Chapter 9

Probiotics and Immunity

Marieta Georgieva, Kaloyan Georgiev and Peter Dobromirov

Additional information is available at the end of the chapter

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Abstract

Probiotics are “living microorganisms” which exert a prophylactic and therapeutic effect by improving the internal microbial balance. Probiotics play a role in defining and maintaining the delicate balance between necessary and excessive defence mechanisms including innate and adaptive immune responses. The beneficial effects of probiotics have been demonstrated in many diseases.

New therapeutic approaches toward several inflammatory diseases are being developed by affecting the microbial composition of the gut immune system. They are based on the fact that this part of immune system is influenced by many factors, including dietary components and commensal bacteria. An understanding of the molecular mechanisms behind the direct and indirect effects on the gut immune response will facilitate better and possibly more efficient therapy for diseases, although probiotics (live microorganisms) have already shown promise as treatments for several diseases in both clinical and animal studies.

Further, the concept of probiotics and the direct and indirect mechanisms by which they can influence gut immunity are described. Emphasis will be placed on the relationship of microbiota and the gut immune system.

A review of the history of *Lactobacillus bulgaricus*, probiotics, and probiotic functional foods is made and legislation and modern challenges are discussed.

Keywords: Probiotics, immunity, probiotic genomic, history, legislation

1. Introduction

Probiotics are defined by the World Health Organization as "live microorganisms, which when administered in adequate amounts, confer a health benefit upon the host." The main benefit of probiotics is that they help restore balance in the intestinal microbiota. Probiotics play a role in defining and maintaining the delicate balance between necessary and excessive defence mechanisms including innate and adaptive immune responses. The immunological mechanisms supporting probiotics and prebiotics effects continue to be better defined with novel mechanisms being described for dendritic cells, epithelial cells, T regulatory cells, effector lymphocytes, natural killer T cells, and B cells [1].

Looking to probiotics to support immune health is nothing new; the idea has existed for more than 100 years [2]. For millennia, humans have consumed microorganisms via fermented foods. Human beings and gut microbiota are in a symbiotic relationship, and the hypothesis of a “super organism” composed of the human organism and microbes has been recently proposed. The gut microbiota performs important metabolic and immunological tasks, and the impairment of its composition might alter homeostasis and lead to the development of microbiota-related diseases [3].

The most common illnesses associated with alterations of the gut microbiota include inflammatory bowel disease, gastrointestinal infections, irritable bowel syndrome and other gastrointestinal functional diseases, colorectal cancer, metabolic syndrome and obesity, liver disorders, allergy, and neurological diseases [4,5].

Neural pathways and central nervous system (CNS) signalling systems, according to new studies, can be activated by bacteria in the gastrointestinal (GI) tract, including commensal, probiotic, and pathogenic bacteria. Novel approaches for prevention and treatment of mental illness, including anxiety and depression [6], may be provided by actual and future animal and clinical studies, aimed at understanding the microbiota–gut–brain axis. In theory, every disease associated with the impairment of intestinal microflora might benefit from the therapeutic modulation of the gut microbiota.

The purpose of this review is to address the most recent findings regarding probiotic regulation of immune health. Probiotic genes and probiotic-derived factors involved in the regulation of host immunity, molecular targets of probiotic action responsible for the host immune responses, and roles and mechanisms of probiotics in prevention and treatment of diseases [7], which are included in clinical applications and mechanisms of action, are of special interest.

The role of specific microorganisms and the overall diversity of the microbiota in many human diseases can be understood to a great extent owing to the rapid growth of metagenomics strategies. Therapies focused on specific effects of different probiotics and prebiotics on the gut microbiota [8] can be helped by the development of this knowledge.

2. Probiotics and immune health

Hippocrates (460-370 EC) stated, “All diseases begin in the gut.” Both microbial diversity and abundance in the gut play an important role in maintaining human health. The attachment, growth, and penetration of pathogenic microorganisms on the gut surface can essentially be prevented by microbiota. Pathogen resistance, both by direct interaction with pathogenic bacteria and by influencing the immune system, is influenced greatly by the intestinal microbiota [9, 10]. Many diseases start with an initial imbalance of human resident microflora

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and the related immunobiological reactivity [11]. One key player in immune health is the gut, a part of the body that is constantly exposed to toxins and foreign antigens, such as those from food and microbes. According to nutrition and immune expert Meydani, "The gut is the largest immune organ in the body, accounting for 25% of the immune cells in the body that provide 50% of the body's immune response." Meydani called intestinal flora "forgotten organ" because of their vital but still underestimated health functions [12]. There are more than 400 species of bacteria residing in the gut, and they have symbiotic relationships with the body. There are 100 trillion bacteria in our intestines. They form an ecosystem like a "flower garden", reported Haruji Sawada, director of the Yakult Central Institute, at the Yakult International Nutrition and Health Conference on May 17, 2010, in Tokyo. The mammalian gut immune system should be viewed as a complex interplay between physical, chemical, and cellular barriers, a vast community of bacteria, and a plethora of host immune cells which mediate innate and adaptive immunity. The intestinal microbiota helps in proper development of the host immune system, which in turn regulates the homeostasis of the microbiota [13]. Accumulating evidence over the last decade indicates that the immune system and microbiota interaction should be finely balanced and any perturbations of this interaction would result in microbiota and immune dysbiosis, leading to inflammatory disorders The rapid surge in the emerging new-age disorders such as inflammatory bowel disease (IBD), rheumatoid arthritis, cardiovascular disease, and metabolic syndrome has driven investigators to explore their etiology in multiple directions such as genetics, diet, and environmental factors, as well as immune system/microbiota interactions. In addition, the practice of strict hygienic and sanitary conditions and consumption of highly processed foods containing high fat, high carbohydrate, and low fiber with numerous food additives and preservatives may account for altered microbial composition, metabolism, and interaction with host immunity. Nearly all the above diseases are characterized by local as well as systemic low-grade chronic or subclinical inflammation in which the inflammation originated in the intestine via the interaction between host immune system and microbiota.

Several beneficial effects of probiotics on the host intestinal mucosal defense system, including blocking pathogenic bacterial effects by producing bactericidal substances and competing with pathogens and toxins for adherence to the intestinal epithelium, have been identified. Probiotics promote intestinal epithelial cell survival, enhance barrier function, and stimulate protective responses from intestinal epithelial cells [14-16] in the case of intestinal epithelial homeostasis. What matters most is that the modulation of the immune system is one of the most reasonable mechanisms underlying the beneficial effects of probiotics on human health. Enhancement of the innate and adaptive immunity and modulation of pathogen-induced inflammation are the discovered effects of probiotics.

Strictly defined strains using genetic, serological, microbiological, and biochemical analyses; lack of pathogenicity, lack of cancerogenicity, presence of beneficial factors; possibility for colonization of the large intestine; viable cultures; acid and bile resistance; proved clinical efficacy are among the requirements for the organisms to be used as probiotics [17].

The established probiotics that meet these criteria are generally lactic acid bacteria (LAB), most commonly *Lactobacillus* and *Bifidobacterium* species, but also *Lactococcus, Streptococcus,* and

Enterococcus species and certain yeast strains. Numerous other LAB have shown probiotic potential in animal studies. For the treatment of IBD, several probiotics have been shown to be efficacious: *Lactobacillus casei, Lactobacillus plantarum, Lactobacillus bulgaricus,* and *Lactobacillus acidophilus*; three strains of *Bifidobacteria* and *S. thermophilus*. In recent years, evidence has accumulated that probiotic strains can exhibit the same activities as commensal bacteria, including immunomodulation [5,11].

Lactic acid bacteria are present in many feeds such as yogurt and are frequently used as probiotics to improve some biological functions of the host. Beneficial effects of the lactobacilli on the body have been identified in the treatment or prevention of acute viral gastroenteritis, after antibiotic-associated diarrhea, certain pediatric allergic diseases, necrotizing enterocolitis, and inflammatory bowel disease such as Crohn's disease and postoperative hernias. Probiotics have been long reported to aid in the treatment of many dysfunctions of the GI tract, and the mechanisms by which probiotics work have recently been elucidated. There are experimental and clinical data [18-24]. Probiotics are described as useful also in combating oxidative stress, improvement in mucosal immunity [25], and general immunity [26]. The desirable changes of the intestinal microbiota were achieved as yogurt was able to attenuate the symptoms of acute inflammation by reducing inflammatory cytokines and increasing regulatory cytokine IL-10-producing cells. The use of murine models demonstrated that the consumption of fermented milks can modulate the immune system and can maintain it in a state of surveillance, which could affront different pathologies such as cancer and intestinal inflammation on its part.

3. Gut's role in immune function

The contributions of the gut microbiota to the development of the immune system have been extensively characterized. One of these characterizations suggests that the host is able to tolerate the large amount of antigens present in the gut [27], owing to the coordinated cross talk between the gut microbiota and the immune system.

Progress in the current knowledge on biodiversity of the intestinal microbiota allows us to understand the mechanisms of how different microorganisms affect the function of the body and the impact of these mechanisms. Altered microflora (dysbiosis) is generally associated with gastrointestinal disorders, but rather a microbial imbalance is associated with common diseases, which are not limited to the gastrointestinal tract [28, 29]. Studies in germ-free (GF) mice give much of the evidence about how microflora forms immune system as GF mice are completely lacking microflora. These mice show profound immune defects, such as fewer and smaller Peyer plaques and mesenteric lymph nodes and also reduced B and T cell immune response [30-32]. Therefore, the serum immunoglobulin (Ig) G and IgA levels in the gut GF mice were reduced [33, 34]. Moreover, many studies in mice and humans show that certain inflammatory diseases are associated with an altered microflora [9, 35, 36].

Inflammatory diseases are often caused by microbial dysbiosis. It was found in a prospective study of children with a high risk of developing asthma that changes in the microbiota occur before the development of the disease [37]. Regulation of immune responses requires certain species of gut commensal microbiota and perturbations in the microbiota could lead to a lack of immune regulation, the outgrowth of more pathogenic microbes, and the promotion of inflammation. The microbial composition of the microbiota in the adult human gut is mainly determined by the microorganism to which the newborn child is exposed during the first years of life. Strategies to manipulate the microbiota during infancy have been shown to prevent development of allergic and atopic diseases later in adult life [38-40]. Thus, the use of probiotics and prebiotics during the early postnatal period has been proposed for the intentional modulation of the microbiota composition. In addition, diet and exposure to microbes during pregnancy may affect the metabolic and immunological profiles of the pregnant uterus and the risk of developing the disease in the offspring [41]. The application of probiotics and prebiotics during pregnancy has been also proposed [42-44]. Differences in this composition are related lo colonization; host factors, such as sex and age; genetic factors; and health state. The dynamic state of the microbe's ecology is increasingly being associated with an expanding number of disorders. New high throughput methodologies such as metagenomics, transcriptomics, proteomics, and metabolomics in the post-genomic era have greatly helped the understanding of the mechanisms, by which the microbiota contributes to host physiology in healthy and ill individuals. Metagenomic studies of the human gut microbiota, for example, have suggested that host metabolism is affected by low bacterial diversity, which is also related to obesity and other diseases [45, 46J.

4. Probiotic modulation of the gastrointestinal mucosal immune system

Perhaps one of the most important aspects of probiotic bacteria is the ability to modulate the host GIT mucosal immune system locally and systemically The interaction between the probiotic microbe with the resident microbiota, gastrointestinal epithelia and gut immune cells to produce an immunomodulatory response is quite complex, and has been reviewed exhaustively [34, 47- 49].

The expression of cytokines and chemokine genes was carried out by activated nuclear factor kappa-B (NF-κB) and mitogen-activated protein kinase signalling cascades, mediated of MAMPs, PRRS (including NOD-like receptor, Toll-like receptors, and C-type lectin receptors). Lipoteichoic acids (DMA), peptidoglycan and S-layer proteins are mostly found MAMPs from probiotic microorganisms. [48, 50] [Figure 1].

Multiple studies have explored the immunomodulatory effect of these MAMPs using functional genomic techniques. Various studies have demonstrated a significant reduction in product ion of proinflammatory cytokines with a simultaneous increase in anti-inflammatory IL-10 and the down regulation of pro-inflammatory IL-12 and TJMF-H. [23, 51J. Microflora in the intestine promotes mucosal barrier function, and also improves the immunity of the host to enteric infection. During the active infection a cytokine normally produced is IL-1β, which is critical for neutrophil restoration and eradication of the pathogen. Microflora play a vital role in the production of homeostatic levels of pro-IL-1β in local intestinal macrophages. The

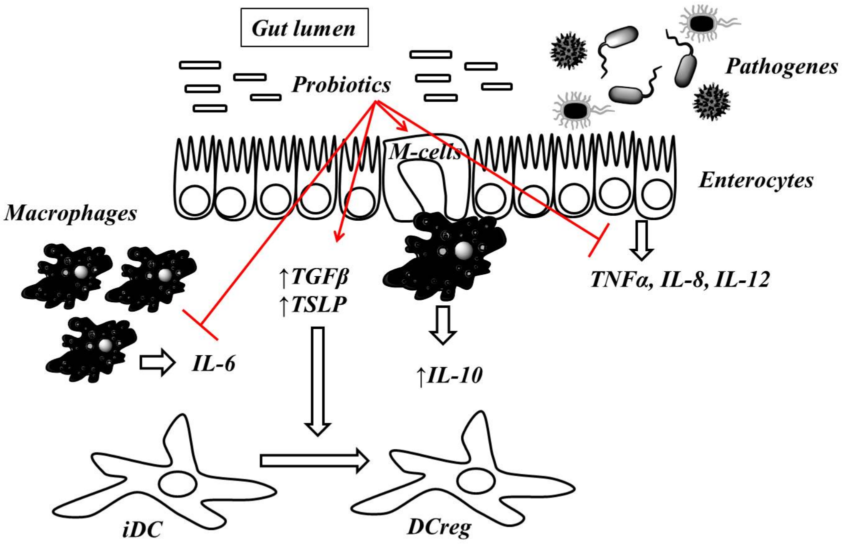

Figure 1. Probiotic modulation of the gastrointestinal mucosal immune system. While intestinal epithelial cells (IECs) exposed to pathogenic microbes or related stimuli produce proinflammatory mediators such as interleukin 8 (IL-8) and tumour necrosis factor a (TNF-a), probiotics suppress the production of these cytokines and instead induce anti-inflammatory mediators such as transforming growth factor b (TGF-b) and thymic stromal lymphopoietin (TSLP), which can promote the differentiation of immature dendritic cells (iDCs) to regulatory dendritic cells (DCregs). Macrophages in the inflamed mucosa produce high amounts of IL-6, and probiotics can decrease their IL-6 production and increase IL-10 production.

gut microbiota can also enhance host immunity through MyD88-independent mechanisms (MyD88 – Myeloid differentiation primary response gene 88). Notably, colonization of GF mice by commensal bacteria induces development of Th-17 cells in the intestine, which is important for protection against *Citrobacter rodentium* infection [52].

5. Immune cells

Probiotics regulate host innate and adaptive immune responses by modulating the functions of dendritic cells, macrophages, and T and B lymphocytes. Probiotics regulate immunomodulalory functions through the activation of toll-like receptors, which is one of the mechanisms of regulation. Recent studies indicate that probiotics activate innate immunity by enhancing adaptive immune response [20, 53]. One of the proposed mechanisms is by activation of toll-like receptors.

Regulatory dendritic cells are the primary professional antigen presenting cells (APCs) modulating adaptive immune responses. Probiotics containing *L. acidophilus, L. casei, L. reuteri, E. bifidium,* and *Streptococcus thermophilus*, stimulate dendritic cells to produce IL-10, TGF-β, COX-2, and indoleamine 2,3-dioxygenase, which in turn increase the formation of CD4 Foxp3 regulatory T cells (Tregs) and the suppressor activity of naturally occurring CD4 CD25 Tregs. They also decrease responsiveness of T and B lymphocytes and the number of T helper (Th) 1, Th2, and Th17 cytokines without inducing apoptosis. This mixture suppressed 2,4,6-

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trinitrobenzenesulfonic acid-induced intestinal inflammation, which was associated with enrichment of CD4 Foxp3 Tregs in the inflamed regions, as was found by in vivo studies. Thus, probiotics that enhance the generation of regulatory dendritic cells to induce Tregs, represent a potential therapeutic approach for inflammatory disorders [50, 54].

Nowadays, the exact mechanism of interaction between probiotic microorganisms and host cells remains elusive. Nevertheless, there is enough gathered information that microbiota in the gut could affect the immune response at both systemic and mucosal levels. Some putative mechanisms include: influence of the microflora itself, amelioration of membrane barrier function, and direct effects of probiotic microorganisms on different epithelial and immune cell types. Many patients with inflammatory bowel disease (IBD) use probiotics to manage this intestinal condition. Downregulation of production of proinflammatory cytokines and other inflammatory mediators seems to constitute important mechanisms for the partial amelioration of colitis, seen with numerous LAB strains in various models. It must also be noted that TNF-α blocking agents are also quite successful in the treatment of patients with CD (Crohn's disease) [55]. However, it should be taken into account that different probiotic bacterial species and strains have various beneficial effects and therefore need to be selected in a more rational manner to treat human diseases.

6. *Lactobacillus bulgaricus* – The contribution to modern healthy nutrition

Lactobacillus bulgaricus is the only probiotic microorganism named after a certain territory and nation. It only multiplies in the region of modern Bulgaria, coinciding with ancient Thrace. It mutates and stops its multiplication after 1-2 fermentations in other regions of the world. Bulgarian traditional food comprises *Lactobacillus bulgaricus. Lactobacillus bulgaricus* is included in the production of Bulgarian food products based on lactic acid, such as yogurt, feta yogurt, white brine cheese, other cheeses and cream, humanized baby food, probiotic functional foods, and whole food supplements [56-58].

Lactobacillus bulgaricus was known to the Thracians – the ancient population that lived in what is at present Bulgarian land, more than 7–8 thousand years ago. The word yogurt is Thracian and means hard, solid milk. During his tour in Thrace, the Greek scientist Herodotus (484–425 BC) wrote that the Thracians prepare special fermented dairy food, which is a gift from their Gods [59].

The father of probiotics and Nobelist – Ilya Metchnikoff (the Russian scientist) attributes the long and healthy life of Bulgarians largely to the yogurt consumption and in particular to the local bacterium in yogurt in his work "Prolongation of Life" [Figure 2].

In 1905 in Geneva, the Bulgarian student Stamen Grigorov isolated *Lactobacillus bulgaricus* from yogurt, brought from Bulgaria [Figure 3].

BSS (Bulgarian State Standard) for yogurt was established in Bulgaria in compliance with European standards of origin. Responding to this standard, the lactic acid fermentation should be accomplished using only *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. Yogurt is a

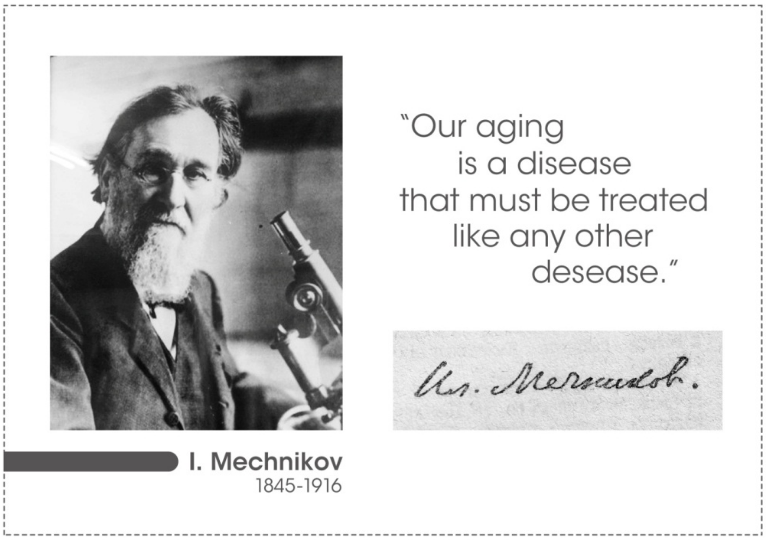

Figure 2. Prof. I. Metchnikoff

Figure 3. Dr Stamen Grigorov

fermented milk product with the typical bacterial cultures *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (the standards of identity, published in the US Code of Federal Regulations).

In Commission Regulation (Elf) No 432/2012 in the list of the permitted health claims made on foods, the only probiotic microorganisms included are *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. According to the claim, in doses over 10^8 colony forming units, they improve lactose digestion [60].

In the 1990s, *Lactobacillus bulgaricus* was used in the production of probiotic functional food for astronauts, which was tested during the flight of the second Bulgarian astronaut in Space [61],

Until recently it was considered that *Lactobacillus bulgaricus* can be isolated from plants – dogwood, rose cup, etc. Bulgarian scientists, N. Alexandrov and D. Petrova have isolated *Lactobacillus bulgaricus* and other fermented milk probiotic microorganisms from spring water in Bulgaria [62, 63]. There is experimental and clinical scientific evidence from around the world of the beneficial effects of *Lactobacillus bulgaricus* in stimulating the immune system, regulating functions and microbial flora of the gastrointestinal tract, including diarrhea and dysbacteriosis, reducing the risk of cancer, radiotherapy protecting effect, regulating cholesterol levels, competitive inhibition of pathogenic strains causing infections, alleviating lactose intolerance, and anti-mutagenic effect. *Lactobacillus bulgaricus* has a beneficial effect on the health of women because of its similarity to the Doederlein flora in the vagina of the woman [64]. *Lactobacillus bulgaricus*, unlike other probiotic microorganisms, which secrete only L (+) lactic acid, secretes L (+) and D (-) lactic acid during fermentation. This determines its powerful antioxidant and anticancer effect. *Lactobacillus bulgaricus* produces antimicrobial substance, known as Bulgariacan, which is resistant to heat and active against highly virulent (pathogenic) strains of microorganisms [56, 57]. It has been shown that *Lactobacillus bulgaricus* adheres best on the colonic mucosa, followed by a rapid reproduction in the body. This is crucial for its more powerful treatment and detoxic effect than other lactic acid bacteria probiotics.

Lactobacillus bulgaricus is reproduced best with *Streptococcus thermophilus*. To gain rapid biomass and biologically active substances and to potentiate their effect, joint cultivation of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* is needed. Lots of scientific studies of yeasts, containing *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, used in yogurt production, were carried out in Bulgaria. The results show that these specific bacteria of yogurt are stable and survive after passage through the human gastrointestinal tract.

Certain Bulgarian companies are using modern biotechnology, and wrap strains of *Lactobacillus bulgaricus* and other probiotic microorganisms with a natural coating, consisting of components of the growing medium, during fermentation. This helps the production of probiotics and the isolation of the new probiotic strains from spring water, which normally survives under changing climatic conditions. Theses probiotic microorganisms should retain their stability and vitality when passing through the gastrointestinal tract, as well as when stored in shops at temperatures of up to 24^{o} C for the entire shelf life.

Lactobacillus bulgaricus is the ancient contribution of mankind to modern science and to the creation of the first healthy foods in the world.

7. Probiotics and probiotic functional foods – Legislation and modern challenges

According to the World Health Organization (WHO) and the Food and Agriculture Organization (FAO), probiotics are live microorganisms which, when administered in adequate amounts, confer a health benefit on the host [65].

For the last decades, there has been a rapid growth in the application of probiotics among both people and animals. They are used in the production of fermented products (milk, cheese), as ingredients in adapted milk for babies and children, in bakery products or confectionery, or as material for the production of food additives. Another modern trend in agricultural science is the use of probiotics in animal breeding and poultry farming to reduce the disease rates with young animals, for maximum utilization of nutrition ingredients of fodder, and fast accumulation of muscle mass without treatment with hormonal preparations and antibiotics, as well as to increase the volume of additional products that animals and poultry give – eggs, milk, etc.

Probiotic microorganisms belong to the *Lactobacillus, Lactococcus, Bifidobacterium, Pediococcus,* or *Bacillus* family, with the microorganisms from Lactobacillus family being most frequently used as probiotics.

According to Claire M. Hassler from the Illinois University, the probiotics from the Lactobacillus family are functional ingredients contained in yogurt and other dairy products, which improve the health condition of the gastrointestinal tract. Many researches confirm the health benefits of probiotics for maintaining gastrointestinal health, avoiding infections through competitive suppression of the development of pathogenic strains, strengthening the immune system, prevention against colorectal cancer, reduction of serum cholesterol, relieving intolerance toward lactose, etc.

Probiotics have healthy effect if taken in specific quantities. According to the Japanese scientists, N. Ishibashi and S. Shimamura, the recommended quantity of live microorganisms in a probiotic product must be at least 107 live microorganisms per gram or per milliliter. According to other scientists, Gomez and Vinderola, the minimum required concentration of live cells in each gram probiotic product at the moment of consumption must be from 10^6 to 10^9 to have any favorable effect.

Today, the market offers a wide range of probiotic products and most of them are in dry forms. A number of factors have influence on the expiry periods of dried probiotic products and their subsequent effect when consumed. The creation of probiotic formulas with lasting durability of the strains and longer expiry periods still continues to be a challenge. Manufacturers of probiotic products constantly apply innovations, which aim to preserve the vitality of probiotic strains during the drying process (lyophilization), rehydration, when passing through the gastrointestinal tract and the strongly alternating pH in the stomach and biliary salts, and during their storage in the sales points. There are numerous researches of the molecular characteristics of probiotic strains and their health effects.

Companies apply different approaches to create new probiotic formulas with preserved vitality of the strains and useful effects. Some Korean and Japanese companies divide probiotic products into four generations:

- First generation – probiotic microorganisms without coating
- Second generation – probiotic microorganisms with single synthetic coating, which protects them when passing through the gastrointestinal tract

- Third generation – microcapsuled probiotic microorganisms with synthetic capsule
- Fourth generation – probiotic microorganisms with two synthetic coatings, protein and mucopolysaccharides, which protect them when passing through the gastrointestinal tract and against high pressure and temperature

Some scientists apply genetic manipulation of known strains to increase their durability. American companies test possible probiotic effects of known spore-carrying microorganisms from the Bacillus family.

Bulgarian manufacturers of probiotics coat the strains of lactic acid microorganisms with natural coating, which consist of ingredients of the food media aiming to raise their durability with the alternating pH and temperature. The Bulgarian scientist, Nikola Alexandrov and his fellow researchers, isolated for the first time in the world 8 completely natural probiotic strains from the Lactobacillus family from pure spring water in Bulgaria [62,63] [Figure 4].

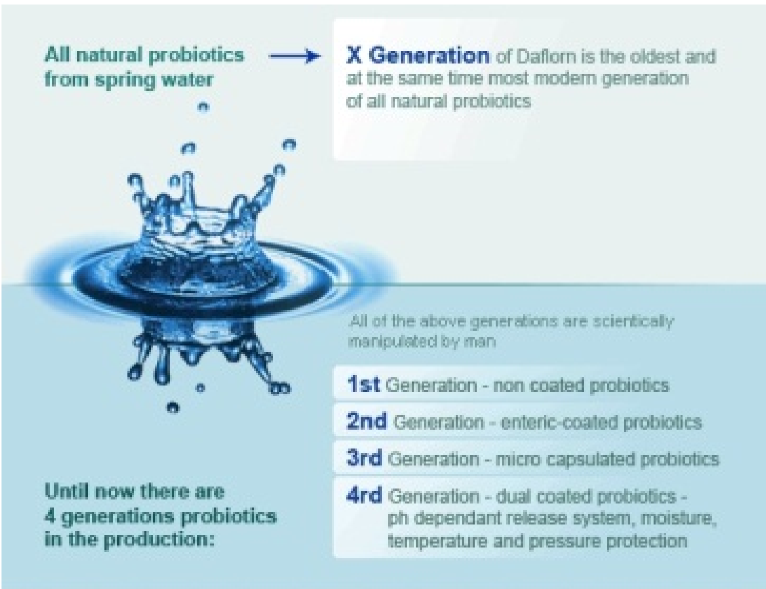

Figure 4. Probiotics from spring water

Research in this field will have major importance for the manufacturers of probiotics, who want to achieve longer term of durability and better health effect of their probiotic products. These researches are also extremely important for the end consumers who demand high-quality functional products to improve the quality of their life.

8. Probiotic functional foods

The oldest concept of functional foods was created by the father of medicine – Hippocrates. He wrote, “Let food be your medicine and medicine – your food.”

Food's major role is to provide sufficient nutritive substances, which will satisfy the need for food of the human organism. Today, nutrition science faces new challenges far beyond the concept of food insufficiency.

There are both socioeconomic changes taking place in the world and changes in the demographic structure of population – growing number of elderly people and increase of the expenses for healthcare. Therefore, governments, researchers, health experts, and the foodstuff industry, in general, direct their efforts to identification and creation of functional foods, which may improve the health of the local population, reduce the risk of diseases with social impact, such as cardiovascular diseases, cancer, osteoporosis, and improve the quality of life for the people. In combination with healthy living, the daily use of these functional foods may have positive impact for the health of both working people in active age and their families and for the elderly. This will result in the sharp reduction of the expenses for healthcare and in the optimization of physical and mental health of the population.

Nowadays scientific evidences proved that some foods and food ingredients have favorable physiological and psychological effects beyond the supply with major food substances. Modern researches focus on the identification of the biologically active components in food, which maintain human health and reduce the risk of diseases. It has been found that many traditional foodstuffs, such as eggs, milk, whole-wheat food, fruits, vegetables, soya, etc. contain components of potential benefit for health. Apart from these foods, there are new products that have been developed to increase or combine these useful components because of their benefits or desired physiological effects.

Functional foods are foods that are consumed as part of the normal nutrition process. They have useful effects when consumed in normal quantities within the dietary regime. Functional food could be:

- Food of natural origin – unskimmed milk, whole-wheat bread, etc.
- Food technologically or biotechnologically enriched with some ingredient
- Food from which ingredient has been extracted technologically or biotechnologically
- Food, in which the nature of one or more substances has been modified
- Food, in which bioavailability of one or more substances has been modified
- Food, which is a combination of two or more of the foods described above

Functional food may be addressed to the overall population or separate groups identified in terms of age (e.g., children or adults), genetic and enzyme modifications (intolerance toward gluten, lactose intolerance), functional status (pregnancy, for example), etc. Functional foods must be in conformity with the overall significance of nutrition – potential interrelations with other food substances and ingredients or possible undesired effects, including allergies and intolerance.

The foundations of the modern science of functional foods were laid in Japan during the 1980. The concept of food of specific health use (FOSHU) was established in 1991. Foods defined as

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FOSHU are approved by the Minister of Health and Social Cares of Japan after providing detailed and sufficient scientific evidence. Such foods are consumed as part of the standard diet [66]. In USA, the requirements for "reduction of the risk of diseases" for specific foods were made binding by law in 1993. The opinions and conclusions related to health are subject to approval by the Food and Drugs Agency on the base of scientific evidence and their goal is to help consumers by providing information about healthy nutrition, thus avoiding the risk of diseases [67]. In the European Union, the development and the approval of functional foods is regulated by a special organization to the European Commission known as FUFOSE (Functional Food Science in Europe) in cooperation with the European ILSI (International Life Science Institute), which works mainly on issues referring to the harmonization of the legislation in the field of functional foods in the EU Member States. This European Commission has developed two types of health requirements for functional foods: "increased function" and "reduction of the risk of disease" [60]. In Bulgaria, the permit for the production of food with special designation is given by the Bulgarian Food Safety Agency (BFSA) after provision of scientific evidence to the Ministry of Health of the Republic of Bulgaria [63, 67].

The biggest challenge before the scientists in the field of nutrition now and in the future is to research the interrelations between a given food or food ingredient and the improved health and reduced risk of various diseases. The provision of accessible and detailed information for the qualities of the functional foods is of exceptional importance for the consumers' choice of food.

9. Conclusion and future directions

Probiotics are reliable resources for prevention and treatment of immune disorders. There are a lot of encouraging data based on analyses of studies in humans and animal models that probiotics possess clinical efficacy in ulcerative colitis and irritable bowel syndrome as well as other intestinal diseases such as infectious diarrhea, antibiotic-associated diarrhea, necrotizing enterocolitis. The most recent studies suggest the exciting possibility that probiotics can be modified for delivery of vaccines and to potentiate the effects of vaccination [68]. While the paradigm of discovery-based genomics in probiotic LAB has uncovered vital aspects of probiotic mechanisms, it has also revealed the complexity of these interactions with the resident microbiota and the mucosal immune system. Nevertheless, a great opportunity was revealed by this challenge. For example, probiotic bacteria are now being explored as suitable models for vaccine/drug production, due to their close association with host immunity and immunomodulatory action [45, 69]. Latest discoveries show that the role of probiotic bacteria and the resident microbiota extend far beyond gastrointestinal health. It has revealed neurochemical importance of intestinal homeostasis. There are studies that show cross talk between gastrointestinal tract and brain (brain–gut axis) [70]. Along with these advancements, it is important that human clinical trials continue with experimental designs that are well-controlled and well-defined, reflecting the great progress that has been made in the field of probiotic and GIT microbiome research [71]. With more than a century passing since Metchnikoff's observations, keen experimental design using integrated genomics has led to a clearer definition of probiotic bacteria, as well as a model for continued discovery. Bulgaria becomes famous in the world with *Lactobacillus bulgaricus* and the Bulgarian territory is still an important reservoir for isolation of natural strains of lactic acid bacteria, which after an additional selection are used as starter cultures for the production of various fermented foods and probiotic products. The biggest challenge for researchers in the field of nutrition, at present and in the future, is the study of the relationship between a certain food or a nutrient and improved health or reduced risk of various diseases. Provision of accessible and comprehensive information about the properties of functional foods is crucial for food selection by consumers.

Author details

Marieta Georgieva[1*], Kaloyan Georgiev[1] and Peter Dobromirov[2]

*Address all correspondence to: marieta_md@yahoo.com

1 Department of Pharmacology and Toxicology, Faculty of Pharmacy, Medical University, Varna, Bulgaria

2 Medical University, Varna, Bulgaria

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