# Approaches to Aging Control

Journal of Spanish Society of Anti-Aging Medicine and Longevity

Nº 24

Special Issue in collaboration with SEMiPyP.

## Microbiota, Probiotics and Prebiotics

December 2020

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# 19th International Congress of Anti-aging Medicine

## XIX Congreso de la Sociedad Española de Medicina Antienvejecimiento y Longevidad

**Madrid, September 30th to October 2nd 2021**

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# Dysbiosis and its modulation through the use of probiotics, prebiotics and other members of the biotics family. General concepts.

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***Keywords:*** *dysbiosis, probiotics, prebiotics, synbiotics, paraprobiotics, postbiotics, biotics*

**ABSTRACT**

The human microbiota may eventually shift to dysbiosis states that are characterized by the loss or underrepresentation of normally dominating species and the substitute display of otherwise minority species, often including potential pathobionts. Members of the biotics family including probiotics, prebiotics, synbiotics, paraprobiotics, postbiotics, etc., can be used under these circumstances to redirect the microbiota towards a state more favorable to the host health. Regarding probiotics, there are recommendations and guidelines for the systematic evaluation of these microorganisms that involve functional selection criteria, safety concerns, and viability requirements, among others. Prebiotics are substrates that are selectively used by host microorganisms and confer a health benefit. When combined with probiotics, they are called synbiotics. Non-viable probiotics and microbial metabolites that can have beneficial health effects are named, respectively, paraprobiotics and postbiotics. Recent progress aiming to modify complex microbial ecosystems in pathological situations has given rise to the transfer of faecal microbiota or, as a safer alternative, the supply of defined microbial communities with the aim of restoring a healthy intestinal microbial community.

## 1. Dysbiosis

In healthy hosts, the different members of the microbiota of a specific niche, such as the colon, live in a balanced state which is characterized by the abundance of species having a relationship of commensalism or mutualism with the host. This situation is often referred to as "eubiosis". In contrast, the term "dysbiosis" is defined by the US National Library of Medicine as an alteration of the qualitative and/or quantitative composition of a microbiota. Some authors have dismissed dysbiosis as a valuable concept in the field of the microbiota and microbiome due to the ambiguity of the definition [1-3]. In the microbiota literature, dysbiosis is usually referred as an "imbalance of the microbiota" or as a "loss of homoeostasis", terms themselves that are not, or only rarely, defined. For these authors, this vague definition

is responsible for the lacking of scientific value associated to the term dysbiosis.

In this context, defining the concept "normal microbiota" is not an easy task since the composition of the microbiota of every niche of the human body is influenced by a wide variety of factors and, therefore, a high interindividual variability seems unavoidable even under physiological conditions [4-6]. However, dysbiosis states are usually characterized by the loss or underrepresentation of normally dominating species and their replacement by otherwise minority species often including potential pathobionts or opportunistic pathogens.

Several factors such as antibiotics and other drugs, stress, genetic factors, diet or lifestyle might trigger dysbiosis. If the dysbiosis-triggering factor is too intense or last for a long time, the process frequently leads to a disease state, which may become chronic or recurrent, and often characterized by inflammation and/or infection by opportunistic microbes. In early life, dysbiosis of the mucosal surfaces of the mother may lead to an altered vertical transfer of the first colonizers and impairing initial acquisition of the microbiota may have short and long-term consequences for the host health [7].

Dysbiosis has been reported to be associated with a long and ever increasing list of illnesses: from most infections occurring in the skin (and associated glands, including mammary glands) and in the digestive, respiratory and genitourinary tracts, to metabolic syndrome; and from almost any disease included among the so-called autoimmune diseases (inflammatory bowel diseases, chronic fatigue syndrome, multiple sclerosis, autism-spectrum disorders, etc.) to a wide varieties of cancer. It must be highlighted that in many cases, evidence is insufficient to distinguish if dysbiosis precedes the disease or if the disease leads to a dysbiosis state [4].

## 2. The "biotics" family

The suffix "-biotics" is used to refer to various nutritional strategies that can be used to lead the microbiota towards a state more favourable to the health of the host. The term "biotic" derives from the Greek word "biȏtikós", which means "belonging to life". Research and commercial development of products specifically designed to modulate the microbiota (probiotics, prebiotics and synbiotics) or derived from certain components of the microbiota (paraprobiotics or postbiotics) have made significant progress in recent years. This advance has been supported by the scientific and clinical achievements that are providing the evidence that supports the health benefits of some of these products (Figure 1).

## 3. Definitions and consensus of probiotics

Towards the end of the 20th century, there was a wide availability of products advertised as probiotics and beneficial to health in the market. This was due to the increase in scientific articles documenting the potential benefits of some strains and, also, to a growing demand by consumers who were increasingly aware of the importance of our microbiota in health. Unfortunately, some companies had taken advantage of this situation to apply the term "probiotic" to products that did not fit this concept or whose alleged benefits lacked any scientific basis. This misuse, intentional or not, had been favored by the absence of an international consensus on the methodology to evaluate the efficacy and safety of these products.

In this context, FAO and WHO convened in 2001 a consultation of international scientific experts who examined the scientific evidence on the functional and safety aspects of probiotics in food. The consultation generated a definition of probiotics as "*live microorganisms that, when administered in adequate amounts, confer a benefit to the health of the host*" [8]. This definition was very slightly modified by the International Scientific Association for Probiotics and Prebiotics (ISAPP) in 2014 [9] and nowadays is unanimously accepted by the scientific community. In 2002, the FAO/WHO committee published a series of recommendations and guidelines for the systematic evaluation of the functional aspects and safety of probiotics, as well as the requirements necessary for naming a

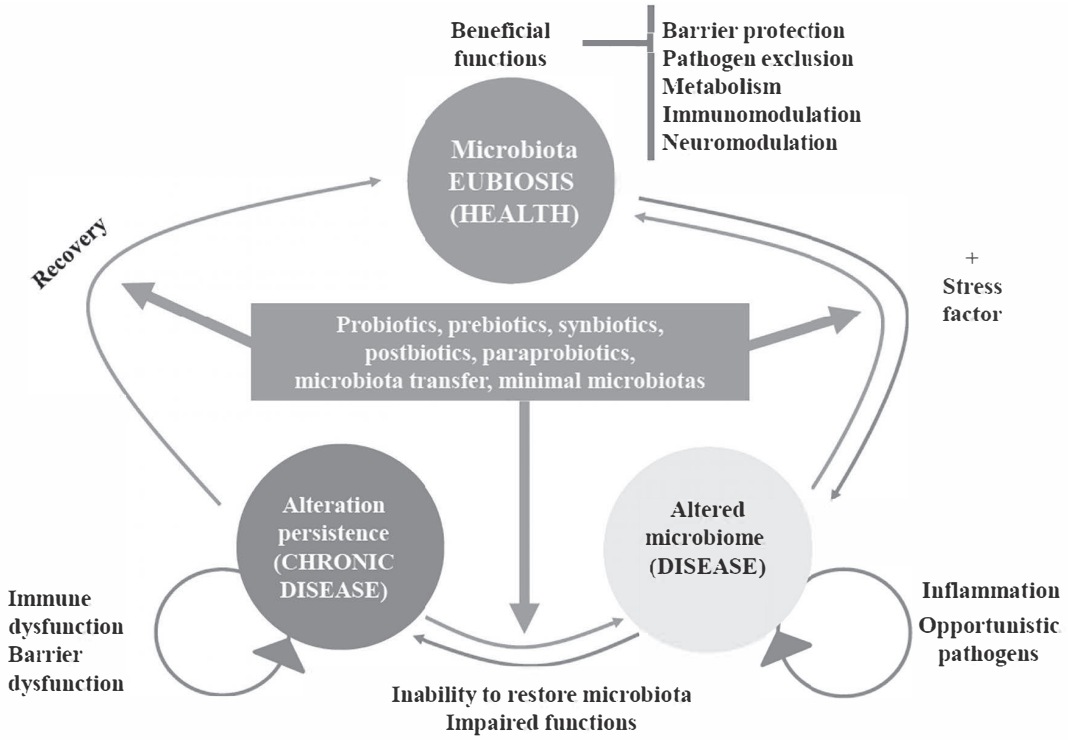


*Figure 1. Schematic representation of the potential consequences of dysbiosis in the human microbiota and the different approaches for its modulation and, eventually, restoration to a eubiosis state.*

microbial strain as "probiotic" and for their use in foods [10].

Sometimes the term "new generation probiotics" is used to refer to those species that are part of the indigenous microbiota from a certain location (e.g., *Faecalibacterium prausnitzii* or *Roseburia intestinalis* in the gut or *Nitrosomonas eutropha* in the case of the skin), where they play relevant roles for our health, but whose requirements for their production and stabilization have made their commercialization to date impossible. In addition, other terms have been proposed to group those probiotics that have specific effects on certain systems or diseases, such as "psychobiotics", "oncobiotics" or "fertibiotics", and the list is very likely to grow rapidly.

Within the Spanish context, in 2009, a consensus was approved on the definition and the characteristics and beneficial properties of probiotics in the frame of the first scientific meeting of the Spanish Society of Microbiota, Probiotics and Prebiotics (SEMiPyP) [11]. The SEMiPyP consensus supported the definition proposed in the joint FAO/WHO report; in addition, it was specified that the viability character of the definition of probiotic must be extended throughout the shelf life of the products, and that the products had to contain the sufficient quantity of microorganisms necessary to exert the indicated benefit. In the commercial production of probiotics, strict quality systems must be implemented so that the identity of the probiotics is verified and the supply of an effective concentration of viable probiotics is guaranteed. The SEMiPyP consensus also excludes components or substances produced by microorganisms from the definition of probiotics, although it has been described that they can exert healthy biological effects. These aspects have given rise to new concepts such as paraprobiotics and postbiotics (see below). Furthermore, the SEMiPyP consensus specifies that the probiotic concept must be associated with spe-

cific strains. Therefore, each probiotic must be identified by genus, species, subspecies (if applicable) and a unique alphanumeric designation. The document also details that the demonstrated benefit for a specific strain is not applicable to another of the same species, until its health benefit is also scientifically demonstrated in that strain. In this sense, it was also agreed that the demonstrated benefit for a strain in a specific health condition (e.g., diarrheal process) is not valid for any other indication (e.g., allergy). Neither the scientific evidence observed on one type of population should be extrapolated to another population that varies in age (e.g., children and the elderly) or in physiological state (e.g., gestation and lactation). The consensus supported the need to carry out the systematic evaluation recommended in the FAO/WHO documents for a strain to acquire the name of probiotic, and emphasizes that clinical trials are absolutely necessary to demonstrate the benefit in human health.

More recently, the ISAPP has also published a consensus document on probiotics [9], which extends the concept to new, well-defined microbial species and consortia from human samples that present sufficient evidence of safety and efficacy. In its consensus document, ISAPP proposes to establish a "probiotic framework" where all the sectors involved are integrated so that the existing knowledge about probiotics is translated into products that represent a benefit for society. This framework should include the following partners: (a) scientists by generating quality studies that provide scientific evidence on the efficacy and safety of the use of probiotics; (b) the industry to obtain products of high quality and with validated and understandable claims; (c) consumers by receiving reliable information to make informed decisions; and (d) regulators protecting the rights of consumers. In the probiotic framework, it is necessary to advance in the knowledge of the mechanisms of action of these microorganisms to be able to select the most effective strains for each condition and, in this way, to provide scientific foundations for the declaration of health benefits of a probiotic.

## 4. Selection and development of probiotics

The process from the initial selection of microbial strains to their commercialization as probiotics involves multiple steps and must take in account a variety of scientific, clinical, technological, regulatory, economic and communicative aspects that are briefly described below [12].

### *4.1. Taxonomic allocation*

The identification of a microorganism at the species and strain levels is an essential requirement for any microorganism that is intended to be commercialized. The microorganisms used as probiotics include yeasts (*Saccharomyces cerevisiae*) and bacteria of different genera (*Lactobacillus*, *Streptococcus*, *Enterococcus*, *Pediococcus*, *Bifidobacterium*, *Propionibacterium*, *Bacillus*, *Escherichia*). The assignment of an isolate to a specific species is an important issue in order to assess its safety in the European Food Safety Authority (EFSA) frame since risk assessment is much easier for those species that, based on a history of safe use, have a Qualified Presumption of Safety (QPS). The QPS list is periodically reviewed, incorporating new taxonomic units if the available data supports it. In this sense, the recent reclassification of the genera *Lactobacillus* and *Leuconostoc* into 25 genera, including 23 novel ones, may mean an important change in the short term [13]. The identification of an isolate at the strain level is essential to enable its traceability in laboratory tests, clinical trials and during production and marketing. Identification at the strain level is also essential if there are beneficial effects specifically associated with that strain and they cannot be extrapolated to other strains of that species.

### *4.2. Safety*

Safety of probiotics is an essential requirement for their use and its assessment has to take in account

the strain, the form of administration, the level of exposure, and the state of health of the host must be taken into account, among other factors [14]. The cases in which it has been possible to establish a relationship between the consumption of a probiotic and an adverse effect are rare and have generally affected people with serious underlying diseases or with a highly altered intestinal barrier. In theory, probiotics could produce four types of adverse effects: (a) pathogenicity; (b) production of undesirable metabolites; (c) excessive immunostimulation or immunosuppression in sensitive individuals; and (d) possibility of transmission of genes that confer resistance to antibiotics [14]. Among them, resistance to antibiotics seems to be particularly relevant due to the increasing rate of (multi)resistant bacteria. Consequently, the possible presence of transmissible genes that may confer antibiotic resistance to other bacteria (including those potentially pathogenic ones) present in the host microbiota is relevant in evaluating the safety of probiotics. The determination of resistance to antibiotics must be carried out according to internationally accepted procedures and respecting the updated criteria of EFSA. The safety evaluation of a probiotic must also take into account the excipients used in the formulation of the final products. In this sense, probiotic products must respect the current regulations regarding the declaration of allergens.

*4.3. Functionality*

From a functional point of view, the criteria for selection of probiotics usually include a series of prerequisites for the strain to reach its place of action in an adequate concentration, including due protection when required. Furthermore, the evaluation usually includes the study of properties that could be associated with a beneficial effect on a host. The functional properties by which a probiotic is selected can be diverse and complex [15]. Ideally, it would be necessary to know the desired function of a probiotic and the population in which it is intended to be applied to employ the most appropriate selection tests.

Assessing the functionality of probiotics requires verification of their efficacy in human trials. The use of animal models allows determining mechanisms of action and biomedical markers. Clinical trials in which it is scientifically determined whether probiotics exert a benefit and their magnitude are typically phase 2 and phase 3. Phase 2 studies evaluate the efficacy of a probiotic versus a placebo, preferably with a randomized and double blind design. The desirable result would be a statistically significant biological improvement relative to well-being, reduction of the risk of illness, faster recovery from illness, milder symptoms during illness or increased recurrence time. Phase 3 studies evaluate the efficacy of a probiotic versus standard therapy used to prevent or treat a given disease. The sample size must be calculated so that it has statistical significance. It would be desirable to carry out more phase 2 and 3 studies to promote the use of probiotics in the prevention and treatment of diseases in those cases where they can replace or complement conventional drugs.

*4.4. Development, production and commercialization*

The production of a high biomass of probiotics on an industrial scale in a cost-effective manner and its subsequent preservation while maintaining a high level of viability can represent a bottleneck in the commercial application of probiotics. To ensure the viability of probiotic strains in the quantities necessary to exert the beneficial effect, all stages of the production cycle (fermentation, concentration, freeze-drying, packaging, distribution and storage) must be taken into account [12]. In addition, the viability depends on the format in which the strains are to be administered; for example, the shelf life of refrigerated probiotic dairy products is markedly shorter than that of lyophilized products sold with a pharma-like presentation. In turn, there are various parameters (oxygen, humidity, temperature, etc.) and formats (microencapsulation, coatings) that play an important role in the stability of the product.

The first phase of the commercial development of probiotics usually consists of depositing the strain in the company bank and checking its identity, as well as evaluating its fermentation capacity and biomass production in mini-fermenters that simulate the conditions of the plant fermenters (temperature, pH, agitation, etc.). The second phase consists of scaling up in a pilot plant to evaluate productivity before and after freezing, lyophilization or spraying process and the study of stability at three months. In general, the goal for the product to be stable is a loss ≤ 0.2 log of colony forming units (cfu) in a mixture of cellulose (or other excipient) packaged in foil sachets stored at constant water activity (<0.2) and at a temperature of 25 °C. In the next stage, the workflow is determined so that the probiotic enters the production phase. After the first production, the price is defined on the basis of the viability of probiotics in the product (cfu/g) and the analytical validation of the mixture is completed. The final objective is the release of the product while long-term stability studies (two years) continue, both in refrigeration and at room temperature (25 °C). In general, the production process is intended to provide a product with a high concentration (> 5 × 1010 cfu/g) that, once dosed in the final packages, has a long shelf life at room temperature. In cases where this is not possible, the product must be kept refrigerated until it is sold.

It is essential to apply the principles of the HACCP (Hazard Analysis and Critical Control Points) system and good manufacturing practices to ensure that probiotic products reach the consumer with the highest quality. It is also relevant to ensure that manufacturing processes do not interfere with the functionality of probiotics. Quality control must take into account the presence of the strains at the appropriate concentration, their viability and stability and the possible contamination of the final product with other microorganisms [16].

The determination of the price can be based on costs, on the elasticity of demand, on the prices set by the competition for similar products, etc. In general, the probiotic products available in pharmacies and parapharmacies are not covered by the public health system and, therefore, tend to have a high final cost. This fact limits the scope of the product to potential users, especially when prolonged treatment is required. In addition, efforts should be increased to facilitate access to probiotics in vulnerable populations in developing countries, for example to prevent and control diarrheal processes in infants.

## 5. Definitions and consensus of prebiotics

Nutrient availability plays a fundamental role on intestinal bacterial composition and metabolism. In the Western diet, the total intake of non-digestible carbohydrates is 10-20 g/day in children younger than 10 years and 15-30 g/day in adolescents and adults [17]. Some of these carbohydrates are capable of modulating the composition and metabolic activities of the intestinal microbiota.

Although in the mid-20th century the existence of a "bifidus factor" present in breast milk was recognized as being responsible for the increase in bifidobacteria in the faeces of children, the term prebiotic was published for the first time in a scientific study in 1995 [18]. In this work, Gibson and Roberfroid defined prebiotics as "*an indigestible food ingredient that benefits the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, improving the health of the host*." Scientific and clinical advances, associated with the development of molecular techniques, have shown that the fermentation of prebiotics is not exclusive to lactobacilli and bifidobacteria, spreading to genera such as *Eubacterium* and *Roseburia*, given their ability to produce high amounts of butyric acid that could exert a protective effect against the development of intestinal diseases. On the other hand, species not recognized until recently as beneficial for gastrointestinal

health such as *Akkermansia muciniphila* and *F. praustnizii* seem to play a fundamental role in the regulation of inflammatory processes. Successive definitions of the prebiotic concept include the requirement that these must be "specific" or "selective" for the bacterial taxonomic groups that promote health or their beneficial metabolic activities, being this distinctive in comparison with other groups of compounds. The fermentation of prebiotic carbohydrates is carried out by a limited number of bacteria, which may form microbial consortia in the case of the fermentation of complex carbohydrates.

In 2015, SEMiPyP published a consensus document on prebiotics, based primarily on non-digestible carbohydrates with known chemical structures that have demonstrated scientific evidence of their benefit in human health [19]. This document emphasizes the notion that the health benefits of prebiotics must be demonstrated through clinical evaluation studies carried out in human populations with adequate scientific methodology and attending to a sufficient concentration to obtain the expected benefit.

Recently, ISAPP has expanded the concept of prebiotic to "*substrate that is selectively used by host microorganisms and confers a health benefit*" [20], expanding the concept of prebiotics to substances other than carbohydrates, such as polyphenols, polyunsaturated fatty acids and its corresponding conjugated fatty acids. Furthermore, it is indicated that modulation of microbial groups can occur at locations in the human body beyond the gastrointestinal tract (e.g., vagina and skin). This consensus maintains the requirement of the selective nature of these compounds over the human microbiota and that the health benefits derived from a specific microbial influence are demonstrated through appropriate clinical studies. The ISAPP consensus on prebiotics also establishes a necessary framework for interaction between different stakeholders, both the consumers who can access the benefit of these compounds from an individualized prescription perspective, and the regulatory authorities in charge of allowing the labelling of a benefit for proven health. In this prebiotic framework, scientists would have the responsibility to decipher research aspects such as the structure of prebiotic compounds, clinically relevant biomarkers of the beneficial effect or mechanisms of action, among others. However, one of the main challenges in generating scientific knowledge around the prebiotic concept is to establish the causality between a change in the structure or function of the microbiota and the determination of a biological effect beneficial to human health.

## 6. Types and characteristics of prebiotic compounds

The carbohydrates on which most characterization and application studies have been carried out as prebiotic compounds are mainly inulin, oligofructose (FOS), galactooligosaccharides (GOS) and lactulose disaccharide, which have been assigned the category of substrates with prebiotic properties scientifically supported by human intervention studies [19]. On the other hand, there are compounds called emerging prebiotics that are currently under study, including αgalactosides, resistant starch, pectooligosaccharides and non-carbohydrate prebiotics like polyphenols. Special mention deserves the human milk oligosaccharides (HMOs), a mixture of extraordinary complexity with more than 1,000 chemical structures described that act beyond mere substrates for the intestinal microbiota and influence numerous beneficial functions for the local and systemic health of the infant [21].

In summary, we can affirm that prebiotic compounds, when focused on gastrointestinal health, must: (a) present resistance and survive, at least partially, to acidic conditions and the digestive process (oral, gastric and intestinal), escaping intestinal absorption; (b) be selectively fermented/metabolized by a number of bacterial species, competitively and/or cooperatively, in the large intestine inducing a modulating effect on

| Prebiotic | Origin | Chemical composition | Health claim, support and mechanism of action |
|---|---|---|---|
| Inulin [34] | Inulin occurs naturally in chicory, onions, asparagus, leek, garlic, wheat and artichoke. | Fructo-oligosaccharides having a degree of polymerization ≥ 10. The fructosyl-glucose and the fructosyl-fructose linkages are β(2→1) and β(1→2), respectively. | Claim: maintenance of normal defecation by increasing stool frequency (provided that is does not the result in diarrhea). Support: six human studies support the claim after consumption of at least 12 g/day of chicory inulin. Mechanism: inulin could exert an effect on stool frequency by stimulating bacterial growth in the gut and by increasing bacteria cell mass and fecal bulk. |
| Lactulose [35] | Disaccharide derived from isomerization of lactose. | 4-O-β-D-galactopyranosyl-D-fructose | Claim: reduction in intestinal transit time within the normal range might be a beneficial physiological effect.<br>Support: several studies have shown a consistent statistically significant effect of lactulose at a dose of 10 g per day on reduction in intestinal transit time.<br>Mechanism: in colon, lactulose is broken down to lactic and formic acid by the action of β-galactosidases from colonic bacteria. This process leads to an increase in osmotic pressure and slight acidification of the colonic content causing an increase in stool water content and softening of the stools. |
| Carbohydrates resistant to digestion [36] | Different botanical sources including legumes, rice, potato and cereals, among others. | Non-starch polysaccharides, oligosaccharides and resistant starch. | Claim: the consumption of food/drinks in which non-digestible carbohydrates replaced sugars (mono- and disaccharides, on a weight-by-weight basis) induced lower postpandrial glycaemic and insulinaemic responses that sugar-containing food/drinks.<br>Support: in the case of FOS, up to three human intervention studies and three mechanistic studies support the health claim.<br>Mechanism: non-digestible carbohydrates are resistant to hydrolysis and absorption and do not contribute to post-pandrial glycaemia. |

*Table 1. Approved health claims of prebiotics by the European Food and Safety Authority (EFSA)*

the intestinal microbiota; and (c) they must confer beneficial effects for health and well-being, and may act locally and/or systemically.

The health benefits associated with the intake of prebiotics described in the scientific literature are very diverse, and include: (a) local effects such as modulation of the intestinal microbiota, resistance to pathogen colonization, improvement of intestinal function and integrity of the intestinal mucosa, as well as an improvement in mineral absorption; and (b) systemic effects such as regulation of the immune system, regulation of appetite, effects on energy metabolism and anticancer properties. However, despite numerous basic and clinical research scientific studies on prebiotic compounds, only a small number of health claims have been approved by EFSA and those include reduced intestinal transit, decreased glycemic index, and increased number of stools (Table 1). This is mainly due to insufficient chemical characterization of prebiotics, the absence of intervention studies in humans, as well as the lack of evidence on biomarkers that demonstrate the specific beneficial effect of probiotic consumption. In this sense, EFSA [22] published a guide document on the scientific requirements necessary to accept health claims on digestive and immune function, in order to facilitate an adequate experimental design in the evaluation of compounds. It is important to highlight that, according to the EFSA criteria, the changes or modulation of the intestinal microbiota must be accompanied by clinical and/or physiological benefits.

## 7. Synbiotics

The term refers to a product that combines at least one probiotic and one prebiotic. Synbiotics are often defined as synergistic mixtures of probiotics and prebiotics that beneficially affect the host by improving the survival and colonization of live beneficial microorganisms in the host. The ISAPP is currently preparing a consensus document on this term that establishes, among other aspects, whether the com-

bination that incorporates probiotics and prebiotics should be evaluated to demonstrate a health benefit of the synbiotic, beyond the benefits that the components have demonstrated separately.

### 8. Paraprobiotics and postbiotics

The concept of probiotics indicates that the viability of microbial cells represents an essential condition to guarantee their beneficial effects. Numerous studies have shown that viability and dose are essential for certain actions or applications of probiotics. However, dead cells (or some components thereof) and certain microbial metabolites can also exert beneficial health effects. Therefore, new terms have been coined to refer to those microbial elements or products that do not require viability to exercise certain beneficial biological functions.

Paraprobiotics, also known as "inactivated probiotics", "non-viable probiotics" or "ghost probiotics", are defined as inactive (non-viable) microbial cells or cell fractions that, when administered in adequate amounts, confer a health benefit to the consumer [23]. Therefore, they are or derive from microorganisms that lost their viability after undergoing processes that have induced structural and metabolic changes in their cells. As reflected in the definition, microbial cells can be intact or lysed, with or without their corresponding cell extracts (which are chemically complex and whose composition is not well defined).

Postbiotics are soluble bioactive factors (metabolic products or by-products) secreted by living microorganisms or released after cell lysis, which confer some physiological benefit to the host [24]. Such soluble factors include short-chain fatty acids, enzymes, peptides, theicoic acids, peptidoglucan-derived peptides, endopolysaccharides and exopolysaccharides, cell surface proteins, vitamins and organic acids. Unlike cell extracts that can accompany paraprobiotics, postbiotics must be chemically defined products. Some authors have suggested expanding the concept of postbiotics to also include paraprobiotics; however, they seem to be two clearly differentiated concepts despite the fact that, on occasions, equivocal terms have been used ("metabiotics", "biogenics", "metabolites of cell-free supernatants", "cell lysates", etc.) that can cause some confusion.

It should be noted that, unlike probiotics and prebiotics, there is still no consensus for the definition of paraprobiotics and postbiotics. Furthermore, there are certain products whose classification can be difficult or ambiguous. For example, probiotic products contain a mixture of living cells (probiotics in the strict sense) and dead cells (paraprobiotics), the proportion of which varies during the shelf life of such products depending on, among other factors, storage conditions. On the other hand, there are compounds that, simultaneously, can behave as prebiotics and postbiotics.

These types of products have the advantage over probiotics that elude the technical challenge of keeping the microorganisms viable and stable in the product at a high dose from when they are produced until they reach the target site in the host. However, the composition of paraprobiotics and postbiotics must be defined and standardized, which is not a simple objective since there are numerous variables to take into account [25]: (a) these types of products can be obtained from a wide range of probiotic species; (b) they can be obtained through a wide range of methods; (c) the composition can be influenced by the methods of obtaining, processing and preserving and, consequently, the host response to these products depends on the production and marketing process; and (d) the methodologies for evaluating the biological and clinical effects can vary substantially.

### 9. Other approaches for modulating the microbiota: microbiota transfer and minimal or synthetic microbiotas

In recent years, transplantation or transfer of faecal microbiota (FMT), also known as "faecal bacterio-

therapy", has aroused great interest as a method to modify complex microbial ecosystems in pathological situations. It consists of administering a suspension of faeces from a healthy person to another person who has a disease characterized by intestinal dysbiosis with the aim of restoring the intestinal microbial community. That is, the gut microbiota is transferred from one person to another, including not only cultivable microorganisms but also those that currently cannot be cultivated and, consequently, cannot be administered in the form of a conventional probiotic [26].

The main reason for the popularity of faecal transplants is the high efficacy it has shown in the treatment of certain diseases, especially in recurrent infection by *Clostridioides difficile* (new taxonomic name for *Clostridium difficile*), with success rates of up to 94% [27]. Despite its popularity, FMT faces significant practical problems stemming from the extraordinary microbiological, immunological and biochemical complexity of faeces, the composition of which can vary even within the same person, depending on numerous factors. For example, faeces could become a source of harmful substances or microorganisms that can pose a health problem in the medium and long term. On the other hand, it is a biological sample that is impossible to standardize, which limits its application on a large scale. Therefore, there is a need to design and develop new biotechnological processes that allow applying the principle of faecal transfer in a reproducible way.

In this sense, the concept of minimal microbial communities, representative for a specific niche, can open new therapeutic avenues to modify the intestinal microbiota of people with various pathologies [28]. A substitute for the intestinal microbiota, elaborated from pure cultures of 33 bacterial species isolated from faeces from a single healthy donor, was successfully applied to treat cases of recurrent *C. difficile* infection in which antibiotic therapy had failed [29]. This pioneering study demonstrated, for the first time, that a designed minimal microbiota in the laboratory it is able to treat antibiotic-resistant infections. The advantages of minimal microbial communities are easily noticeable since they allow: (a) control the composition of the mixture of strains; (b) guarantee the absence of harmful substances and pathogens, including viruses; and (c) manufacturing on an industrial scale and reproducibly using biotechnological processes. In fact, the aseptic production of mixtures of viable strains (lyophilized or frozen) in industrial fermenters is a well-known technology that is used in the production of starter or probiotic cultures. On the other hand, some of the bacterial species that should be included in faecal minimal microbiotas are strict anaerobes which remain largely non-cultivable at present. As a result, technological improvements will be required before minimal microbiotas can be commercialized.

The success of the FMT has motivated the study of other forms of microbiota transfer, including skin microbiota transplantation [30] and vaginal microbiota transfer, both for the initial colonization of babies born by caesarean section, [31] and for the treatment of bacterial vaginosis refractory to antibiotic treatment [32]. Similar approaches have also been suggested for transfer of the microbiota from human milk. Sometimes mothers of premature infants are unable to produce enough milk to fully meet the nutritional requirements of their babies, and the infants' diet needs to be supplemented with donated human milk, which is pasteurized and, consequently, loses its microbiota. However, the small amounts that the mother can produce serve to inoculate pasteurized milk from a donor that, after incubation, largely recovers the microbiota that characterizes human milk [33].

The implementation of these synthetic microbial communities in state-of-the-art therapies would be of great benefit to patients and, furthermore, would allow us to advance our understanding of the human microbiome. However, many studies will be necessary to define the functions that the components of a minimal microbiome must play in the prevention or treatment of specific diseases.

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