Probiotics and Prebiotics Concepts

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Contributor: Andrea Ballini , Ioannis Alexandros Charitos , Stefania Cantore , Skender Topi , Lucrezia Bottalico , Luigi Santacroce

Probiotics are microorganisms which, if taken in sufficient quantities, contribute positively to the health of the host and are the focus of both scientific studies and commercial companies.

intestinal microbiota dysbiosis functional foods probiotics prebiotics

1. Introduction

Nowadays, consumers have turned to the purchase of functional foods that could prevent the onset of dysbiosis of the human microbiota and, therefore, of diseases connected to it. A human's daily nutrition in the context of diet has an impact on his health and requires the presence of foods with more benefits than the simple supply of energy, mineral salts, trace elements and vitamins-the so-called functional foods [1]2. More specifically, the concept of promoting functional foods began in Japan in 1984 because studies had demonstrated the connection between nutrition, taste satisfaction, activation of physiological systems (such as immunity) and food fortification. Subsequently, research on the design of these products moved to Europe and America ^[3]. A characteristic of these foods is that various definitions exist. An "official" definition of functional foods is as follows: a food can be defined as functional if, together with its basic nutritional action, it has a beneficial effect on one or more physiological functions, such as to improve general health and/or to reduce the risk of developing disease. Therefore, they are foods that, in addition to nutritional value, offer health benefits. These foods have nutrient-rich ingredients (such as fruits and vegetables) but can also be enriched with vitamins, minerals, probiotics, prebiotics, and fibers ^{[4][5]}. The form of food and the required intake should be intended for different purposes; therefore, they cannot be available in pill or capsule form. In simple words, they are foods that fulfill the body's nutritional needs and, at the same time, have a beneficial effect on the body, if consumed in reasonable guantities and always according to the principles of proper nutrition ^[6]. Probiotics are microorganisms which, if taken in sufficient quantities, contribute positively to the health of the host and are the focus of both scientific studies and commercial companies. The market in the field of functional foods and, specifically, those containing probiotic bacteria, which constitute 60% of all functional foods, is constantly expanding.

2. Probiotics and Prebiotics Concepts

The Nobel Prize winner for Medicine in 1908, Elie Metchnikoff, argued that the cause of aging is toxin released by the decaying of certain bacteria in the intestine, or, by the degradation of components through the release of proteolytic enzymes from *Clostridium* spp. ^[7]. In fact, he stated that "*the dependence of friendly bacteria on food*

allows measures to be taken to modify the microbial composition of our body and thus replace the harmful ones". Metchnikoff's scientific hypothesis on fermented milk obtained from *Bacillus bulgaricus* (actually called *Lactobacillus delbrueckii subsp. Bulgaricus*) laid the foundation for the development of the first dairy industry ^{[Z][8]}. In addition to the application to whey and food, probiotics began to be used to improve the health of patients. In 1989, Fuller defined a probiotic as a dietary supplement with live microbes which has a positive effect on the host by improving the microbial balance of the host's gut. Subsequently, in 1991 he defined probiotics as single or mixed cultures of live microorganisms that have a beneficial effect when administered to humans or animals and contribute to the improvement of the properties of their acquired endogenous microbial presence ^[9]. In 1998, a group of scientists proposed that probiotics are food components composed of living microorganisms that have a beneficial effect on health ^[10]. One of the simplest and most accepted explanations was that proposed by a committee of experts composed of members of the Food and Agriculture Organization of the United Nations and the World Health Organization which states: "*Probiotics are microorganisms which, when administered sufficient quantities, confer a beneficial action on the health of the patient*". Thus, they prevent and aid the patient in avoiding the dysbiosis and lead to the gut microbiota's eubiosis (**Figure 1**).



Figure 1. The gut microbiota/host axis depends on the favorable balance between the microorganisms that constitute it. Credits: Original figure by I.A. Charitos.

Recently, probiotics have been characterized as live microorganisms that show resistance to gastric, biliary and pancreatic secretions, adhere to epithelial cells, and colonize the human intestine. Therefore, probiotics are bacteria which are beneficial to health, which now also have clinical effects ^[11]. Probiotic microorganisms must usually be of human origin, and therefore safe, and they must maintain their vitality, both during the technological processes that the food undergoes and during their passage through the gastrointestinal tract ^{[8][9][10][11][12]}. Equally

desirable are the possibilities of their immobilization in the intestinal epithelium, the competitive action against pathogenic microorganisms, and their resistance to antibiotic substances. Probiotic bacteria are kept alive both in freeze-dried form and when injected into fermented products (**Table 1**) ^[7].

Table 1. ⊤	he main	probiotic	strains.
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Lastabasillasaaa	Probiotic Bacteria	Othor
Lactobacillaceae Lactobacillus acidophilus Lactobacillus johnsonii Lactobacillus helveticus Lactobacillus crispatus Lactobacillus gasseri Lacticaseibacillus casei Lacticaseibacillus rhamnosus Lactiplantibacillus plantarum subsp. plantarum Limosilactobacillus reuteri Ligilactobacillus salivarius Enterococcus faecium	Bifidobacterium animalis Bifidobacterium breve Bifidobacterium infantis Bifidobacterium longum Bifidobacterium adolescentis Bifidobacterim lactis Bifidobacterim bifidum	Other Saccharomyces boulardii Saccharomyces cerevisiae Aspergillus niger Aspergillus oryzue Clostridium butyricum Escherichia coli

The most used probiotic species are from *Lactobacillaceae* family, *Bifidobacteria* spp. and yeasts such as *Saccharomyces boulardii* [8][9].

Probiotics can be taken through specific formulations (supplements), through dairy functional foods (such as yoghurt, cheese, ice cream and other) and through non-dairy products [11]. Since probiotics are sensitive to environmental factors, such as the inhospitable environment of the gastrointestinal tract, various techniques are used to protect them; mainly the micro-encapsulation technique is used, so that they maintain the best performance. In general, various species from Lactobacillaceae family (such as Lactobacillus acidophilus, Lacticaseibacillus casei, Lacticaseibacillus rhamnosus. and Lactobacillus helveticus) have been extensively studied for the prevention of certain health disturbances, such as the non-communicable diseases (NCDs) [12]. However, some tests have shown adverse effects associated with the administration of probiotics, as is common with any preparation. Most often, side effects are mild and include symptoms such as nausea, indigestion and abdominal discomfort (such as flatulence, and constipation). Less commonly, infections can develop; the most serious side effects that have been reported are endocarditis and septicemia ^[10]. A positive attitude toward probiotics has been observed by healthcare professionals, and also by consumers, as they are not considered medicines. It should be noted, however, that the safety and efficacy of probiotics must be determined by considering the quantity and dosage of the probiotics, the characteristics of the consumer (including the research of the metabolic profile of his intestinal microbiota) and the reason for the intake of probiotics. These reasons make it necessary to research an individualized probiotic treatment for each individual [11][12][13].

Prebiotics are products of food digestion that have a positive effect on the health of the host. Thus, their purpose is to modify the composition of the intestinal microbiota, in order to favor the growth of probiotic bacteria and inhibit the growth of unfriendly or unwanted microorganisms ^{[13][14]}. In 2008, the FAO (Food and Agriculture Organization of the United Nations) defined prebiotics as "*a non-vital food ingredient that benefits the health of the host associated with microbiological modulation*" ^[15].

Prebiotics are functional alimentary ingredients that are found naturally in vegetable foods or can be produced by synthetic production through enzymatic conversion of sugars ^[10]. These alimentary compounds are generally structures of carbohydrates or soluble alimentary fibers, which are selectively metabolized by the human microbiota. The most used prebiotics in Europe are galattooligosaccarides (GOS) and inulin derivatives, such as fructooligosaccarides (FOS) [13]. The GOS derived from lactose is found in human milk and vaccines, but are also present as additives in many other foods, such as cereals and dairy products [16]. GOS favors the proliferation strains from the Lactobacillaceae and Bifidobacteriaceae families, which are highly beneficial for the host's health ^[13]. Moreover, these prebiotics can prevent infection by pathogenic microorganisms, because they can structurally imitate their binding sites and prevent their adhesion to epithelial cells $\frac{17}{2}$. FOS are fructans, hydrolytic derivatives of inulin, with a small number of fructose monomers. They are found in high percentages in plant foods, such as onion, asparagus, wheat and artichoke. [16][17][18]. Prebiotics, such as cellulose, lignin and oligosaccharides, present in foods such as raw oats, soybeans and chicory roots, must resist gastric acids and be able to reach the large intestine in order to be fermented by the intestinal microbiota, which favors the development of beneficial intestinal species ^[9]. Recent studies have shown that non-digestible carbohydrates, such as resistant starch and fiber, are not metabolized in the small intestine and for this reason they can reach the large intestine, where they are fermented by the intestinal microbiome [11]. In this context, non-digestible carbohydrates can potentially act as prebiotics, stimulating the growth of some species that contribute to the health of the host ^[19].

Several studies have shown that prebiotics are able to influence metabolic and immune factors such as IL-6, insulin resistance and the amount of glucose in the blood. These data indicate that the intestinal microbiota is able to regulate the host's metabolism and immune response as a function of the diet, thus contributing to the maintenance of the host's state of health ^[20].

Prebiotics, therefore, provide benefits to the host, including strengthening the integrity of the intestinal mucosal barrier, increasing host mucosal immunity, lowering pH and the production of Short Chain Fatty Acids (SCFAs) and inhibiting the growth of pathogenic microorganisms ^[21]. The use of prebiotics also plays a role in the treatment of obesity. The presence of prebiotics in the gut is associated with the production of both protective mucus and SCFAs, as well as with the production of anti-inflammatory cytokines. Furthermore, it is associated with the secretion of satiety hormones and thus prevents overeating. ^[22]. Fructose-containing oligosaccharides are mainly used as prebiotic substances. Thus, they have a favorable effect on the growth of probiotic bacteria by limiting the surface area that would otherwise be occupied by pathogenic microorganisms (such as *Escherichia coli*) ^[22]. It is also possible to use other oligomers of specific sugars, such as lactulose, soy and maltose, as well as oligosaccharides containing xylose, mannose, and galactose. Finally, honey is considered a prebiotic food. Prebiotic oligosaccharides can be obtained by (a) isolation from plant raw materials, (b) biotechnological production

or enzymatic synthesis and (c) enzymatic hydrolysis of polysaccharides. Many prebiotic oligosaccharides are produced on an industrial scale and are commercially available $\frac{13}{13}$. Indeed, $\beta(1-2)$ fructans (such as inulin and/or fructo-oligosaccharides) are the best studied prebiotics and are present in various foods (such as leeks, onions, garlic, artichokes, asparagus, shallots, bananas and wheat) ^[19]. Their average consumption, in an ordinary diet, is estimated at a few grams per day and they have been recognized as dietary fibers in most countries. Industrially, inulin is derived from hot water extraction of radish roots, followed by refining and spray drying. High inulin (>90%) is commercially available as a white powder. Like oligofructose, it is obtained industrially by partial enzymatic hydrolysis of inulin using a special endoinulase $\frac{17}{2}$. Furthermore, fructooligosaccharides (FOS) can also be produced by transfructosylation of sucrose. Commercial preparations of oligofructose include various contents, up to 95%, and are in the form of white powders and sticky syrups ^[23]. As for soy oligosaccharides, they are obtained by direct extraction and purification from soy milk whey, a by-product of soy protein concentrate production. They are marketed as a syrup containing 6% raffinose and 18% stachyose, as well as sucrose, glucose and fructose (52%)^[23]. Some galactooligosaccharides (GOS) components have been reported to occur naturally in human milk at an amount of about 3mg/L^[13]. Industrially transgalactosylated oligosaccharides are produced from the synthesis of lactose by using a β -galactosidase ^[15]. In practice, the combined use of probiotics and prebiotic ingredients is usually applied due to their synergistic action in foods. In this way, symbiotic products are created that benefit the consumer through the survival and establishment of selected live microorganisms in the digestive system [23][24].

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