Review Bioscience of Microbiota, Food and Health Vol. 32 (3), **–**, 2013

Gut Microbiota, Probiotics, and Human Health

Alexander SUVOROV[1]

[1]*Department of Molecular Microbiology, Institute for Experimental Medicine, Acad. Pavlov Street 12, Saint-Petersburg 197376, Russia*

Received November 30, 2012; Accepted April 2, 2013

The review is devoted to the problems of microbiota and the ways of it correction employing beneficial life bacteria-probiotics. It covers the issues related to the functioning of human microbiota and its importance for the health, individual variability of microbial content, functioning of the probiotics in the human organism and the history of probiotic studies with particular focus on the microbiological investigations in the USSR. The article discusses the safety issues related to probiotics and the problems with probiotic therapy, trying to explain the reasons for the side effects caused by probiotics. The necessity of personified selection of the probiotic strain or individual microbial therapy autoprobiotics is also discussed.

Key words: microbiota, probiotic, enterotype

INTRODUCTION

The entire concept of the human organism being located at the top of the evolutionary tree is deeply rooted in the brain of many people due to traditional, cultural or religious modes of thinking. This concept was reanalyzed deeply due to the recent findings of the damages caused by the modern civilization to the outer environment and general public health. Serious ecological catastrophes, global warming, nuclear waste contamination and chemical leaks are accompanied by the appearance of novel important bacterial or viral pathogens, spread of antibiotic resistance strains and the dramatic increase in cancer or cardiovascular diseases. All these exo- and endoecological changes lead to novel modes of thinking and seeing of the human being as a complex organism tightly bound to its outer world and its endoecology.

Human civilization witnessed the negative effects of its own behavior long ago: extensive animal breeding in the Sahara and destructive and deadly epidemics of the Middle Ages in cities with poor sanitary conditions are the small examples of the importance of equilibrium between the "outer" nature and "inner" bacterial world. However, the impact of modern technologies on the surrounding world and human health surpass all the previously noticed negative effects. The role of bacteria as factors influencing human health has never been fully understood but was always intuitively acknowledged by human habits and tradition. Many of the social restrictions regarding food and diets were and are based on negative effects of bacterial food contamination or inability to store certain products properly. In other cases the beneficial health effects of fermented food products were noticed ages ago and were sometimes considered sacred. The current review is devoted to the role of the microbiota in maintaining health and application of health beneficial bacteria in medical practice.

Corresponding author. Mailing address: Alexander Suvorov, Department of Molecular Microbiology, Institute for Experimental Medicine, Acad. Pavlov Street 12, Saint-Petersburg 197376, Russia. E-mail: alexander_suvorov1@hotmail.com

HUMAN MICROBIOTA AS SEEN NOW

The concept of the human microbiota and its role in human health underwent significant changes in the eyes of the scientific community, physicians and common people. The former attitude of microorganisms as something alien to humans or even dangerous changed into the understanding that bacteria (more correct would be the term "microbiota," including viruses, bacteria, archaea and some eukaryotes) are normal and even necessary for proper functioning of the human organism, populating the entire body with large a prevalence of microbes in such loci as the gut, skin, mouth and urogenital system. The gut is the human organ the most populated by bacteria, the number of which exceeds by at least by two orders of magnitude the total number of human body cells [1, 2]. This understanding gradually allowed change the entire concept of the indigenous microbiota as a vitally important part of the body and its role in the maintenance of human health. At present with the advent of new sequencing technologies and the joint effort of American and European microbiota analysis programs (Human Microbiome Project - www.hmpdacc.org and MetaHIT

- www.metahit.eu) the composition and the major dominant bacterial *phyla*, representing human microbiota were identified in contrast to the previous studies based on classical bacteriology [1]. It is established that bacterial content of human gut microbiota is composed mainly from *Firmicutes, Bacteriodetes,* Actinobacteria, Proteobacteria*, Fusobacteria* and *Archaea* with predominance of *Firmicutes* and *Bacteriodetes* [3, 4]. Indigenous gut microbiota tend to form a complex multispecies biofilm covering entire mucus layer with only few bacterial species reaching the very gut epithelium [5]. Composition of human microbiota depends on the diet preferences of the host but also depend on the individual peculiarities of the host genetics and his/her innate immune system. Individual microbial content seems to be stable during the life span remaining as it was established quite early in life [6]. Interestingly even the neonates seem to differ by the predominance of either *Bacteroidetes* or *Bifidobacteriaceae* [6]. These individual features change gradually during life, switching from bifidobacteria being predominant in the breast-feeding period to the dominance of *Bacteriodetes* and *Firmicutes* in the later stages of life [7]. These discoveries agree with the finding that the normal microbiota in adults, being highly individual, has a significant degree of stability and tends to recover after temporary dysbiotic conditions. [8]. The significant amount of data on the microbiota sequencing followed by bioinformatic analysis allowed generation of a concept of enterotypes. According to the suggestion of Arumugam et al. [9], the human gut microbiome can be partitioned into three enterotypes: one with the prevalence of *Bacteroides*, another with *Prevotella* and a third that is almost completely affiliated with phylum *Firmicutes* with slightly higher levels of *Ruminococcus*. This distribution was found to be independent from the diet preferences, body mass index, race or gender. It implied a host-controlled microbiota composition. Almost instantly, this concept of the magic "three" was challenged by other studies, in which the existence of two or four enterotypes was found [10, 11]. This fairly artificial bioinformatics- based approach of enterotyping humans, boosted dramatically the research in the field because it provided the scientific community for the first time with a simple and easily accessible tool for the analysis of the results of studies of microbiota. It is already clear that these relatively stable microbiota compositions (two, three or four) are providing similar solutions for the organism of the human host at the level of the metobolome.

The functional role of gut microbiota as an additional vitally important para-/meta-organ is almost impossible to overestimate. The gut microbiome participates in almost all metabolisms of incoming nutrients, is involved in vitamin synthesis, in cholesterol catabolism, shapes numerous immune reactions related to the innate and adaptive immunity, and modulates the relationship of the human being with pathogenic microorganisms [12, 13].

Indigenous bacteria hydrolyse exogenous and endogenous substrates. Mucins enable them to obtain an uninterrupted supply of carbon and energy despite differences in the human diet. In return bacteria produce short chain fatty acids (such as butyrate), amines, phenols, indols, and gases [14]**.** Even the development of immune system or the brain depends on the host microbiota [15, 16]. It is also established that many gastrointestinal and somatic diseases develop as result of microbiota changes (dysbiosis) inflicted by the stress, intoxication, radiation or antibiotic treatment. Dysbiosis, defined as deregulation of the normal homeostasis of the intestinal microbiota, is involved in the pathogenesis of various diseases including (but not limited to) antibiotic-associated diarrhea (AAD), *Clostridium difficile*-associated disease (CDAD), inflammatory bowel disease (IBD), acquired immune deficiency syndrome (AIDS) and obesity [17]. Dysbiotic conditions depending on the degree of the microbiota disturbances either disappear themselves or transform into different pathologies, which require specific microbial (probiotic) treatments.

HISTORY OF PROBIOTICS AND "RUSSIAN CONNECTION"

Most likely, the first reason why humans started selecting certain bacterial stocks for their use was the need for food preservation. When the access to the food was sporadic, the ability to preserve the aliments in fermented form was the only way to prevent hunger. Fermented milk or meats in the form of cheeses or different kinds of processed meat (Spanish Jamon Serrano as an example) were able to preserve the nutritious properties of food for several months. That was vitally important for farmers and shepherds, allowing them to make distant journeys and enhancing dissemination of humankind around the Earth. Natural selection of the best strains allowed choosing the best strains and those that were most advantageous regarding the prevention of food spoilage and preservation of the nutritional food properties.

During the evolution of human societies, some direct healing properties of lactic acid bacteria (LAB) strains were selected based on their health benefits. Yogurts, kefirs, matsoni, kumis, airan and many other fermented milk products became known and were sometimes

Table 1. Probiotic drugs and food products produced and distributed in Russian Federation

Species included	Name of the product	Type of product	Company
Bifidobacterium bifidum Nº1 or *Bifidobacterium bifidum 791*	Bifidumbacterin Bifidumbacterin forte	Freeze dried powder 10^8 CFU/ml, 10^7 CFU/g	Biomed Metchnikoff JSC, FSUC "SIC "Microgen", Patrner LTD
Bifidobacterium bifidum Nº1 + Lysozym	Bifilis	Freeze dried powder, 10^6 CFU/ml	Ferment, LTD
Lactobacillus plantarum or *Lactobacillus fermentum*	Lactobacterin	Freeze dried powder, 10^7 CFU/ml, in 10 ml flasks, tablets, vaginal suppositories	Biomed Metchnikoff JSC, FSUC "SIC "Microgen"IM-Bio
Enterococcus faecium L3	Laminolact	Bon-bons with contact dried bacteria 10^6 CFU/g in 200g boxes	Avena, LTD
Lactobacillus acidophilus	Acilact	Vaginal suppositories 10^7 CFU/ml	Lekko, LTD
Bacillus cereus IP 5832	Bactisubtil	Freeze dried powder, 10^9 CFU/g in capsules	Aventis Pharma International, France
Lactobacillus acidophilus D-76, D-75	Vitaflor	Freeze dried powder, 10^7	State Institute of Fine pure Biochemicals
Escherichia coli M-17	Colibacterin	Freeze dried powder, 10^7 CFU/ml, in 10 ml flasks	FSUC "SIC "Microgen"
Lactobacillus acidophilus, *Bifidobacterium infantis, Enterococcus faecium*	Linex	Freeze dried powder, 1.2×10^7 CFU/g in capsules	Sandoz, Lec, Slovenia
Bifidobacterium bifidum bifidum Nº1 and *E. coli* M-17).	Bificol	10^7 CFU/ml, 10^7 CFU/ml in 10 ml flasks	Biomed Metchnikoff JSC, FSUC "SIC "Microgen"
Bifidobacterium longum Enterococcus faecium SF68	Bifiform	10^7 CFU/ml, 10^7 CFU/ml, in capsules	Ferrosan, Denmark

thought to posses mystical powers because of their health benefits and life-extending properties. At the end of the 19th century, Nobel prize winner Ilia Metchnikoff was the first to study LAB scientifically. Metchnikoff noticed the correlation between the longevity of Bulgarian shepherds and their yogurt diet. In the results of his studies he was the first to suggest that humans could live significantly longer and healthier if they consume beneficial bacteria [18]. This simple idea happened to be quite sound. In order to find the bacteria thriving in yogurts, Metchnikoff isolated several strains of lactobacilli, which he called *Lactobacillus bulgaricus*. He proved that it is possible to make eatable fermented milk products using pure cultures of *L. bulgaricus*. According to Metchnikoff's hypothesis, lactobacilli were eliminating pathogenic toxin- producing bacteria from the colon - what he considered the main reason for life shortening. His collaborators at the Pasteur Institute were also the first to perform experiments on germ-free animals, starting gnotobiology as a new branch of biological science. Metchnikoff was not only the first to study bacteria in fermented milk; he also promoted production of the first bacterial drug, Lactobacillin, which was manufactured in Saint Petersburg starting 1912. That was long before Nissle in 1917 suggested his *Escherichia. coli* product wrongfully cited as the first probiotic [http://www.probiotics-help.com/mutaflor.html].

Metchnikoff's studies were later overshadowed by the development of antibacterial drugs after the discovery of antibiotics, and Soviet Union remained the only country in which scientists continued selecting and analyzing health beneficial strains and certifying them as drugs sold in pharmacies (Table 1).

Studies of several brilliant Soviet scientists such as Tsiklinskaia P., Peretz L., Ugolev A., Kiselev P. and Shenderof B. made a significant impact in understanding of the action of health-promoting bacteria in the human organism and in launch of production of several strains of health beneficial bacteria, belonging to the species of lactobacilli, enterococci, bifidobacteria and *E.coli*, on an industrial scale [19–22].

Products containing LAB approved as "drugs" with the commercial names Lactobacterin, Bifidumbacterin and Colibacterin are still on the market of Russian Federation. For example, Bifidumbacterin – a drug containing bifidobacteria was designed in 1966, and industrial production of it started in 1972 [23]. "Lactobacterin" (probiotic drug containing *Lactobacillus plantarum* strain 8P-A3) production also was stared in early 70s. The term probiotic meening food or drugs containing life health beneficial bacteria, appeared in world literature much later, in the 80s, after the revival of interest in these beneficial bacteria [14]. Around that time, a significant

amount of studies has been already accomplished in the USSR regarding the selection of probiotic strains, their antagonistic activities, vitamin production and specific influence on the intestinal microbiota. The main health benefits of intestinal bacteria such as antagonistic activities, vitamin production, enzymatic activities and immunomodulation were postulated by Leonid Peretz already in 1955 [19].

PROBIOTICS AND THEIR FUNCTIONS IN THE HOST

Use of probiotics as health beneficial products or ingredients containing live bacteria is huge, and there is a constantly growing number of different functional foods and pharmaceuticals.

Most of the commonly used probiotic strains belong to the group of LAB and bifidobacteria. LAB include several different genera including *Streptococcus, Staphylococcus, Lactococcus, Pediococcus, Lactobacillus, Enterococcus, Leuconostoc* and some others. LAB had acquired the ability to recognize several sugars, such as for instance xylose, cellobiose, ribose, arabinose, glucose, and fructose before they developed the ability to ferment lactose to lactate. They firstly colonized fruit and vegetable ecological niches, and later cheese, wine, and especially milk, which reflected their preference for habitats rich in lactose [24]. Starting with Metchnikoff, studies of LAB and their use as probiotics have predominantly focused on the genus of *Lactobacillus*. *Enterococcus*-based probiotics are well represented in the post-Soviet and Eastern European market and are less common in Western Europe and the United States. For example, the *Enterococcus*-containing drugs Linex and Bifiform are comprise more than 80% of the Russian market for probiotics (www.gidrm.ru/includes/mktng/marketing).

Among the other probiotic strains, one should mention bifidobacteria as the dominant microbiota in breast-fed children which are also prominent as components of both: probiotic drugs and food products. Other probiotics on the market belong to different species of bacilli, *E.coli*, saccharomyces and some clostridial strains [25, 26].

At present time, a large number of relevant clinical studies with probiotics have been performed and even analyzed employing meta-analysis. Some of these studies aimed at treatment of gastrointestinal diseases

Table 2. Some probiotic strains used in clinical practice

Probiotic strain (preparation)	Disease	References
VSL#3 *(Streptococcus thermophilus* *Bifidobacterium breve* *Bifidobacterium longum* *Bifidobacterium infantis* *Lactobacillus acidophilus* *Lactobacillus plantarum* *Lactobacillus casei* *Lactobacillus bulgaricus)*	Ulcerative colitis	[59–61]
Escherichia coli Nissle	Ulcerative colitis	[62]
Lactobacillus GG	Ulcerative colitis	[63]
VSL#3	Pouchitis	[64]
Lactobacillus GG	Crohn's disease	[65, 66]
Saccharomyces boulardii	Crohn's disease	[67]
Lactobacillus GG	Irritable bowel syndrome	[68]
Bifidobacterium animalis DN-173 010	Irritable bowel syndrome	[69]
Bifidobacterium infantis 35624	Irritable bowel syndrome	[70]
Escherichia coli (DSM17252)	Irritable bowel syndrome	[71]
Lactobacillus plantarum MF1298	Irritable bowel syndrome	[27]
Lactobacillus plantarum 299v	Irritable bowel syndrome	[72]
Lactobacillus reuteri ATCC 55730	Irritable bowel syndrome	[73, 74]
Bifidobacterium bifidum CECT 7366 *Lactobacillus spp*	*H. pylori* infection	[75, 76]
Enterococcus faecium L3	*H. pylori* infection	[77, 78]
Clostridium butyricum	*H. pylori* infection	[79]

such as irritable bowel syndrome, Crohn's disease, pouchitis and ulcerative colitis, are listed in Table 2. The positive outcomes of probiotic treatment in most of the studies reflect the effectiveness of probiotics in clinical practice. However, the results of treatments employing different or even the very same probiotic strain vary from study to study. For example, in the case of irritable bowel syndrome (IBS) treatment together with studies demonstrating positive effects of probiotic therapy, some studies showed no differences compared with the control or even the aggravation of pathologies

[27–30]. In a recent study on patients with IBS, intake of *L. plantarum* MF 1298 was associated with a significant aggravation of symptoms, but neither intake of *L. plantarum* MF 1298 nor symptoms were associated with the composition of the fecal microbiota [27]. What was most striking in this respect was results of a clinical study of patients with acute pancreatitis, in which 16% of patients in the probiotics group died, compared with 6% in the control group [31].

This discrepancy in the results of clinical studies reflects the fact that the probiotic bacteria (sometimes poorly studied) administered to the individual patients with their own unique microbiota might interact with the host tissues or their own microbiota in different ways. Medical doctors and scientists who made decisions regarding the clinical studies in many cases neglected the endoecological aspects of introduction of bacteria into the gut of patients. These possible side effects of microbial therapy, which have been proved as effective in most of the studies, are also postulated by Matsushima and Takagi in the editorial titled "Is it effective?" to "How to use it?": the era has changed in probiotics and functional food products against *Helicobacter pylori* infection [32]. However, accurate prediction of the functioning of probiotics in the gut is impossible without understanding the physiology of probiotic strains and the mode of their interactions with the host.

MECHANISMS OF PROBIOTIC ACTION

In numerous reviews describing the use of probiotics, several features of the strains included into the preparations were mentioned. Probiotics should be of human or animal origins depending on their intended uses. They should have the ability to survive in sufficient numbers as well as to pass through the gut (bile and acid tolerant), be safe for consumption, and be adhesive to the intestinal mucosa. They should exert an antagonistic effect against pathogens, and interfere with the translocation of the pathogenic bacteria and modulate the immune system [14, 27–30, 33]. However, none of the probiotic strains meet these criteria in full or the studies showing this are not convincing. First, the relevance of the probiotic strain to the host is often questionable due to the fact that most of the historically selected LAB probiotic strains including Metchnikoff *Lactobacillus delbrueckii* subsp. *bulgaricus* most likely originated from the cattle microbiota. Three things regarding probiotic functions are most obvious: antagonistic potential, the influence of the probiotics on the process of digestion and immunomodulation.

Antagonistic activity of most probiotic strains can be studied outside the host, allowing evaluation of the range of the affected opportunistic/pathogenic bacteria. Different mechanisms of antibacterial action are involved, but synthesis of organic acids and antimicrobial peptides (bacteriocins) are the most common weapons of bacterial wars for colonization locus and for nutrients. Expression of many bacteriocins of lactobacilli, enterococci or bifidobacteria is strictly regulated by the complex genetic regulatory systems involving three-component signaling and pheromone activation by the quorum sensing mechanism [34–36]. The majority of bacteriocin-producing strains generate peptides inhibiting growth of a narrow range of bacteria with similar colonization preferences; however, some probiotics such as *L. plantarum* 8P-A3 or *E. faecium* L3 synthesize multiple bacteriocins with extremely high inhibitory activities against gram-positive and gram-negative pathogens [35, 36].

Similar effects were determined in studies with the other bacteriocins, isolated from LAB [37, 38]. Appearance of probiotics in the gut induces noticeable metabolic effects on the organism such as lowering of the cholesterol level, vitamin production, diabetes or obesity [33, 39–41]. However, it is usually difficult to distinguish the effects of relatively small amounts of bacteria being introduced into the total microbiome. These reactions are better monitored in gnotobiotic animals or animals with artificially induced dysbiosis [42]. On the other hand, a healthy microbiota is usually resistant to colonization by external microorganisms [43]. Objective evaluation of the immunomodulatory functions of probiotics presents similar problems because the tests are usually performed either on the organisms with established microbiota or gnotobionts known to have a defective innate immune system. Both these models have their weaknesses. It has been established that probiotics do influence the innate and adaptive immune functions involving toll-like receptors (TLRs) and their downstream systems including NF-κB, JAKSTAT, MAPK, and SAPK/JNK pathways. These reactions are followed by interleukin

and defensin differential expression, which can vary depending on the type of probiotic used. For example, the most common reactions to probiotic lactobacilli or enterococci are downregulation of NF-κB and IL-8 expression and induction of IL-10 [16, 44–47]. However, these effects are very strain dependant. Different strains belonging to the same species can modulate the immune response quite differently by helper T (Th1/Th2) cell polarization.

Another probiotic feature, which has been under intensive investigation lately, is their influence on epithelium integrity. Probiotics belonging to different species can influence protein expression in tight junctions blocking the process of bacterial translocation [48]. These effects were more visible in the case when the microbiota of the experimental animals was in an artificially induced dysbiotic condition [48–50].

PROBIOTICS AND SAFETY

Many scientists and especially physicians active in this field are considering only lactobacilli or bifidobacteria as safe probiotics meeting generally regarded as safe (GRAS) criteria. They are completely ignoring the fact that many probiotics including the GRAS strains bear putative pathogenicity factors and mobile genetic elements in their genomes. On the other hand the strains with a long history of being successfully used as probiotics belonging to such species as *E.coli*, enterococci or *Bacillus subtilis* are regarded as potentially hazardous. However, this point of view has nothing to do with microbial ecology or with common sense and in reality harms the entire concept of the clinical usage of probiotics. Bacteria being highly plastic and adaptive to different environments do not "respect any human moral values" or do not particularly target the humans. The only thing they can do and will do is propagate in the presence of appropriate nutrients and in certain environments. Many strains of *Lactobacillus salivarius* used in several probiotic preparations in reality express a fibrinogen-binding protein encoded by the gene CCUG_2371. The presence of this virulence factor in the strain can cause platelet aggregation facilitating a septic infection [51]. The most used and studied probiotic strain, *Lactobacillus rhamnosus* GG, carries vancomicin resistance genes and 5 timidly called "genomic islands" (in other organisms they are named pathogenicity islands or the PAI) with several bacteriophages and genes for 3 surface expressed LPXTG-like pilins (spaCBA) and a pilin-dedicated sortase [52]. These genomic findings are considered an explanation of the probiotic features of the strain [52]. However, the very same genetic features in other species such as enterococci are considered virulence factors. This is a good example of a pseudoscientific approach with double standards that has propagated under the pressure of large industrial corporations selling certain types of probiotics. On the other hand this mode of thinking reflects a natural desire to follow the pattern of commonly accepted stereotypes.

AUTOPROBIOTICS AND FECAL TRANSPLANTATION

It is of general agreement that at least some health benefits of probiotics occur as result of the interactions of the probiotic strains or strain composition with the host microbiota. It also established that the beneficial effects of probiotic are most evident under dysbiotic conditions and are not seen in the healthy microbiota. Other solutions for restoring the microbiota back to normal are fecal transplantation or autoprobiotic therapy. Fecal transplantation is a medical procedure based on the replacement of the host microbiota with the microbiota of a donor. This procedure had been evaluated in several clinical studies on patients with inflammatory bowel disease (IBD) or for the treatment of *Clostridium difficile* infection [53, 54]. Besides being fairly unhealthy way to introduce bacterial biomass (through the nose or the rectum), this approach has Achilles' heels such as the donor microbiota, which may carry opportunistic bacteria able to cause problems in the treated patient. In our previous study of healthy individuals, about 50% of the indigenous enterococci carried several putative virulence factors in their genome [55]. Also, the enterococci are clearly not the most dangerous bacteria in the gut.

Another approach is based on the indigenous bacteria used for restoring the normal microbiota in the case of a dysbiotic condition [20]. This approach, named as autoprobiotic technology, can be based on LAB or bifidobacteria previously stored in cryobanks, isolation of individual strains from the microbiota and returning the bacteria back into the gut after propagating them outside the organism, allowing analysis of each individual strain and return of it to the host. Usually it takes a week to prepare autoprobiotic yogurt for the patient. In our clinical studies of patients with IBS, ulcerative colitis and pneumonia autoprobiotics introduced to patients by employing a randomized placebo-controlled approach provided significant positive effects as judged from the majority of clinical parameters and life quality [56].

CONCLUSIONS

Contemporary science is collecting more and more data regarding the human microbiota, which functions as an important "organ" tightly bound to the other organs of the body. Previous dogmas of clinical microbiology, which were trying to divide the microbial world into hazardous and beneficial microorganisms, are questioned by the new genomic and metabolomic data. The contemporary crisis of pharmacology being unable to produce and bring new antibiotics into the market [57] is giving human race a chance to see the problem of human health from the level of microecology, moving away from the simple eradication strategy. The emotional appeal of Blaser, "Stop the killing of beneficial bacteria," needs to attract more attention from the scientific and medical community [58]. It is obvious that the tight systemic links between the microbiota and the cells of the human body are highly individualized and need to be restored when the microbiota changes due to various reasons, with antibiotic treatments being number one. Dysbiotic conditions lay underneath many infectious and somatic diseases of our contemporaries. It is obvious that microbial therapy should be much better implemented in the arsenal of medical doctors; however, a significant amount of studies needs to be done before this kind of therapy will become really common.

Despite the great number of different probiotics on the world market and permanently growing sales of probiotics, there is no agreement in the scientific community regarding their mode of functioning and interpretation of the results of the clinical studies. The main reason for this is simply based on the lack of the relevant studies and extremely complex microbiota of each individual. There is no common agreement on the expected features or the composition of probiotic strains. Only several things about probiotics are obvious: we want them to pass alive to the target locus of the organism, interact with the host microbiota and the host immune system and they should not cause an infection.

On the other hand there are a lot of things they are supposed to do: they supposedly must deplete a number of opportunistic bacteria, somehow modulate the immune system, most likely consume internal nutrients and produce their own metabolites, strengthen the epithelial barriers, colonize sites in the organism or disappear from the host. There is no agreement: regarding the issues of the preferred period of colonization, ability of probiotics to adhere to the host epithelium, affiliation of probiotics to the indigenous human microbiota, and the features regarding the safety of the probiotic strains. Most of these issues of scientific disagreement are being minor but at first glance require clarification. For example, the ability to colonize the epithelium in bacteria is often correlated with the presence of the adhesins, which are considered virulence factors on bacterial surfaces. Thus the presence of the adhesions or fimbriae on the surface of the probiotic bacteria can be judged differently.

There is no agreement regarding the preferred time of colonization and very limited data on monitoring the fate of probiotic strains inside the organism. The preferred dosage of probiotic bacteria is not clear too. Most likely, the optimal amount of consumed probiotic bacteria is strain specific and depends on the survival of the probiotic bacteria in the host.

It is unclear what is better: one probiotic strain or a multistrain composition. The interrelationship between the strains of such probiotic compositions is the mostly poorly studied. In any case, the more alien strains are introduced into the gut, the more chances there are that one of the members of the consortiums will cause an unpredicted reaction.

In this respect the idea using indigenous strains as probiotics looks quite attractive. Autoprobiotic strains have better chances relative to probiotics to colonize the host and thus normalize the host microbiota. However, autoprobiotics as medical therapies require further study.

In spite of the obstacles and the problems with microbial therapy stated in the present overview, the body of evidence concerning the use of probiotics in medicine is substantial, and better solutions for returning the individual microbiota back to normal are not on the horizon.

ACKNOWLEDGEMENTS

I am thankful to my colleagues for constructive advise regarding this review.

Major help was provided by Thomas Haertle from INRA, France, who kindly edited the text and gave many important critical remarks. I am also thankful to my Russian colleagues Ekaterina Kisseleva from the Institute of Experimental Medicine, Galina Alechina from Avena Ltd and Vetchaslav Melnikov from ITSC for the constructive criticism and some suggestions. Some experimental data sited in the review were collected in the Department of Molecular microbiology, Institute Experimental Medicine Russia. This work was supported by RFBR grant 10-04-00750a.

REFERENCES

1. Tannock G., editor. 1995. Normal Microflora. Chapman & Hall, London.
2. Zoetendal EG, Vaughan E, de Vos W. 2006. A microbial

world within us MicroReview. Mol Microbiol 59: 1639–1650. [Medline] [CrossRef]
3. Available at http://mbio.asm.org/content/3/5/e00376-12.long (accessed 2012-10-23)
4. Van den Abbeele P, Grootaert C, Marzorati M, Possemiers S, Verstraete W, Ge'rard P, Rabot S, Bruneau A, El Aidy S, Derrien M, Zoetendal E, Kleerebezem M, Smidt H, Van de Wiele T. 2010. Microbial Community Development in a Dynamic Gut Model is Reproducible, Colon Region Specific, and Selective for Bacteroidetes and Clostridium Cluster IX. Appl Environ Microbiol 76: 5237–5246.
5. Swidsinski A, Loening-Baucke V, Lochs H, Hale L. 2005. Spatial organization of bacterial flora in normal and inflamed intestine: a fluorescence in situ hybridization study in mice. World J Gastroenterol 11: 1131–1140. [Medline]
6. Jost T, Lacroix C, Braegger C, Chassard C. 2012. New insights in gut microbiota establishment in healthy breast fed neonates. PLoS One 7:e44595. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22957008 (accessed 2012-08-30).
7. Biagi E, Nylund L, Candela M, Ostan R, Bucci L, Pini E, Nikkı J, Monti D, Satokari R, Franceschi C, Brigidi P, De Vos W. 2010. Through Ageing, and Beyond: Gut Microbiota and Inflammatory Status in Seniors and Centenarians PLoS ONE 5:e10667. Available at: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2871786/ (accessed 2010-05-17)
8. Budding AE, Grasman M, Lin F, Bogaards J, Soeltan-Kaersenhout D, Vandenbroucke-Grauls C, van Bodegraven A, Savelkoul P. 2010. IS-pro: high-throughput molecular fingerprinting of the intestinal microbiota. FASEB J 24: 4556–4564. [Medline] [CrossRef]
9. Arumugam M, Raes J, Pelletier E, Le Paslier D, Yamada T, Mende DR, Fernandes GR, Tap J, Bruls T, Batto JM, Bertalan M, Borruel N, Casellas F, Fernandez L, Gautier L, Hansen T, Hattori M, Hayashi T, Kleerebezem M, Kurokawa K, Leclerc M, Levenez F, Manichanh C, Nielsen HB, Nielsen T, Pons N, Poulain J, Qin J, Sicheritz-Ponten T, Tims S, Torrents D, Ugarte E, Zoetendal EG, Wang J, Guarner F, Pedersen O, de Vos WM, Brunak S, Doré J, MetaHIT Consortium, Antolín M, Artiguenave F, Blottiere HM, Almeida M, Brechot C, Cara C, Chervaux C, Cultrone A, Delorme C, Denariaz G, Dervyn R, Foerstner KU, Friss C, van de Guchte M, Guedon E, Haimet F, Huber W, van Hylckama-Vlieg J, Jamet A, Juste C, Kaci G, Knol J, Lakhdari O, Layec S, Le Roux K, Maguin E, Mérieux A, Melo Minardi R, M'rini C, Muller J, Oozeer R, Parkhill J, Renault P, Rescigno M, Sanchez N, Sunagawa S, Torrejon A, Turner K, Vandemeulebrouck G, Varela E, Winogradsky Y, Zeller G, Weissenbach J, Ehrlich SD, Bork P. 2011. Enterotypes of the human gut microbiome. Nature 473: 174–180.
10. Wu GD, Chen J, Hoffmann C, Bittinger K, Chen Y, Keilbaugh S, Bewtra M, Knights D, Walters WA, Knight R, Sinha R, Gilroy E, Gupta K, Baldassano R, Nessel L, Li H, Bushman F, Lewis J. 2011. Linking Long-Term Dietary Patterns with Gut Microbial Enterotypes. Science 334: 105–108. [Medline] [CrossRef]
11. Holmes I, Harris K, Quince C. 2012. Dirichlet multinomial mixtures: generative models for microbial metagenomics. PLoS One 7(2):e30126. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22319561 (accessed 2012-02-3)
12. Pessione E. 2012. Lactic acid bacteria contribution to gut microbiota complexity: lights and shadows. Front Cell Infect Microbiol 2: 86. [Medline] [CrossRef]
13. Kumar M, Nagpal R, Kumar R, Hemalatha R, Verma V, Kumar A, Chakraborty C, Singh B, Marotta F, Jain S, Yadav H. 2012. Cholesterol-lowering probiotics as potential biotherapeutics for metabolic diseases. Exp Diabetes Res 2012: 902917. [Medline] [CrossRef]
14. Tannock GW. 2010.The Bowel Microbiota and Inflammatory Bowel Diseases. Int J Inflam 5:954051. Available at: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3004003/ (accessed 2010-08-5)
15. Manco M. 2012. Gut microbiota and developmental programming of the brain: from evidence in behavioral endophenotypes to novel perspective in obesity. Front Cell Infect Microbiol 2: 109. [Medline] [CrossRef]
16. Hooper LV, Littman D, Macpherson A. 2012. Interactions between the microbiota and the immune system. Science 336: 1268–1273. [Medline] [CrossRef]
17. Malo MS, Alam S, Mostafa G, Zeller S, Johnson P, Mohammad N, Chen K, Moss A, Ramasamy S, Faruqui A, Hodin S, Malo P, Ebrahimi F, Biswas B, Narisawa S, Millán J, Warren H, Kaplan J, Kitts C, Hohmann E, Hodin R. 2010. Intestinal alkaline phosphatase preserves the normal homeostasis of gut microbiota. Gut 59: 1476–1484. [Medline] [CrossRef]
18. Metchnikoff E. 1908. The prolongation of the life. Optimistic study. G.P. Putnam's sons, New York and London.
19. Peretz L. 1955. Role of the normal microbiota for the organism of human, Medgis, Moscow (in Russian).
20. Shenderov B., editor. 2011. Probiotics and functional foods, in Food Engineering, Eolss Publishers, Oxford.
21. Ugolev AM, Ivashkin V. 1992. Theory of universal functional blocks and fundamental biomedical problems. Klin Med (Mosk) 70: 8–14 (in Russian). [Medline]
22. Kiselev PN, Shutko T. 1968. On mechanisms of the cellular self-defence against the action of microbial toxins. Tsitologiia 10: 1068–1073 (in Russian). [Medline]
23. Aleshkin V, Amerhanova A, Pospelova V, Afanasiev S, Shenderov B. 2008. History, present situation, and prospects of probiotic research conducted in the G.N. Gabrichevsky Institute for Epidemiology and

Microbiology. Microbial Ecology in Health and Disease 20: 113–115. [CrossRef]
24. Carr FJ, Chill D, Maida N. 2002. The lactic acid bacteria: a literature survey. Crit Rev Microbiol 28: 281–370. [Medline] [CrossRef]
25. Hempel S, Newberry S, Maher A, Wang Z, Miles J, Shanman R, Johnsen B, Shekelle P. 2012. Probiotics for the prevention and treatment of antibiotic-associated diarrhea: a systematic review and meta-analysis. JAMA 307: 1959–1969. [Medline] [CrossRef]
26. Woo TD, Oka K, Takahashi M, Hojo F, Osaki T, Hanawa T, Kurata S, Yonezawa H, Kamiya S. 2011. Inhibition of the cytotoxic effect of *Clostridium difficile* in vitro by *Clostridium butyricum* MIYAIRI 588 strain. J Med Microbiol 60: 1617–1625. [Medline] [CrossRef]
27. Ritchie M, Romanuk T. 2012. A Meta-Analysis of Probiotic Efficacy for Gastrointestinal. Diseases PLoS One. 7: e34938. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22529959 (accessed 2012-04-18)
28. Farup PG, Jacobsen M, Ligaarden SC, Rudi K. 2012. Probiotics, symptoms, and gut microbiota: what are the relations? A randomized controlled trial in subjects with irritable bowel syndrome. Gastroenterol Res Pract 2012:214102. Available at: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3415104/ (accessed 2012-07-31)
29. Lee BJ, Bak Y. 2011. Irritable Bowel Syndrome, Gut Microbiota and Probiotics. J Neurogastroenterol Motil 17: 252–266. [Medline] [CrossRef]
30. McFarland LV, Dublin S. 2008. Meta-analysis of probiotics for the treatment of irritable bowel syndrome. World J Gastroenterol 14: 2650–2661. [Medline] [CrossRef]
31. Besselink MG, van Santvoort H, Buskens E, Boermeester M, van Goor H, Timmerman H, Nieuwenhuijs V, Bollen T, van Ramshorst B, Witteman B, Rosman C, Ploeg R, Brink M, Schaapherder A, Dejong C, Wahab P, van Laarhoven C, van der Harst E, van Eijck C, Cuesta M, Akkermans L, Gooszen H, Dutch Acute Pancreatitis Study Group 2008. Probiotic prophylaxis in predicted severe acute pancreatitis: a randomised, double-blind, placebo-controlled trial. Lancet 371: 651–659. [Medline] [CrossRef]
32. Matsushima M, Takagi AJ. 2012. "Is it effective?" to "How to use it?": the era has changed in probiotics and functional food products against *Helicobacter pylori* infection. J Gastroenterol Hepatol 27: 851–853. [Medline] [CrossRef]
33. Wallace TC, Guarner F, Madsen K, Cabana M, Gibson G, Hentges E, Sanders ME. 2011. Human gut microbiota and its relationship to health and disease. Nutr Rev 69: 392–403. [Medline] [CrossRef]
34. Dobson A, Cotter P, Ross RP, Hill C. 2012. Bacteriocin production: a probiotic trait? Appl Environ Microbiol 78: 1–6. [Medline] [CrossRef]
35. Tsapieva A, Duplik N, Suvorov A. 2011. Structure of plantaricin locus of *Lactobacillus plantarum* 8P-A3. Benef Microbes 2: 255–261. [Medline] [CrossRef]
36. Yermolenko E, Kolobov A, Chernysh A, Suvorov A. 2011. Influence of synthetic peptide inductors on antibacterial activity of enterococci. Beneficial Microbes 1: 253–257.
37. Batdorj B, Dalgalarrondo M, Choiset Y, Pedroche J, Métro F, Prévost H, Chobert JM, Haertlé T. 2006. Purification and characterization of two bacteriocins produced by lactic acid bacteria isolated from Mongolian airag. J Appl Microbiol 101: 837–848. [Medline] [CrossRef]
38. Atanassova M, Choiset Y, Dalgalarrondo M, Chobert JM, Dousset X, Ivanova I, Haertlé T. 2003. Isolation and partial biochemical characterization of a proteinaceous anti-yeast compound produced by *Lactobacillus paracasei subsp. paracasei* strain M3 from Bulgarian yellow cheese. Int J Food Microbiol 87: 63–73. [Medline] [CrossRef]
39. Machado MV, Cortez-Pinto H. 2012. Gut microbiota and nonalcoholic fatty liver disease. Ann Hepatol 11: 440–449. [Medline]
40. Nakamura Y, Omaye S. 2012. Metabolic diseases and pro- and prebiotics: Mechanistic insights. Nutr Metab (Lond) 19:60 Available at: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3464869/ (accessed 2012-06-19)
41. Cani PD, Delzenne N. 2009. Interplay between obesity and associated metabolic disorders: new insights into the gut microbiota. Curr Opin Pharmacol 9: 737–743. [Medline] [CrossRef]
42. Macho Fernandez E, Valenti V, Rockel C, Hermann C, Pot B, Boneca IG, Grangette C. 2011. Antiinflammatory capacity of selected lactobacilli in experimental colitis is driven by NOD2-mediated recognition of a specific peptidoglycan derived muropeptide. Gut 60: 1050–1059. [Medline] [CrossRef]
43. Mangalat N, Liu Y, Fatheree NY, Ferris MJ, Van Arsdall MR, Chen Z, Rahbar MH, Gleason WA, Norori J, Tran DQ, Rhoads JM. 2012. Safety and Tolerability of *Lactobacillus reuteri* DSM 17938 and Effects on Biomarkers in Healthy Adults: Results from a Randomized Masked Trial. PLoS One 7:e43910. Available at: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3435331/ (accessed 2012-09-6)
44. Petrof E, Claud E, Sun J, Abramova T, Guo Y, Waypa T, He S, Nakagawa Y, Chang E. 2009. Bacteria-free solution derived from *Lactobacillus plantarum* inhibits multiple NFkappaB pathways and inhibits proteasome function. Inflamm Bowel Dis 15: 1537–1547. [CrossRef]
45. Schlee M, Harder J, Köten B, Stange E, Wehkamp J, Fellermann K. 2008. Probiotic lactobacilli and VSL#3 induce enterocyte β-defensin 2. Clin Exp Immunol 151: 528–535. [Medline] [CrossRef]
46. Yoon SS, Sun J. 2011. Probiotics, nuclear receptor signaling, and anti-inflammatory pathways. Gastroenterol Res Pract 2011: 971–938. [Medline]

47. Tarasova E, Yermolenko E, Donets V, Sundukova Z, Bochkareva A, Borschev I, Suvorova M, Ilyasov I, Simanenkov V, Suvorov A. 2010. The influence of probiotic enetrococci on the microbiota and cytokines expression in rats with dysbiosis induced by antibiotics. Beneficial Microbes 1: 265–270. [Medline] [CrossRef]
48. Khailova L, Dvorak K, Arganbright KM, Halpern MD, Kinouchi T, Yajima M, Dvorak B. 2009. *Bifidobacterium bifidum* improves intestinal integrity in a rat model of necrotizing enterocolitis. Am J Physiol Gastrointest Liver Physiol 297: G940–G949. [Medline] [CrossRef]
49. Mennigen R, Nolte K, Rijcken E, Utech M, Loefler B, Senninger N, Brewer M. 2009. Probiotic mixture VSL#3 protects the epithelial barrier by maintaining tight junction protein expression and preventing apoptosis in a murine model of colitis. Am J Physiol 296: 1140–1149.
50. Ukena S, Singh A, Dringenberg U, Engelhardt R, Seidler U, Hansen W, Bleich A, Bruder D, Franzke A, Rogler G, Suerbaum S, Buer J, Gunzer F, Westendorf AM. 2007. Probiotic Escherichia coli Nissle 1917 inhibits leaky gut by enhancing mucosal integrity," PLoS ONE, 2: IDe1308. Available at: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2110898/ (accessed 2007-12-12)
51. Collins J, van Pijkeren J, Svensson L, Claesson M, Sturme M, Li Y, Cooney J, van Sinderen D, Walker AW, Parkhill J, Shannon O, O'Toole P. 2012. Fibrinogen-binding and platelet-aggregation activities of a *Lactobacillus salivarius* septicaemia isolate are mediated by a novel fibrinogen-binding protein. Mol Microbiol 85: 862–877. [Medline] [CrossRef]
52. Kankainen M, Paulin L, Tynkkynen S, von Ossowski I, Reunanen J, Partanen P, Satokari R, Vesterlund S, Hendrickx AP, Lebeer S, De Keersmaecker SC, Vanderleyden J, Hämäläinen T, Laukkanen S, Salovuori N, Ritari J, Alatalo E, Korpela R, Mattila-Sandholm T, Lassig A, Hatakka K, Kinnunen KT, Karjalainen H, Saxelin M, Laakso K, Surakka A, Palva A, Salusjärvi T, Auvinen P, de Vos WM. 2009. Comparative genomic analysis of *Lactobacillus rhamnosus* GG reveals pili containing a human- mucus binding protein. Proc Natl Acad Sci USA 106: 17193–17198. [Medline] [CrossRef]
53. Damman CJ, Miller SI, Surawicz CM, Zisman TL. 2012. The microbiome and inflammatory bowel disease: is there a therapeutic role for fecal microbiota transplantation? Am J Gastroenterol 107: 1452–1459. [Medline] [CrossRef]
54. Brandt LJ. 2012. Fecal transplantation for the treatment of *Clostridium difficile* infection. Gastroenterol Hepatol N Y 8: 191–194. [Medline]
55. Bondarenko V, Suvorov A., editors. 2007. Symbiotic enterococci and the problem of enterococcal infection, Sandoz, Moscow (in Russian).
56. Suvorov A, Simanenkov V, Gromova L, Kolodjieva V, Tsapieva A, Chernish A, Solovieva O, Ermolenko E. 2011. Enterococci as probiotics or autoprobiotics. In Prebiotics and probiotics potential for human health, Ivanova I (ed), Paisi Hilendarski, Sofia, pp. 104–112.
57. Projan SJ. 2003. Why is big Pharma getting out of antibacterial drug discovery? Curr Opin Microbiol 6: 427–430. [Medline] [CrossRef]
58. Blaser M. 2011. Antibiotic overuse: Stop the killing of beneficial bacteria. Nature 476: 393–394. [Medline] [CrossRef]
59. Tursi A, Brandimarte G, Papa A, Giglio A, Elisei W, Giorgetti GM, Forti G, Morini S, Hassan C, Pistoia MA, Modeo ME, Rodino' S, D'Amico T, Sebkova L, Sacca' N, Di Giulio E, Luzza F, Imeneo M, Larussa T, Di Rosa S, Annese V, Danese S, Gasbarrini A. 2010. Treatment of relapsing mild-to-moderate ulcerative colitis with the probiotic VSL#3 as adjunctive to a standard pharmaceutical treatment: a double-blind, randomized, placebo-controlled study. Am J Gastroenterol 105: 2218–2227. [Medline] [CrossRef]
60. Miele E, Pascarella F, Giannetti E, Quaglietta L, Baldassano RN, Staiano A. 2009. Effect of a probiotic preparation (VSL#3) on induction and maintenance of remission in children with ulcerative colitis. Am J Gastroenterol 104: 437–443. [Medline] [CrossRef]
61. Bibiloni R, Fedorak R, Tannock G, Madsen K, Gionchetti P, Campieri M, De Simone C, Sartor R. 2005. VSL#3 probiotic-mixture induces remission in patients with active ulcerative colitis. Am J Gastroenterol 100: 1539–1546. [Medline] [CrossRef]
62. Adam B, Liebregts T, Holtmann G. 2006. Maintaining remission of ulcerative colitis with the probiotic *Escherichia Coli* Nissle 1917 is as effective as with standard mesalazine. Z Gastroenterol 44: 267–269. [Medline] [CrossRef]
63. Zocco MA, dal Verme L, Cremonini F, Piscaglia A, Nista E, Candelli M, Novi M, Rigante D, Cazzato IA, Ojetti V, Armuzzi A, Gasbarrini G, Gasbarrini A. 2006. Efficacy of *Lactobacillus* GG in maintaining remission of ulcerative colitis. Aliment Pharmacol Ther 23: 1567–1574. [Medline] [CrossRef]
64. Mimura T, Rizzello F, Helwig U, Poggioli G, Schreiber S, Talbot IC, Nicholls RJ, Gionchetti P, Campieri M, Kamm MA. 2004. Once daily high dose probiotic therapy (VSL#3) for maintaining remission in recurrent or refractory pouchitis. Gut 53: 108–114. [Medline] [CrossRef]
65. Prantera C, Scribano M, Falasco G, Andreoli A, Luzi C. 2002. Ineffectiveness of probiotics in preventing recurrence after curative resection for Crohn's disease: a randomized controlled trial with *Lactobacillus* GG. Gut 51: 405–409. [Medline] [CrossRef]
66. Bousvaros A, Guandalini S, Baldassano RN, Botelho C, Evans J, Ferry GD, Goldin B, Hartigan L, Kugathasan S, Levy J, Murray KF, Oliva-Hemker M, Rosh JR, Tolia V, Zholudev A, Vanderhoof JA, Hibberd PL. 2005. A randomized, double-blind trial of *Lactobacillus* GG

versus placebo in addition to standard maintenance therapy for children with Crohn's disease inflammatory bowel diseases. Inflamm Bowel Dis 11: 833–839. [Medline] [CrossRef]

67. Garcia Vilela E, Ferrari M, Torres H, Guerra Pinto A, Carolina Carneiro Aguirre A, Paiva Martins F, Marcos Andrade Goulart E, Sales Da Cunha A. 2008. Influence of *Saccharomyces boulardii* on the intestinal permeability of patients with Crohn's disease in remission. Scand J Gastroenterol 43: 842–848. [Medline] [CrossRef]
68. Bausserman M, Michail S. 2005. The use of *Lactobacillus* GG in irritable bowel syndrome in children: a double-blind randomized control trial. J Pediatr 147: 197–201. [Medline] [CrossRef]
69. Guyonnet D, Chassany O, Ducrotte P, Picard C, Mouret M, Mercier CH, Matuchansky C. 2007. Effect of a fermented milk containing *Bifidobacterium animalis* DN-173 010 on the health-related quality of life and symptoms in irritable bowel syndrome in adults in primary care: a multicentre, randomized, double blind, controlled trial. Aliment Pharmacol Ther 26: 475–486. [Medline] [CrossRef]
70. Whorwell PJ, Altringer L, Morel J, Bond Y, Charbonneau D, O'Mahony L, Kiely B, Shanahan F, Quigley EM. 2006. Efficacy of an encapsulated probiotic *Bifidobacterium infantis* 35624 in women with irritable bowel syndrome. Am J Gastroenterol 101: 1581–1590. [Medline] [CrossRef]
71. Enck P, Zimmermann K, Menke G, Klosterhalfen S. 2009. Randomized controlled treatment trial of irritable bowel syndrome with a probiotic E.-coli preparation (DSM17252) compared to placebo. Z Gastroenterol 47: 209–214. [Medline] [CrossRef]
72. Sen S, Mullan M, Parker T, Woolner J, Tarry S, Hunter J. 2002. Effect of *Lactobacillus plantarum* 299v on colonic fermentation and symptoms of irritable bowel syndrome. Dig Dis Sci 47: 2615–2620. [Medline] [CrossRef]
73. Niv E, Naftali T, Hallak R, Vaisman N. 2005. The efficacy of *Lactobacillus reuteri* ATCC 55730 in the treatment of patients with irritable bowel syndrome - a double blind, placebo-controlled, randomized study. Clin Nutr 24: 925–931. [Medline] [CrossRef]
74. Drouault-Holowacz S, Bieuvelet S, Burckel A, Cazaubiel M, Dray X, Marteau P. 2008. A double blind randomized controlled trial of a probiotic combination in 100 patients with irritable bowel syndrome. Gastroenterol Clin Biol 32: 147–152. [Medline] [CrossRef]
75. Chenoll E, Casinos B, Bataller E, Astals P, Echevarría J, Iglesias JR, Balbarie P, Ramón D, Genovés S. 2011. Novel probiotic *Bifidobacterium bifidum* CECT 7366 strain active against the pathogenic bacterium Helicobacter pylori. Appl Environ Microbiol 77: 1335–1343. [Medline] [CrossRef]
76. García CA, Henríquez A, Retamal R, Pineda C, Delgado Sen C, González C. 2009. Probiotic properties of Lactobacillus spp isolated from gastric biopsies of *Helicobacter pylori* infected and non-infected individuals. Rev Med Chil 137: 369–376. [Medline]
77. Zakharova NV. 2006. Methods for increasing the efficacy and safety of *Helicobacter pylori* eradication regimens. Eksp Klin Gastroenterol 4: 59–66 (in Russian). [Medline]
78. Baryshnikova NV, Suvorov A, Tkachenko E, Uspenskiĭ IU. 2009. The role of genetic features of Helicobacter pylori in pathogenesis of digestive system diseases: from theory to practice. Eksp Klin Gastroenterol 1: 12–19 (in Russian). [Medline]
79. Imase K, Takahashi M, Tanaka A, Tokunaga K, Sugano H, Tanaka M, Ishida H, Kamiya S, Takahashi S. 2008. Efficacy of *Clostridium butyricum* preparation concomitantly with *Helicobacter pylori* eradication therapy in relation to changes in the intestinal microbiota. Microbiol Immunol 52: 156–161. [Medline] [CrossRef]

Comments