsules, rectal and vaginal suppositories, ointments and creams.

There is an expanded production of biological forms of drugs in the complexation with interferon, immunoglobulins and vitamins. One of the promising areas today is creation of new generation of probiotics – multivalent or combined with immobilized bacteria of a different taxonomic unit on the assumption that probiotic strains must be biocompatible and have synergistic effect [20].

Our interest and knowledge in sphere of clinical use of probiotics is constantly growing. The accumulated convincing information shows that probiotics penetrating intestinal canal change its microfloral structure and function [5−9, 21−36]. According to what we know, there are three lines of clinical and model determinations contributing to the study of the biological effects of probiotics. It should be noted that strengthening of the scientific base and the need for well-designed and conducted studies of specific factors that are critical in the probiotic therapy are both of major importance.

Tb. 2 shows systemized directions and key questions about the role of probiotic organisms in the development of antibacterial effect, reinforcement of barrier function of epithelial tissue and modulation of immune response [15].

Probiotic intensification of barrier function of epithelial tissue. There is evidence that *S. thermophilus* and *L. acidophilus* inhibited the adhesion and invasion of human enteroinvasive *Escherichia coli.* In epithelial cells, which contacted with those probiotic bacteria, scientists observe an increase in actinine and occludin phosphorylation in dense cell connections. [37].

In Caco-2 cell culture there was a strengthening or weakening of dense cell contacts in response to the cell-free supernatants varying in content of released elements. It is found that *E. coli* O157:H7 significantly increased the permeability of Caco-2 cells and released elements, while *B. Lactis* significantly reduced it [38]. *Lactobacillus rhamnosus* GG prevented cytokine-induced apoptosis in intestinal epithelial cell model by inhibiting activation of proapoptotic p38/ mutagen activating protein kinase [19].

As has been mentioned, the intestinal epithelium is covered by a transparent viscoelastic gel adhered to the mucosa. Ultrastructure of mucus is presented by muco-glycoprotein polymeric compounds. Mucus is considered to be one of the main factors controlling the intestinal microbiocenosis. Recent studies demonstrate that *L. rhamnosus* (GG) and *Lactobacillus plantarum* boost *in vitro* mucin gene expression (*MUC2*, *MUC3*) in colonocytes HT-29, which are able to inhibit the adhesion of pathogenic bacteria [39].

It was shown that the adhesion process of lactobacilli and bifidus bacteria to the intestinal epithelium is implemented by homofimbrial structures and certain components of the cytoderm (lipoteichoic acid, proteins and outer membrane phospholipids) [34, 36]. Nowadays there is objective evidence that *L. rhamnosus* (GG) has a specific feature of interacting with enterocytes: it concerns the mucin-binding pilidium – distinctive pilomotor structures (pili structure), with the help of which *LGG* can be firmly attached to the intestinal mucosa [40].

Moreover, during the study of strain-specific capacity of probiotics by comparing Nucleotide genomes it was found that only one of the LGG genomic "islands" encodes the synthesis of the three LPXTG-like adhesive mucin-binding pili and pili-associated sortase [18].

These materials show good LGG strain adhesive properties to the enterocytes, which provide extremely high capacity for probiotic transient colonization and therefore adequate stimulation of the immune function of the gastrointestinal tract.

Antibacterial probiotic effect. The antiseptic properties of probiotics are associated with the production of anti-microbial factors: organic acids and bacteriocins inhibitory proteins. It is important to mention that organic acids appear to be the "weak acids"; more than 90% of their molecules in the bowel lumen are in the anion dissociated form. These natural metabolites are important for regulating metabolism and absorption in the colon. Under certain physiological and pathological conditions they initially rapidly penetrate through the membrane tentative-pathogenic (pathogenic) bacteria that colonize the intestinal mucosa, alter intracellular pH, reduce the potential energy, and accumulate toxic anions lead to ultrastructural defects of bacterial cells, which ultimately suppresses its vital functions [41, 42]. There is evidence that the inhibitory effect of organic acids directly depends on the pH index; still, there is evidence that at low values stronger antimicrobial activity is observed in lactic and propionic acid, at pH > 4.5 -

in acetic acid [32, 35]. Potential activity in respect to the major tentative-pathogenic microorganisms of such substances as hydrogen peroxide, diacetyl and bacteriocins is a well-known fact. It was emphasized that high bacteriocins inhibit the closely related species of bacteria that live in the same biotope, and microcins (low molecular weight metabolites) have a wide range of antimicrobial activity, and therefore have more significant bacteriostatic action [35, 41−46].

The involvement of nitric oxide (NO) in the development of physiological and pathological conditions is also a dynamic theme of investigations. Many testings show that NO is related to a key signal molecules of gastrointestinal tract; it is synthesized not by the cells of the human body, but also by some commensal germs (*E. coli*, *Lactobacillus*) [47, 48]. NO cytotoxic effect is enhanced by association with the acidic environment (bifidus bacteria lower pH down to 5.0, lactobacillus – down to 4.0). Only in this case nitrites can be generated – these are highly toxic endogenous metabolites disrupting the normal functioning of many tentative-pathogenic and pathogenic microorganisms, besides the ability of normal microflora to be resistant to these compounds has been proved [49]. The peculiarity of nitrites lies also in their ability to potentiate the simultaneous antibacterial effect of hydrogen peroxide and lactic acid bacteria which are formed by saccharolytic bacteria [48].

Probiotic mechanisms of immunomodulation. Special place is occupied by studies examining the capability of probiotics to affect immunological recovery of body using such physiological processes as improvement of the functional capacity of engulfing cells and cytotoxic activity of macrophages, stimulation of the gut-associated lymphoid tissue and the effect on the immunocompetent T and B cells [25−31, 34].

To date, three main paths of physiologic immune response are discussed. The first one is manifested in the fact that adhesion of probiotic bacteria to the epithelial cells of the intestinal biotype causes the release of cytokines, trapped dendritic cells. Thus, intestinal canal epithelial cells play crucial role in processing the signals that act on the common signaling pathways. Passage of probiotics in the gut lumen can be enough for implementing intercellular communication [50]. The second way is also connected with the mechanisms of cellular effects and consists in the following: M cells in the follicle-associated epithelium of the Peyer's patches at the surface provide delivery of probiotic bacteria in subepithelial area for subsequent contact with immune cells (macrophages and dendritic cells). There they are recognized by receptors (TLR, CLR, NLR); this leads to the release of cytokines and the expression of co-stimulatory molecules for T cells [11]. The third way is the connection of microorganisms pushed into the lumen appendages of dendritic cells located in the mucous coat [11].

The dialectics of complex relationship between the state of intestinal microflora and production of secretory immunoglobulin is of particular interest. Joint data show that stimulation of immunoglobulin (Ig) is accompanied by the increased expression of adhesion receptors and bactericidal activity, thereby forming specific protection. It is assumed that secretory immunoglobulins play an important role in the local immune response. For instance, IgA1 - hapten-specific antibodies with heavy chains having affinity with mucositis ensure the formation of an immunoglobulin monolayer on the surface of mucous coat. Other Ig (subclass A2), having no relationship with the mucous membrane, migrate into the lumen and provide the first line of immune defense against infection. It should be taken into consideration that process-specific adhesion of tentative-pathogenic and pathogenic microorganisms to the mucosa may be inhibited by the presence of other factors, like presence of IgA and lysozyme, which in turn contribute to receptors' adhesion of bifidus bacteria and lactobacilli [39]. More detailed research of IgA role in prevention of the mucosal coat colonization by extraneous bacteria assumed that more than 99% of bifidus bacteria and lactobacilli are not covered by secretory immunoglobulin. On the contrary, the surface of Enterobacteriaceae, staphylococci, and other opportunistic and saprophytic microorganisms is completely lined with IgA. The available information suggests that immunological tolerance of normal flora is in the basis of this phenomenon.

Important advantage of microflora in the development of immune response should be considered at some point as its universal immunomodulatory effects, including both immune stimulation and immunosuppression [51, 52].

According to the recent experimental studies, probiotics can be attributed to antiendotoxin means. It has been convincingly showed that bifidus bacteria have endotoxin binding capacity; they also reduce endotoxin dependent induction and interleukin (IL) 8 release [39]. There exists another point of view that bacterial lipopolysaccharide and peptidoglycan that are part of different normal flora strains have immunoregulatory effects. In line, it was found that the key meaning of antiendoxive immunity consists not in absolute body protection against endotoxin, but in limitation of its concentration and biological activity to the level required for the physiological functioning of the immune system [53].

The best-studied industrial species and strains in the composition of probiotics. It is obvious that prescription of prebiotic medications should be viewed as pathogenetically justified under different conditions and illnesses, so the range of their application is clearly defined: acute stage of the disease, periods of recovery and prevention therapy. The purpose of such a therapeutic intervention is the *proved ability* of probiotics to exert a protective effect on the intestinal barrier, antagonistic action – on tentative-pathogenic microorganisms and stimulatory action – on the immune system [54−56].

It should be reminded that the term “evidence-based medicine” was introduced in 1990 by a group of Canadian scientists from McMaster University (Toronto) and it suggests such approach to medical practice in which decisions on the use of medical preventive and diagnostic measures are taken on the basis of available evidence of their effectiveness and safety.

In this context, we emphasize that systematic review, meta-analysis and randomized comparative clinical trials are of the best evidence, in contrast to other research options (non-randomized comparative studies, prospective comparative surveillance studies, retrospective comparative surveillance studies, non-comparative study and expert knowledge). The *in vitro* studies conducted on animal models are certainly important for determining clinical strategy, but they are not sufficient for approving probiotics’ utility for human health. Tb. 3 summarizes clinical studies data in which the use of probiotics rendered better results [57−74].

Recommendation list of basic probiotics includes drugs containing the representatives of only one bacteria kind (monostrain), association strains of one (multistrain) or several types of microorganisms (multi-species), self-eliminating antagonists, combined probiotics and synbiotics. According to numerous scientific testimonies, positive effect on human health can be attributed only to an exact strain (strains), but not species or a group of probiotics [3, 75].

In recent years, the science literature provides general opinion about a probiotics’ viable usage in clinical practice, however, according to the recent meta-analyses, medications based on lactobacilli and bifidus bacteria do not always have a positive effect on the bacillary environment and biotransformation in the large intestine. Exogenous probiotic survivability changes under the influence of hydrochloric acid of gastric juice, bile acids and digestive enzymes.

The study of these problems showed that different strains of microorganisms’ survival rate is estimated at 20-40% [3]. For better understanding of these events one should consider the recent information about the presence of probiotic resistance to an acid-base healthy environment. *In vitro* tests showed the 3-5 ordinal reduction in the number of viable bifidus bacteria and lactobacilli firstly in the acid, then – in an acid-based healthy environment simulating human digestion [76]. Subsequently, these data were confirmed by *in vitro* experiments, in which the model environment was used instead of gastric juice and human duodenal contents. Completed studies have shown that the number of probiotic microorganisms is reduced to hundreds of microbial cells [77]. The results of previous research have been confirmed by direct experiments on animals using labeled probiotics [78]. The final conclusion can be presented as follows: it is necessary to use additional forms of probiotic protection, such as acid-resistant capsules or sorbents to keep probiotic potential, which may be leveled by a number of factors (antibiotics, acidic, acid-base environment, digestive secretions of the gastrointestinal tract, etc.) [77, 79].

Fundamentally important from a practical point of view is the question of the optimal single and course doses of probiotic intake. With the expansion of the range of drugs used, we accumulated a lot of evidence that probiotics in high doses and during long therapy courses may lead to side effects [80−84]. For the first time there is evidence that with intrajejunal administration of high doses of probiotic microorganisms test animals have malconditions that in some cases lead to death [80]. There have been several publications indicating that exceeding the daily dose of certified probiotic preparations 5, 10, 100 times is accompanied by progressive increase of dead lymphocytes [81]. Medical practice confirms the ability of different probiotics when used in excessive doses to induce cytokine imbalance, which is manifested by fever, arthritis, hepatitis, increased actual or manifestation of latent autoimmune disorders [85−87]. These proofs make us think of optimizing the duration of probiotic therapy and the possibility of its individualization.

The next important step in the study of positive effects of probiotics is studies comparing features of monostrain, multistrain and multi-species drugs. There is a debatable question of the optimal probiotic culture, which, according to experts, should be mixed. Mixed probiotic strains complement each other's actions on the human body, that is, exhibit synergistic properties. Many studies have served as basis for such a statement, showing that intestinal microbiocenosis is a complex association of bacteria, so topical application with the adhesion will be more successful in multi-species probiotic strain [88−90]. However, one should bear in mind the well-known feature of modern diseases, which is their multi-factor ability to develop. It becomes evident on this premise that rationally combined probiotics with a wide range of physiological effects should be offered as drugs of choice [8, 87]. Such probiotics provide a fundamentally new opportunity to prevent or reduce the risk of multifactor diseases, as probiotic properties are strain-specified [86].

As a practical illustration, we can cite a study made by G. Zoppi et al. [91], who examined the efficacy and effects of 6 commercial probiotic drugs on intestinal microbiocenosis. More than 50 children treated with the help of ceftriaxone therapy were put under the supervision. Probiotic lyophilized drugs in capsules or sachets were prescribed as additional. Three monostrain probiotics were used: *S. boulardii*, *E. faecium* SF68 and *L. rhamnosus* GG. Among multistrain probiotics studies were conducted of a multistrain drug, containing three different strains of lactobacilli: *L. rhamnosus* GG + *L. acidophilus* + *Lactobacillus bifidus*; multistrain drug, containing two strains of lactic acid bacteria: *Bifidobacterium bifidus* + *L. acidophilus* and multi-species probiotic under the title VSL#3, comprised of a high concentration of bacteria belonging to 9 different strains: *S. thermophilus*, *E. faecium*, *Bifidobacterium breve*, *B. infantis*, *B. longum*, *L. acidophilus*, *L. plantarum*, *L. casei*, *Lactobacillus delbrueckii,* ssp of

bulgaricus. In accordance with the received data, *S. boulardii* prescription does not lead to the restoration of the intestinal microbiocenosis. *E. faecium* SF68 treatment did not eliminate, but exacerbated the existence of dysbiosis aggravated by increase in the number of anaerobic cocci. Only L. rhamnosus GG showed high activity towards the major tentative-pathogenic microorganisms. As it turned out, in groups where S. boulardii and L. rhamnosus GG were applied there was an increase in the potential risk of bacterial resistance to β-lactam antibiotics: in particular, the number of samples with positive results to presence of β-lactamase rose to 83%. All the studied probiotics led to a decrease in fecal pH and only for multi-species this result was statistically significant. The latter fact should be regarded as positive, as the acidic environment inhibits the growth of pathogenic microorganisms and reduces the activity of proteolytic bacteria. The proof of success of probiotics of multi-species treatment of antibiotic-associated dysbiosis is the data, showing a significant reduction in the risk of gastrointestinal symptoms, normalization of aerobic/anaerobic microbial populations' balance and the absence of antibiotic resistance of fecal microflora. Hence, multistrain probiotics are more promising than traditional monostrain ones, because they show high effectiveness in preventing antibiotic-associated dysbiosis among children. Other clinical studies have shown that prescription of multi-species probiotic VSL#3 for patients with ulcerative colitis and ileitis reduces the need in antibacterial agents and prevents the development of bacterial complications that monostrain probiotics are not capable of [12, 13, 89, 92, 93].

Another example of an experimental study of anti-infective focus of probiotics. By design, laboratory mice were randomly assigned to receive skimmed milk diluted with drinking water (control set), based on *L. acidophilus*, or *L casei,* or combinations of both strains for 8 days. Then all the animals were infected with Salmonella typhimurium, and then at various time intervals the number of viable pathogenic bacteria in the liver and spleen was microbiologically determined, serum antibody concentrations to *S. typhimurium* were analyzed; mice were observed for 21 days. It appeared that milk fermented with probiotic monostrain did not increase pathogen test resistance, although the initial survivability of animals was higher than in the set group. Fermented milk product enriched with strain L. casei caused a significant decrease in the number of Salmonella in liver and spleen on the 10^{th} day after infection and led to the better marked specific antibody production than in the set group.

At the same time, the lowest antibody titer was observed in animals treated with the *L. acidophilus.* The results are interesting, because the combination of *L. acidophilus* and *L. casei,* activating immune defense factors, contributes to the effective elimination of pathogen and creates conditions preventing the spread of acute intestinal infection in mice and premature death of laboratory animals. Thus, the period of liver and spleen debridement was 7 days, in the same

time period high values of serum antibodies to S. typhimurium were established, and by the end of the experiment the survival and recovery levels of all laboratory animals were stated [94].

Similar results were obtained in other experimental studies, according to which the most promising probiotic to demonstrate its antagonistic activity against enterotoxigenic E. coli (*E. coli* O157:H7) and salmonella (*Salmonella enteritidis*, *S. typhimurium*) appears to be multi-species probiotic drugs [95−97].

Based on the detailed review of clinical and experimental studies with analysis of multi-species probiotic drug properties (tb. 4), it was found that the latter will have the highest possible survival rates, as these rates for the ingested probiotics is different for separate genera, species and strains of bacteria [87−88]. Probiotic strains may cause local reduction in acidity of intestinal contents, creating a favorable environment for the formation of the colonization capacity, particularly of acidophilus bacteria. Certain probiotic strains have the properties of the substrates disposed to form organic acids that have a beneficial effect on the intestinal mucosa coat. For example, Lactobacilli produce lactate, which is metabolized by propionibacterium into propionic acid [98].

In vitro tests prove that some probiotic strains (*S. Thermophilus)* create anaerobic conditions which allow strict anaerobic bacteria such as bifidus bacteria reproducing on the mucosal surface and remaining viable during passage through the gastrointestinal tract [99].

Furthermore, it is known that a wide range of physiological effects of probiotics is related to their ability to adhere to mucus and epithelium of the mucous membrane of the intestines. Interesting and quite unexpected results were obtained during the *in vitro* properties' study. It was found that L. rhamnosus GG or L. delbrueckii subspecies bulgaricus increased adhesion of Bifidobacterium animalis BB12 more than twice. A similar situation was determined for Propionibacterium freudenreichii P6, which increases the adhesion more than 3 times in the presence of L. rhamnosus GG and almost twice - in the presence of B. animalis BB12 [100, 101]. These examples demonstrate that the stimulation of adhesion of one strain to another optimizes multi-species probiotics colonization process. Moreover, until now, propionibacterium representing part of the normal human microflora has never been used as a probiotic due to the low adhesiveness. The above data will critically review the quality, functional activity, synergistic effects of microorganisms belonging to the fixed multi-species probiotic combination. This can also be applied to the promising types of probiotic bacteria, including propionibacterium.

Results of a clinical study of gluten-hydrolyzing strains of probiotics (*L. acidophilus* 311, L. acidophilus 180, *L. cas*ei 925a, *L. casei* 4628, *B. longum* 17 xs and *Propionibacterium avidum* 1) in 25 patients with celiac disease have become convincing evidence. High efficiency

of such strains could be observed in comparison with therapy using commercial probiotic equivalent dose. It has been shown that multi-species strain is accompanied by a significant probiotic therapy is accompanied by significant duration decrease of clinical symptoms of the disease, gastrointestinal physiology and intestinal microbiocenosis recovery [102].

Clinical work was preceded by a trial period during which the high rate of enzymatic proteolysis of wheat gluten with proteases tested *in vitro* strains was proved [103].

Undoubtedly important is the question of the ability of probiotic bacteria to multiply in specific locations. One should take into account the functional place of probiotics when selecting "candidates", because intestinal commensals maximally display their metabolic activity, i.e. have a beneficial effect on human health, only in their particular ecological niches. The joint data shows that members of the genus Lactobacillus providing an environmental protecting barrier of the human body are widespread in the biotope of the gastrointestinal tract, but dominate in the proximal part of the small intestine, whereas bifidus bacteria are prevalent in the colon [104−106]. It is natural that priority should be given to multi-species strains or drugs.

Given the shortage of data on exact mechanisms of probiotic products' effectiveness, data on the functional activity of strains is constantly being refined; functional activity can be stimulated by possible symbiotic relationship.

It is known that *L. acidophilus* and representatives of the genus *Bifidobacterium* grow slowly in milk, because they do not decompose proteins due to the almost complete absence of bacterial proteases. Addition of typical yoghurt strains, particularly *L. delbrueckii* subspecies bulgaricus, is the most physiological way of increasing the number of source types of bacterial cells. This is partly due to the potential impact of cooperative interaction with the exchange of the products of bacterial metabolism: amino acids, peptides, free, formate and CO2 [107].

Experimental data published showed the marked increase in the level of *B. animalis* in the presence of *L. acidophilus*, which hydrolyzes milk casein using extracellular proteases, thus forming amino acids and peptides able to stimulate breeding population of *B. animalis* [108].

According to another study, analogous situation was proved in respect to the same species of bacteria, only from the other side: the growth of L. acidophilus strain was amplified by *B. animalis*, probably, due to a metabolite such as acetate [109].

These arguments suggest that symbiotic relationship in most cases occurs not on the level of species, but rather on the strain level. It is well known today that strains belonging to the genera of *Lactobacillus*, *Lactococcus*, *Streptococcus*, *Bifidobacterium* и *Propionibacterium*, demonstrate symbiotic relationship with each other, which, of course, is essential in determining the structure of communities in the microflora and metabolic activity of gut bacteria, which, if possible, should be benign.

It is advisable to emphasize that the use of combined drugs with combined additive or synergistic strain-specific effects should refer to the current tendencies in the probiotic therapy.

There is an obvious need in further research related to the creation of individual probiotics based on autostrains and autoassociations of symbiotic microorganisms [110]. Today we have documentally proved value of microbial exometabolites that are actively involved in the restoration of human intestinal microflora. These data provide a strong impetus to the development of biotechnology of new generation standardized probiotics [111].

As a practical illustration, one can cite a number of innovative probiotic RioFlora complexes with full scientific dossier justifying the differential control of microbial indigenous microflora cells. Until now, there have been 2 probiotics of the type: both multi-species and multi-strain probiotic drugs that are able to fulfill a specific function of the intestinal microflora in different clinical situations. One of the most important characteristics of these probiotics is their purposeful selection of strains and the presence of a special matrix, which simulates intestinal biofilm, thus ensuring conservation of the number of viable microorganisms when passing through the gastrointestinal tract, thus ensuring probiotic potential as well. 2 probiotic complexes are represented in the Russian Federation, designed for adults and children over the age of 3.

RioFlora Balance Neo is a probiotic that has a fixed combination of 8 viable bacteria from following production strains: *B. bifidum* W23, *B. lactis* W51, *L. acidophilus* W37, *L. acidophilus* W55, *Lactobacillus paracasei* W20, *L. plantarum* W62, *L. rhamnosus* W71, *Lactobacillus salivarius* W24.

Each enteric capsule contains at least 5 × 108 CFU / caps. microorganisms which have a wide range of physiological effects. Currently, the major research centers in Russia have begun clinical trials in order to obtain their own experience on the use of drugs considered to treat patients with a various disease nosologies and course severity of the process. This will allow standardizing approaches to treatment based on current scientific evidence for all doctors.

Table 1. The studied probiotic microorganisms

Lactobateria	bifidus bacteria	Other	Fungi
L. acidophilus *L. casei* *L. delbrueckii, ssp*	*B. bifidum* *B. infantis* *B.*	*S. thermophilus* *Enterococcus faecium* *Propionibacterium freudenreichii*	*Saccharomyces cerevisiae* *Saccharomyces boulardii*

bulgaricus *L. reuteri* *L. brevis* *L. cellobiosus* *L. fermentum* *L. plantarum* *L. rhamnosus* (GG) *L. salivarius* *L. gasseri* *L. johnsonii* *L. helviticus* *L. farciminis*	*longum* *B. thermophilum* *B. adolescents* *B. lactis* *B. animalis* *B. breve*	*Escherichia coli Nissle 1917* *Bacillus clausii* *Bacillus oligonitrophilis*	

Table 2. The clearest evidence of the biological probiotic microorganisms' effects

Antibacterial effect	**Reinforcement of the epithelial barrier function**	**Modulation of immune response of the host**
< pH of the intestinal lumen Stimulation of the secretion of defensins The secretion of antimicrobial peptides Inhibition of pathogenic invasion Blockade of bacterial adhesion to epithelial cells Formation of nitric oxide	Protein phosphorylation of dense cell contacts Increase in mucus production Increased glycosylation of membranes' components of epithelial cells The increase in sIgA production	Stimulation of antibody production Stimulation of NK-cells Modulation of functional activity of dendritic cells Modulation of gene expression regulators: NF-kB and AP-1 Change in cytokine production The induction of regulatory T cells PPAR γ Induction Modulation of apoptosis Inhibition of proteasome

		activity

Table 3. Indications based on the evidence for the use of probiotics in gastroenterology

Indications	Probiotic strain	Dose prescribed
Treatment of acute intestinal infections (AII) in children	*L. rhamnosus* GG *L. reuteri* ATTTC 55730 *L. acidophilus* + *B. infantis* *S. cerevisiae* (*boulardii*)	10^{10}–10^{11} BID 10^{10}–10^{11} BID 10^9 TID 200mg TID
Treatment of AII in adults	*Enterococcus faecium* LAB SF 68	10^8 TID
Complete prevention of (AAD) in children	*S. cerevisiae* (*boulardii*) *L. rhamnosus* GG *B. lactis* BB12 + *S. thermophilus*	250mg TID 10^{10} OD/BID 10^7+10^6
Prevention of AAD in adults	*Enterococcus faecium* LAB SF 68 *S. cerevisiae* (*boulardii*) *L. rhamnosus* GG *L. casei* DN-114 001 in fermented milk with *L. bulgaricus* + *S. thermophilus* *L. acidophilus* CL1285 + *L. casei* Lbc80r *Bacillus clausii*	10^8 BID 1g per day 10^{10}–10^{11} BID 10^{10} BID 5×10^{10} BID 2×10^9 TID
Prevention of nosocomial diarrhea in children	*L. rhamnosus* GG *B. lactis* BB12 + *S. thermophilus*	10^{10}–10^{11} BID $10^8 + 10^7$

	B. lactis BB12 *L. reuteri* ATTTC 55730	10^9 BID 10^9 BID
Prevention of diarrhea caused by C. difficile in adults	*L. casei* DN-114 001 in fermented milk with *L. bulgaricus* + *S. thermophilus* *L. acidophilus* + *B. bifidum* *S. cerevisiae* (*boulardii*)	10^{10} BID 2×10^{10} OD 2×10^{10} OD
Adjuvant therapy at eradication of *H. pylori*	*L. rhamnosus* GG *L. casei* DN-114 001 in fermented milk with *L. bulgaricus* + *S. thermophilus* *S. cerevisiae* (*boulardii*) *Bacillus clausii*	6×10^9 BID 10^{10} BID 1g per day 2×10^9 TID
The decrease of some symptoms at irritable bowel syndrome	*L. rhamnosus* GG *B. infantis* 35624 VSL#3 mixture *L. rhamnosus* GG, *L. rhamnosus* LC705, *B. breve* BB99, *Propionibacterium freudenreichii ssp. shermanii* *B. animalis* DN-173 010 in fermented milk with *L. bulgaricus* + *S. thermophilus*	6×10^9 BID 10^8 per day $4{,}5\times10^{11}$ BID 10^{10} per day

		10^{10} BID
Remission maintenance at ulcerative colitis	*E. coli Nissle* 1917	5×10^{10} BID
Prevention and remission maintenance at puoschitis	VSL#3 mixture of 8 strains (1 *S. thermophilus*, 4 *Lactobacillus*, 3 *Bifidobacterium*)	$4{,}5\times10^{11}$ BID
Prevention of necrotizing enterocolitis in preterm infants	*B. infantis*, *S. thermophilus*, *B. bifidum* *L. acidophillus* + *B. infantis*	$0{,}35\times10^{9}$ of each strain BID 10^{9} of each strain BID

Table 4. Differences between monostrain and multi-species probiotics.

Monostrain probiotic	**Multi-species probiotic**
Successful colonization	
Survival depends on the specific properties of the strain: The strain should independently overcome all stress barriers of gastrointestinal tract	Different strains with specific traits are more likely to colonize: • reduction in antagonistic activity of the endogenous microflora against sensitive strains • creation of optimal pH • creation of an anaerobic niche

	• improvement of bacteria adhesion
Probiotic effect on the body	
The probiotic effect is limited to the properties of the strain	The probiotic effect is reinforced through a combination of the properties of the strain: • additive effect of the specific properties of the strain (colonization of different niches) • synergistic effects of different strains (common probiotic effect may be more pronounced than the sum of the individual stimulant effects) Positive relationship between the strains increasing their biological activity: • symbiosis between different strains, for example, through the metabolites exchange

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