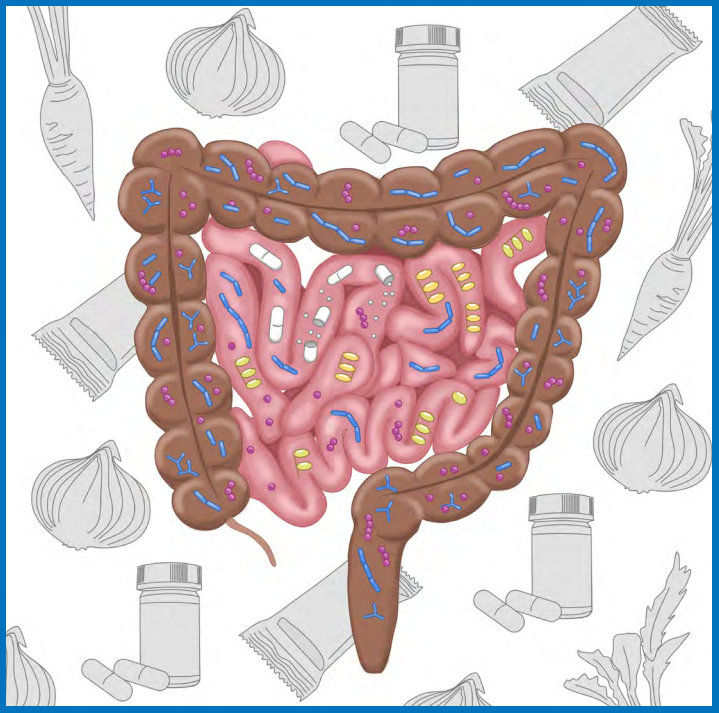
ILSI EUROPE CONCISE MONOGRAPH SERIES

DIETARY PROBIOTICS, PREBIOTICS AND THE GUT MICROBIOTA IN HUMAN HEALTH



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International Life Sciences Institute

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This publication is made possible by support of the ILSI Europe Prebiotics and Probiotics Task Forces. Industry members of these task forces, as well as the composition of the Board of Directors and the Scientific Advisory Committee are listed on the ILSI Europe website at www.ilsi.eu.

DIETARY PROBIOTICS, PREBIOTICS AND THE GUT MICROBIOTA IN HUMAN HEALTH

Revised Concise Monograph

ILSI Europe Concise Monograph. Probiotics, Prebiotics and the Gut Microbiota. 2013:1-32.
Commissioned by the Prebiotics and Probiotics Task Forces.

The image on the front cover is produced by Pinja Kettunen, SciArt.

ISBN: 978-2-9603031-0-0

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ILSI Europe thanks the following for revising this monograph:
Cath Mersh, editor; Nathalie Delzenne, Paul de Vos, Mary Ellen Sanders, Kristin Verbeke, scientific reviewers.

FOREWORD

Since the first edition of this monograph was published in 2013, there has been a growing interest in prebiotics, probiotics and, more recently, synbiotics. Prebiotics and probiotics are now commonly found in a range of food products and dietary supplements for infants, children, adults and seniors, along with specific groups such as sportspeople and expectant mothers. Although also being researched for applications in pharmaceuticals, animal feeds and non-dietary applications for humans, the focus of this monograph is food applications.

Research to understand the composition and function of the microbiota has expanded dramatically in recent years with the development of ever-more sensitive analytical techniques and increased computer power. These tools have facilitated data mining to better understanding the microbiota's relationship to physiology and health. The role of prebiotics and probiotics in human health has also been investigated in greater depth, contributing to a better understanding of known health benefits and the discovery of new target health benefits. Therefore, an update on these findings is appropriate and timely.

The popular first edition of this monograph highlighted the need for an easily understandable and objective source of information for interested non-specialists. On this basis, the ILSI Europe task forces on prebiotics and probiotics agreed to produce this revised second edition, drawing on input from experts in the respective fields to reflect recent advances. The objective is to provide an easily accessible introduction to the abundant scientific knowledge on prebiotics, probiotics and the intestinal microbiota and how they impact the human host. For this reason, the monograph does not address detailed regulatory aspects, which vary between countries and regions.

The challenge in nutritional sciences is develop knowledge that can better enable consumers maintain health, support normal bodily functions and reduce the risk of disease through good nutrition. Instead of testing clinical endpoints of disease, validated markers of health or disease risk are assessed through nutritional intervention studies. Influencing biomarkers of disease risk often requires an in-depth understanding of the underlying mechanisms. This is where future research in prebiotics and probiotic science will add to existing knowledge and evidence. Due to the complexity of the systems with which they interact, such as the intestinal microbiota and the immune system, understanding the mechanisms that drive observed health benefits is a scientific challenge.

The scientific understanding of prebiotic and probiotic mechanisms has grown substantially in recent years. Although effects are often strain and product specific, some prebiotic and probiotic benefits may be driven by common, shared mechanisms and may therefore be generalizable. The use of emerging physiological and analytical tools in a multidisciplinary research setting will enable the elucidation of further mechanisms. In this way, it will be possible to improve the understanding of prebiotic, probiotic and synbiotic health effects.

Based on recent sound scientific evidence, the mono-

graph is a valuable reference work, aimed at informing a wide audience about the intestinal microbiota and the prebiotic and probiotic nutritional concepts. Although this new edition is thoroughly revised and updated, we remain indebted to those who contributed to the first edition, including the author (Nino Binns), editors (Glenn R. Gibson and Mary Ellen Sanders), reviewers (Nathalie Delzenne, Lorenzo Morelli) and others who laid the foundation for this second edition.

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INTRODUCTION

Microbes, or microorganisms, include bacteria, fungi, yeasts and microalgae. They exist everywhere on earth, including hostile environments such as volcanoes, the ocean bed, the ice of the Arctic and Antarctic and in deserts. Incredibly diverse, they have adapted to their own particular niches over billions of years. To many people, microbes are best known for their role in causing disease, but they do much more than cause disease. In fact, they are essential to our planet and more recently there also is increasing evidence for a profound impact on our health. For millennia, mankind has harnessed their power in the production of fermented foods, including dairy and vegetable products, bread, wine and beer. Owing to their potential for very selective action, microbes are crucial to the development and production of pharmaceuticals, such as antibiotics, and to the production of food ingredients, such as vitamins, citric acid and acetic acid. They are also involved in the production of many other chemicals and enzymes and used in waste processing.

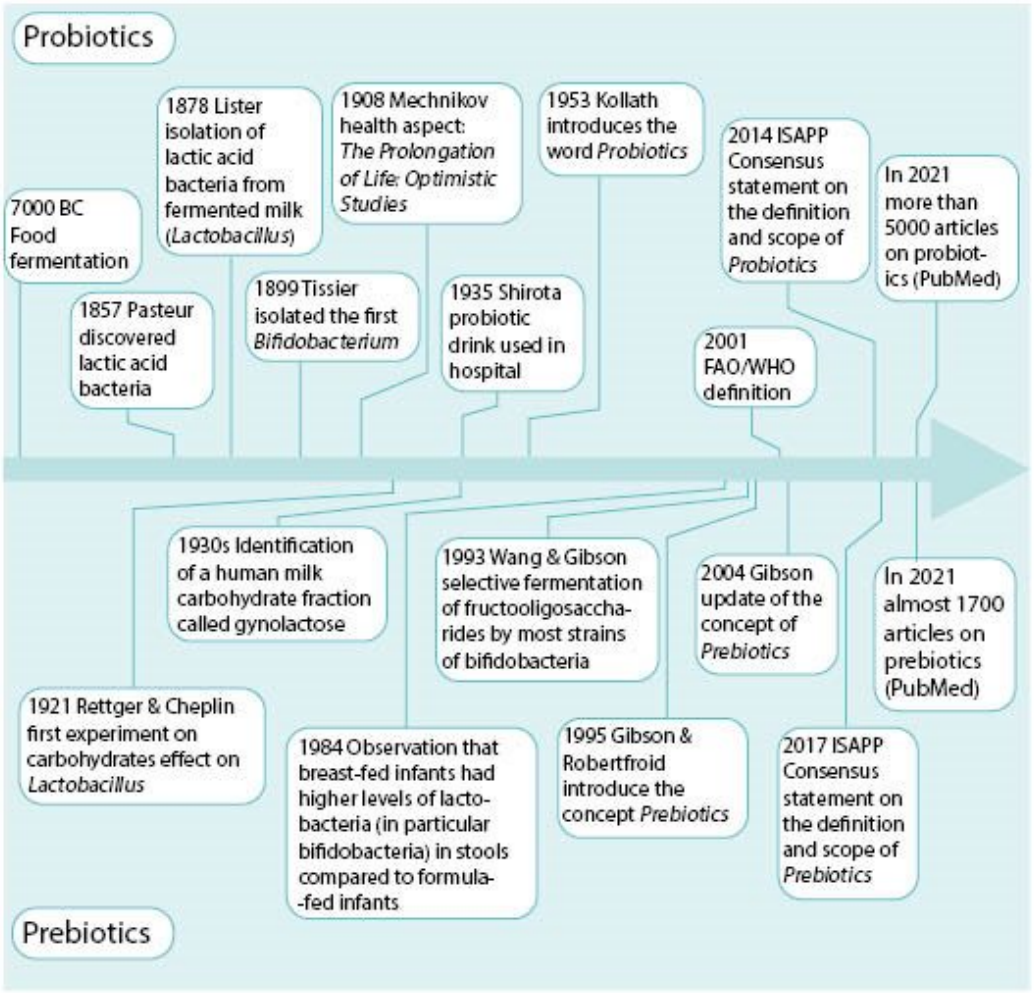
Most of the 1013 bacteria in the gut are present in the large intestine, or colon. In recent decades, interest in the gut microbial population – the microbiota – and its environment has intensified. Numerous research studies have shown that, far from being passive inhabitants of the gastrointestinal (GI) tract, the habitual residents of the gut (commensal microorganisms) interact with their host in an intricate manner. They may modulate the effect of potentially harmful bacteria or impact the host's GI tract physiology and digestion. More recently, they have been increasingly implicated in functions beyond the gut, such as glucose homeostasis, fat metabolism, immunity and mental health.

The idea that food-borne bacteria may be beneficial to health emerged at the turn of the 20th century and is usually attributed to Russian scientist Ilya Metchnikoff, winner of the Nobel Prize (Figure 1). He hypothesised that consumption of large amounts of fermented milk products – soured milk – could prolong and improve quality of life due to their content of lactic acid bacteria which limit the activity of undesirable microbes in the colon. Metchnikoff saw the intestinal tract as an organ that could be manipulated to improve health by adding the right types of bacteria. As a result, commercial yogurts and fermented milks gained some popularity after the First World War, but it was not until the 1980s that sales of products containing probiotics began to grow rapidly – first in Japan and then in Europe during the 1990s.

Probiotic bacteria are defined as "*Live microorganisms that, when administered in adequate amounts, confer a health benefit on the host*". They can interact with commensal bacteria and have a direct impact on the host. Disentangling these interactions is one of the key challenges for future research. Other key challenges are to understand their mechanisms of action, to map which probiotic strains confer specific health benefits and to define the necessary intake levels to achieve those effects.

The prebiotic concept developed more recently (Figure 1). The Japanese were the first to recognise the value

FIGURE 1.
Timeline with milestones in probiotic and prebiotic research.



of non-digestible oligosaccharides, initially in animals where their addition to piglet feed helped relieve and prevent diarrhoea. Japanese researchers also recognised the value of oligosaccharides in human milk and later demonstrated that consumption of fructo-oligosaccharides and galacto-oligosaccharides led to an increase in intestinal bifidobacteria and stimulated their growth in the human gut. However, it was not until 1995 that the scientific concept for human gut microbiota modulation by 'prebiotics' was introduced and since then, a wealth of research information has accumulated. The most recent definition of prebiotic by International Scientific Association of Probiotics and Prebiotics (ISAPP) is *"a substrate that is selectively utilized by host microorganisms conferring a health benefit"*.

Today, digestive health is the target of more than 60% of functional food products around the world, with prebiotic and probiotic products the most widespread. Although probiotics and prebiotics may be aimed at any site within the body, most take the form of food ingredients that function in the intestinal tract. From here, they target the host by distinct and complementary mechanisms of action.

This concise monograph will describe the concepts of probiotics and prebiotics for use in the human diet and will explore the scientific basis for potential human health benefits. Current research indicates that these food ingredients offer possible health benefits with reasonable certainly of no harm to the general population of healthy consumers. Indeed, a range of naturally occurring prebiotics and a number of probiotics, primarily from the former *Lactobacillus* genus and *Bifidobacterium*, have long been consumed throughout the world either as part of a traditional diet or in the form of modern functional foods and supplements. This is also the case with microbes like *Saccharomyces*. This monograph does not cover new probiotics being developed from population-wide microbiota research, which have not been historically used. These new generation probiotics are often linked to the treatment or prevention of disease and often fall under the rubric of drugs or live biotherapeutic products.

ROLE OF THE GI TRACT MICROBIOTA IN HEALTH AND DISEASE

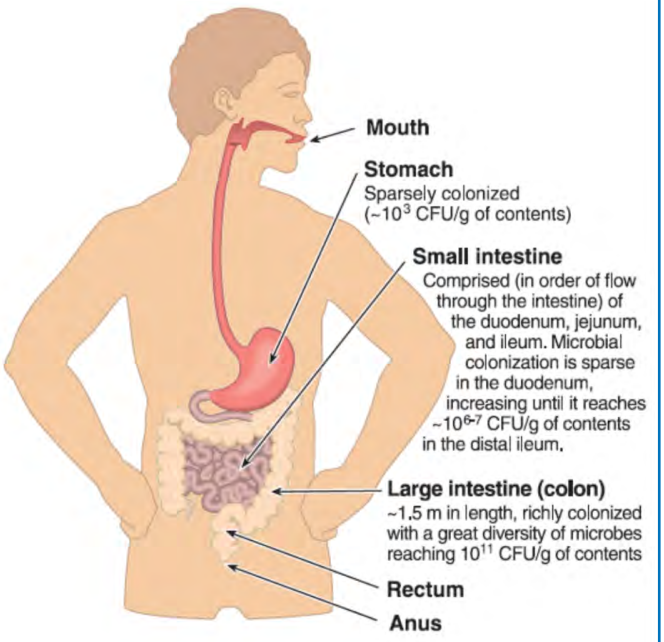
Microbiota of the GI Tract

Humans are home to many microbes, which are associated with tissues such as the skin, the vaginal tract, the respiratory tract and the GI tract. Existing throughout the GI tract, microbes differ in composition and number depending on the region (Figure 2), with the majority residing in the colon.

Streptococci are the most common of the numerous bacteria in the oral cavity. While bacteria do not colonise the stomach in high numbers due to the low pH and rapid transit, a healthy adult stomach may still contain around 10^3 bacteria in every ml of stomach contents, the main inhabitants being lactobacilli, enterococci, *Helicobacter* and bacilli. The duodenum also tends to be acidic, characterised by a rapid transit and pancreatic secretions and bile that create a hostile environment for microbes. Here, lactobacilli and streptococci predominate will cell counts of 10^2-10^4 per ml. Along the jejunum and, particularly, the ileum there is a gradual increase in the numbers and diversity of bacteria. Finally, the colon contains the majority of GI microbes, with as many as 10^{11} organisms per ml of intestinal content.

Prior to birth, microorganisms are absent from the GI tract but quickly colonise it during and after birth. The precise composition of the microbiota depends on factors such as the method of delivery and the environment in which birth takes place, the mother's microbiota and the manner of feeding. In healthy breast-fed infants, bifidobacteria dominate the faecal microbiota. Healthy formula-fed infants, on the other hand, previously had a wider range of organisms present. Along with bifidobacteria, these included bacteroidetes, clostridia, enterobacteria and streptococci. Today, however, the supplementation of infant formulae with prebiotics results in a similar bifidogenic effect to breast milk. After weaning, the number and diversity of the gut microbiota gradually change to resemble that of an adult. Once the adult-like microbiota of children is established at the age of two to three, they are relatively stable but subject to influence by lifestyle factors, such as diet, disease, antibiotics and other medication, and ageing. Gut microbes may be commensal (a person's native, colonising microbes) or transient (microbes just passing

FIGURE 2.
The human gastrointestinal tract.



Source: Binns N (2013).

through), and they can be beneficial, potentially harmful or pathogenic. Microbes considered to be beneficial usually ferment carbohydrates, do not produce toxins and may, for example, interact with the immune system or inhibit pathogens by competitive exclusion. Such microbes include *Bifidobacterium*, *Eubacterium* and lactobacilli.

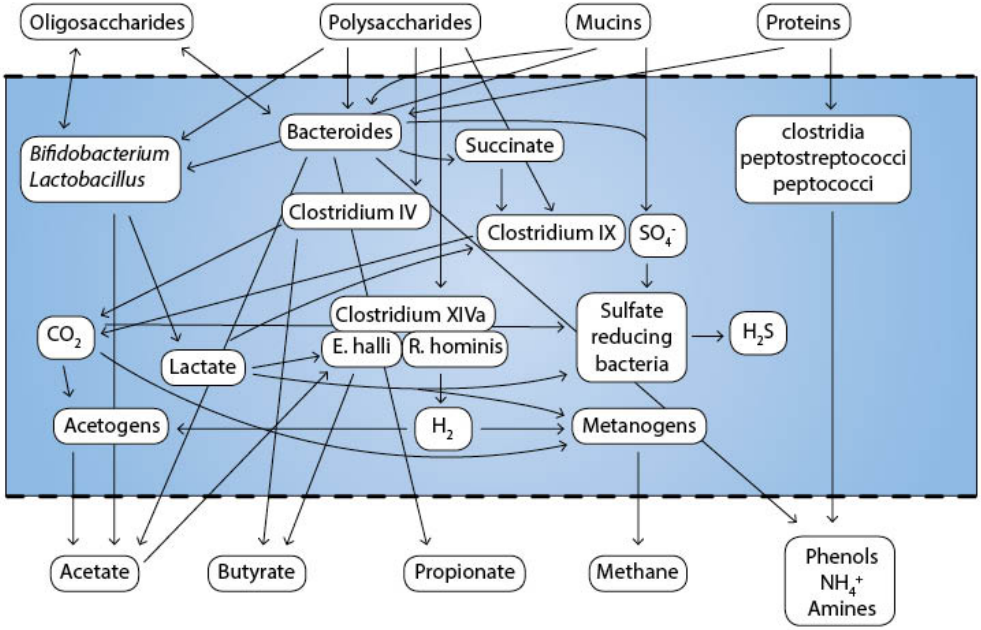
The human colon contains around a thousand anaerobic species, including the dominant bacterial phyla of Bacteroidetes and Firmicutes, minor phyla Actinobacteria, Proteobacteria, and Verrucomicrobia, and the Archaea kingdom. Numerous global projects are investigating the human microbiota, the microbiome (the microbiota plus its genes) and their relation to health status. Clearly, the gut microbiota has evolved with humans over millions of years and are essential for normal postnatal development and adult health. As the following sections describe, the microbes themselves and their anaerobic fermentation of undigested foodstuffs, fibres and prebiotics to short chain and branched chain fatty acids (SCFA and BCFA), as well as indole derivates and other fermentation products from proteins, play a key role in our health.

Due to the considerable microbiota variation between individuals, the numerous factors that affect the composition, insufficient knowledge on functions of individual microbiota species and the challenge to study what is happening inside the GI tract, it is not yet possible to define a 'healthy' or 'normal' microbiota. Nevertheless, deviations in richness, composition or function from the usual microbiota, known as dysbiosis, have been observed in numerous disease states. Whether the microbiota causes or partly causes the disease state, or if the change in microbes is a result of the disease itself, is still under investigation. Various approaches are being used to address this, such as human gut microbiota transfer to germ-free or antibiotic-treated animal models. Recent research also suggests that the normal microbiota is not simply a collection of microorganisms but reflects an inter-relationship between different bacterial groups that may work together to the benefit of the host. Based on the current evidence, a rich diversity of organisms in the GI tract is generally beneficial to the host.

Changes in the microbiota may result from many factors such as diet (high fibre, protein or fat, etc.), environment (stress), genetics, GI infection or use of oral antibiotics to treat a disease. Some alterations may be quite rapidly corrected without intervention, causing the microbiota to return to 'normal' for that individual. It is possible that some circumstances, for example repeated antibiotic use and/or certain diets (malnutrition or overnutrition), may result in a permanently disrupted microbiota. The capacity of prebiotics and probiotics to hasten or improve the correction of the microbiota following an insult is a subject of research.

All individuals harbour microbes that have opportunistic, pathogenic potential. The small intestine is the main target of many exogenous infections such as rotavirus, *Salmonella enterica subsp. enterica serovar Typhimurium* and some *Escherichia coli* types, which are usually contracted from contaminated water or food. Present in the colon, *Clostridioides difficile* is among the most

FIGURE 3.
Schematic diagram of the principle metabolic activity in the colon.



Source: Prof. R. Rastall, University of Reading.

important and may cause serious diarrhoea and inflammation when conditions in the gut are altered by illness or medication, allowing it to proliferate. Other undesirable colonic microbes such as proteolytic bacteria and sulphate-reducing bacteria do not cause acute disease but may be associated with the production of toxins, pre-carcinogens, carcinogens and toxic gases, such as hydrogen sulphide. This may result in the host becoming more susceptible to transient pathogens, antibiotic-associated diarrhoea and, possibly, inflammatory bowel disease and irritable bowel syndrome.

Bacterial Fermentation and Metabolism

As living organisms, all microbes require a source of energy in order to grow and reproduce. Many ferment carbohydrates (saccharolytic fermentation), a capability harnessed by humans in the production of various food or drink products. For example, in wine production, yeast ferments the sugars in grape juice to yield alcohol. In yogurt production, bacteria such as lactobacilli and streptococci ferment milk sugar (lactose), producing lactic acid which provides the characteristic tart

flavour. In sauerkraut production, bacteria naturally present in cabbage ferment sugars to form lactic acid in the absence of oxygen and the presence of 2-3% salt.

In like manner, microbes in the first part of the colon meet their energy needs by fermenting dietary and endogenous residues that have escaped digestion and absorption in the upper GI tract (Figure 3). Many metabolise carbohydrates and dietary fibre including polysaccharides (such as pectins, hemicelluloses, acacia and other gums, inulin and resistant starches), oligosaccharides (such as raffinose, stachyose, fructo-oligosaccharides, galacto-oligosaccharides and resistant dextrins), sugars (lactulose, non-absorbed lactose and non-absorbed fructose) and polyols (such as mannitol, lactitol, maltitol and isomalt). The main species in the colonic microbiota that ferment carbohydrates belong to the genera *Bacteroides, Bifidobacterium, Ruminococcus, Eubacterium* and lactobacilli. This microbial action results in the production of short chain fatty acids (SCFA), mainly acetic, propionic and butyric acids, lactic acid, which is mostly converted to acetic and propionic acid by gut microbes, and gases. The gases produced, H_2, CH_4 and CO_2, may contribute to the equilibrium of the microbiota. The nature of the fermentation products depends partly on the substrates as well as on the type of bacteria (Figure 3) and other individual host factors. SCFAs are absorbed, enhancing the uptake of water and salts and providing a source of energy for the host, while the gases are either metabolised by other microbes, absorbed, released as flatus or exhaled.

Bacteria also metabolise other components found in their environment (Figure 3). In addition to foodstuffs consumed by the host and not fully digested, substrates for bacterial growth include degraded bacterial cells, host-derived mucins, enzymes and sloughed-off intestinal cells. Peptococci and clostridia species metabolise proteins as a source of nitrogen for growth and yield branched chain fatty acids, such as isobutyrate and isovalerate, as well as a range of nitrogenous and sulphur-containing compounds, some of which may be harmful. For example, ammonia, amines, and phenolic compounds can, under certain conditions, lead to the formation of carcinogens, particularly in the left, descending colon where putrefactive conditions can prevail. Phytochemicals, such as isoflavones and polyphenols, are also metabolised to yield smaller components like equol and smaller phenolic molecules that are more readily absorbed. The impact of this microbial activity on human health is still under investigation.

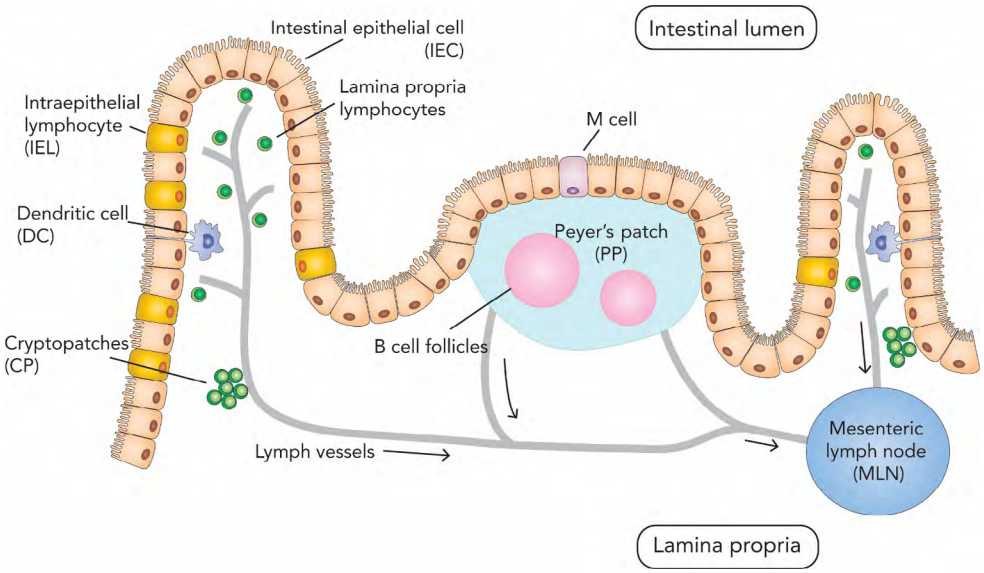
As bacteria grow in number, they comprise a portion of the bulk of the stools that form in the rectum. High stool bulk is related to a shorter gut transit time and to a lower risk of constipation and bowel cancer. If the dietary intake of fermentable fibre is suddenly increased, this can lead to intestinal discomfort, including abdominal distention, pain and looser stools. However, habituation will usually happen, and these symptoms tend to disappear. Although non-fermentable dietary fibre sources, such as wheat bran fibre, are the most important contributors to stool bulk, bacterial mass resulting from the fermentation of more soluble dietary fibres and carbohydrate residues also contributes to stool consistency and bulk.

The GI Epithelial Barrier and Immune System

The GI tract is sometimes described as the body's largest immune organ. It represents the host's greatest area of mucosal contact with the environment and contains as many as 80% of all immune cells. The intestinal microbiota is also a vital part of the body's defence system.

In a newborn infant, the GI tract is believed to be essentially sterile and tolerogenic, as it should not reject maternal cells or products. The immune system only becomes functionally mature and responsive following immune cell exposure to the myriad of foreign substances in the intestinal tract. Studies on animals raised in germ-free conditions have shown that the immune system is poorly developed in such animals and that they have

FIGURE 4. Schematic overview of the lymphoid elements of the gut associated lymphatic system.



Peyer's patches (PP) and mesenteric lymph nodes (MLN) are organised intestinal lymphoid follicles. (A–C) Pathways of intestinal antigen uptake: luminal antigen can be taken up by (A) intestinal epithelial cells, (B) interdigitating lamina propria dendritic cells, and by (C) M cells. The lymphatic drainage of PP and villus lamina propria goes to the MLNs (direction of lymph flow indicated by arrow).

lower levels of immunoglobulins and fewer specialised immune cells in their intestinal mucosa. Germ-free animals are, thus, much more susceptible to diseases compared to those that are conventionally reared. It is also known from these studies that microbial antigens, derived from the intestinal microbiota as well as the environment, play a crucial role in the maturation of the immune system.

The gut immune system is composed of scattered immune cells that are aligned between gut epithelial cells and the gut-associated lymphoid tissue (GALT). The GALT is organised into different compartments such as lymph nodes, lymph follicles and Peyer's Patches (Figure 4). The GALT is responsible for regulating adequate immune responses, which implies a strong well-regulated response to unwanted intruders and more tolerogenic responses towards desired microorganisms and food components. To perform these tasks, specialised cells, such as the M cells covering the Peyer's patches and the dendritic cells, which act as sentinels along the mucosa, allow passage of specific antigens – minute samples of viable or dead bacteria and protein and peptide fragments. The antigens are transferred to dendritic cells from the M cells. Acting as so-called antigen-presenting cells (APCs), these dendritic cells process and present the antigens to lymphocytes, a type of immune cell. In this way, the APCs are very important in stimulating a balanced immune response and, as is increasingly documented, having an impact beyond the gut (see Cross-Talk with the Host on page 33). It has been hypothesised that reduced exposure to microbes in industrialised countries has led to increased incidence of chronic immune dysfunction, leading to atopic (allergic) and auto-immune disorders or inflammatory bowel disease because of changes in the way the immune system has matured. This is known as the 'hygiene hypothesis'.

The integrity of the epithelial lining of the GI tract is critical to health. A disrupted intestinal barrier, also called a leaky gut, is implicated in a variety of diseases. In a healthy state, the epithelial cells form a tight barrier which is a first line of defence against pathogens. Proteins known as occludins and claudins help police the small intercellular space (tight junction) between cells to control access by foreign molecules and particles. Another cell type responsible for barrier function is the goblet cell, which can be found between the epithelial cells. Goblet cells secrete mucins – high molecular weight glycoproteins, which are the major component of mucus. The mucus layer helps protect the underlying epithelial cells from mechanical damage and the direct action of chemical compounds that are ingested or endogenously derived from gut secretions. Mucus is also a source of energy for many gut microorganisms.

The SCFA butyric acid helps to keep the gut barrier intact by serving as a major source of energy for the epithelial cells lining the colon and stimulating the growth and differentiation of epithelial cells. Butyrate is further known to stimulate mucous production by goblet cells. The amount and composition of mucus produced by the gut varies by site. The small intestine has a thick, quite mobile layer of mucus, while the colon has two layers: one mobile layer similar to the small intestine and a second thinner layer that is much more viscous and less permeable. Although microbes reside

predominantly in the lumen of the GI tract, they are also associated with the mucous layer. If the mucous layer is compromised, microbes may adhere to the cells that line certain areas of the small intestine. This is where beneficial microbes may compete with pathogens. Together, the epithelium and mucus form a barrier against pathogens, which is reinforced by specialised Paneth cells. Located in the crypts of the small intestine, the Paneth cells produce antibacterial peptides known as defensins, defensive enzymes such as lysozyme, and cytokines. The gut barrier function is maintained in close collaboration with the human microbiota.

The SCFAs produced by the microbiota are key mediators in the generation of tolerogenic lymphocytes. Furthermore, these microbiota-derived SCFAs can attenuate inflammatory mediators in the body and prevent excessive immune responses, for example by binding to special receptors, called G protein coupled receptors which migrate around the body. In addition, butyrate can regulate the expression of hundreds of our human genes via inhibition of histone deacetylase, which also modulates inflammation in the body. SCFAs can, for instance, act on immature blood cells in the bone marrow, a major site of innate and adaptive immune cell development, to promote the generation and development of specialised immune cells. In this way, diet and microbiota are linked to the gut-lung axis and can impact airway inflammation and respiratory infection outcomes. Emerging evidence also shows that SCFAs act on cells in the brain to attenuate proinflammatory and depression-accelerating mediators. This supports the notion that diet and microbiota are linked to the gut-brain axis and can impact behaviour and the sense of well-being.

Techniques to Explore the GI Microbiota

In the past, whether they were derived from foods, blood, tissues or excreta, microbes obtained from their initial source were characterised by culturing them in a laboratory. The cultured microorganisms could then be counted and identified by microscopy, biochemical observations and other taxonomic identification tests.

Faecal sampling has always been the mainstay of analyses of the human gut microbiota, especially given the limited accessibility of other GI sites. An inherent limitation of this approach is that the microorganisms expelled in the faeces and cultured in the laboratory do not necessarily accurately reflect what can be found in different segments of the gut, particularly the upper gut. Even colonic biopsy samples may not accurately reflect the actual microbiota since, prior to their excision, the colon is cleared with laxatives, disturbing the endogenous microbiota. Another challenge in understanding the composition of the gut microbiota is that numerous microbes have not yet been successfully cultivated under laboratory conditions.

In the early 1990s, research scientists developed a technique called fluorescence in situ hybridization (FISH). By using fluorescent probes directed at highly variable regions of the 16S ribosomal ribonucleic acid (rRNA) within the bacterial cells, different species and even sub-species of bacteria could be identified and quanti-

fied. From the mid-1990s, the introduction of sequence analysis of 16S ribosomal DNA, often obtained by polymerase chain reaction (PCR), enabled microbiologists to detect and identify microorganisms without the need to culture them. These techniques have allowed more accurate detection and identification of a far greater diversity of species, especially ones that were previously unknown or difficult to culture from faecal or intestinal samples. Culture-independent analysis of faecal samples has, thus, led to an increased understanding of the complexity of the intestinal microbiota. Modern techniques also allow very high numbers of samples to be analysed in parallel, increasing knowledge of the inter-individual variation and stability of the microbiota within individuals.

The co-development of high-throughput DNA sequencing technology and bio-informatics has enabled clustering and analysis of large amounts of data. With these tools, researchers have embarked upon major new projects to study the human microbiome – the collective genomes of all microorganisms in or on the human body. Large research consortia have started to study and characterise the complete microbial population of the human intestines and other parts of the body with the aim of associating the composition and function of the microbiome with health and disease. Notable projects include the US-led Human Microbiome Project, Europe-led MetaHIT project, Flemish Gut Flora project, Dutch Microbiome Project, American Gut Project and Million Microbiome of Humans Project (MMHP). A great deal of current research on probiotics and prebiotics interfaces with these research programmes on commensal bacteria. All these projects will help to shed light on the role of microbes, both commensal and ingested, in human health.

Analyses of the intestinal microbiota have made tremendous progress over the past two decades. Various molecular techniques make it possible to investigate the unknown members of the microbiota and their functionality and to follow specific strains. A number of challenges remain, however. As mentioned earlier, analysis primarily remains restricted to faecal samples that may not be representative of the microbiota higher up the GI tract or the mucosal microbiota. On the analytical side, new techniques enable accurate and quantitative analysis of the microbiota. Although the detection limits may currently still be too high to capture all the minor components of the intestinal microbiota, it is reasonable to assume this will improve in the future. More powerful computers and new statistical algorithms will also be required to deal with the ever-increasing amount of data.

THE PROBIOTIC CONCEPT

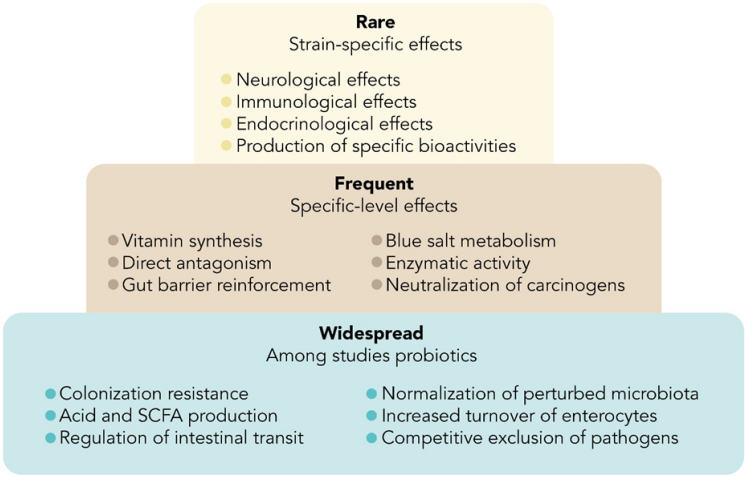
Definition and History

The word 'probiotic' (origins: Latin 'pro' = for and Greek 'bios' = life) was first used in 1954 to indicate substances required for a healthy life. The most widely used and accepted definition is one proposed by ISAPP *"Live microorganisms that, when administered in adequate amounts, confer a health benefit on the host"*. This definition is a grammatically-corrected version of the definition proposed by a 2001 FAO/WHO expert consultation.

The strain specificity of probiotic function is a well-accepted cornerstone of the probiotic field and refers to the need to link specific probiotic benefits with specific strains and doses. However, while certain benefits may be unique to specific strains, some of the mechanisms that drive probiotic benefits may be widespread among certain taxonomic groups. This is illustrated in the pyramid presented in Figure 5.

As mentioned, the original proposal that certain bacteria may benefit human health is usually attributed to Ilya

FIGURE 5. Probiotic effects are considered to be strain specific. They cannot be extrapolated to strains within the same species. There are, however, widespread effects seen across multiple probiotic strains of different species.



Metchnikoff, who worked at the Pasteur Institute at the beginning of the 20th century. His insights still resonate today: *"The dependence of the intestinal microbes on the food makes it possible to adopt measures to modify the flora in our bodies and to replace the harmful microbes by useful microbes"* and *"systematic investigations should be made on the relation of gut microbes to precocious old age, and on the influence of diets which prevent intestinal putrefaction in prolonging life and maintaining the forces of the body."* A French paediatrician, Henry Tissier, also published information at around the same time about his work on young children with diarrhoea. Finding that their stools contained fewer unusual Y-shaped (bifid) bacteria than were present in stools from their healthy peers, he suggested that patients with diarrhoea could be treated with these 'bifid' bacteria to help restore a healthy gut microbiota.

Until recently, high-quality scientific research supporting the purported benefits of probiotics was somewhat limited because the complexity of the gut ecosystem was largely underestimated. In the last three decades, however, research has progressed. With the application of molecular techniques, major advances have been made, both in the characterisation of specific probiotics and in our understanding of their mechanisms of action and health effects.

The Selection of Probiotic Candidates

Beyond safety, the selection of a probiotic strain is driven primarily by its potential to confer a health benefit on humans. In food and dietary supplement applications, it is commonly accepted that probiotics must survive until they reach the part of the GI tract where they exert their intended effect. For example, to be active in the colon, probiotics must resist salivary enzymes, stomach acid, small intestinal secretions of bile and enzymes as well as the pH changes and chemical milieu of other foods and beverages encountered during their passage along the GI tract. In addition, they need to compete with the resident microbiota. Finally, a selected strain has to fulfil a number of technological requirements, such as culturability on a large scale, genetic stability and viability in a food product or supplement. Thus, the development of suitable probiotic strains worthy of further study is a very complex and detailed process that can take substantial research effort.

TABLE 1.
Criteria to qualify as a probiotic

Taxonomic characterisation of strain that has been deposited in an international culture collection under the Budapest Treaty
Demonstration of safety of the strain, for its intended use
A well-defined health benefit, demonstrated from at least one relevant human study
Sufficient viability at the end of the shelf life of the product to deliver a health benefit

The most commonly used probiotics in foods are species from the former genus *Lactobacillus* and the genus

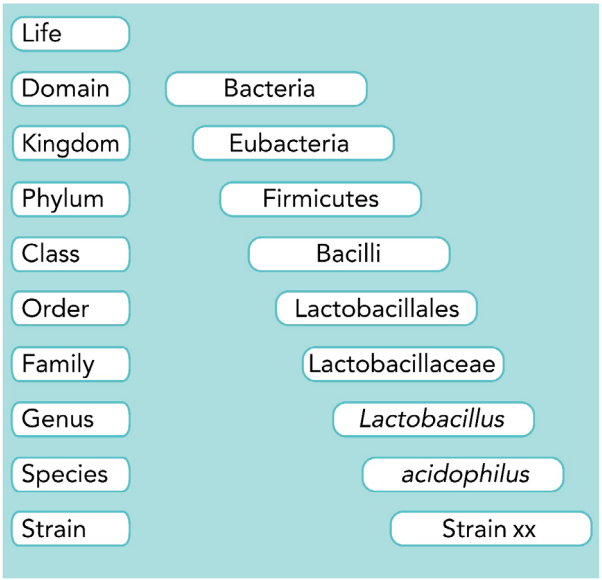
Bifidobacterium, but *Escherichia coli*, bacilli and yeasts such as *Saccharomyces* spp., have also been used. Probiotics have been isolated from healthy human commensal microorganisms, the environment or from foods, especially fermented foods. Some, but not all, probiotics are able to replicate and persist in the gut at least temporarily but disappear a few days after consumption has ceased. There are a number of important steps required to characterise each strain, as recommended by organisations including ISAPP and IPA. These criteria are summarised in Table 1.

Characterisation and Taxonomy

The determination of genus, species and strain is essential for full characterisation of a microbe. Using current methodologies, the phenotype and genotype of a microbe can be determined, leading to its correct assignment to a genus, species and possibly subspecies, or to become the basis for the description of a new taxon, Further, different strains of the same species can be distinguished by unique genetic and physiological properties.

Taxonomy provides a first view of the organism's main physiological and metabolic properties, including any potential safety concerns. Full taxonomic characterisation of probiotics is necessary for proper identification and naming of any strain. This ensures suitable description of the probiotic intervention so that clinical trials can be repeated, and health claim dossiers can be evaluated. Modern molecular methods are far more reliable than phenotypic methods for species and strain identification. Thanks to recent technological progress, sequencing the full genome of a strain is no longer very expensive or time consuming, and the information obtained can provide the expected detailed level of strain characterisation and enables a comparison with taxonomically related strains.

FIGURE 6.
Code of Nomenclature Example



(https://www.bacterio.net/)

The International Code of Nomenclature has to be followed in naming all microorganisms (Figure 6). In 2020, a taxonomic revision of the former Lactobacillus genus was published in which the genus now comprises 25 genera. A tool is available where the old and new

names comprising the former Lactobacillus genus can be found easily (http://lactobacillus.uantwerpen.be/).

Safety

Many probiotic organisms belong to genera represented in the functional group of bacteria known as lactic acid bacteria, which have been safely consumed for many years and, as such, are presumed to be safe food ingredients. To formalise and underwrite this principle, the European Food Safety Authority has developed a pre-market safety assessment system by which microorganisms can obtain qualified presumption of safety (QPS) status. Briefly explained, this enables a safety assessment of selected groups of microorganisms from a defined taxonomic group (e.g. genus or group of related species) to be made based on four pillars of information: identity, body of knowledge, possible pathogenicity and end use. If the taxonomic group and characterisation to strain level do not raise safety concerns, or if any safety concerns can be defined and excluded, the organism may be granted QPS status. Then, for any strain of microorganism that is unequivocally demonstrated to be from a qualified QPS group such as lactobacilli or *Bifidobacterium*, further safety assessment is limited to tests for antibiotic resistance. If a microbe is not covered by QPS, then a comprehensive safety assessment is likely to be required before it can be used in food. In the US, the safety of probiotic strains used in foods can be assessed using the Generally Recognized as Safe (GRAS) process or in dietary supplements using the New Dietary Ingredient (NDI) process. Both processes can result in notification to the Food and Drug Administration (FDA), although the burden of safe use rests with the manufacturer.

Applications of Probiotics in Food

Probiotic organisms are used in a variety of foods, the main category being dairy products, or as food supplements in capsule, powder or tablet form. Since viability is an essential property of a probiotic, the final product must contain an adequate amount of living probiotics to deliver the documented health benefit until the end of its shelf life. Addition of probiotics to foods or food supplements requires documentation of the benefits by good quality human trials of the relevant food product including the specific strain. These studies should also be able to demonstrate the safe, effective dose of the probiotic organism in food. Like legislation on food safety, the regulation of health claims on foods varies by country or region. Claims on commercial products containing probiotics must adhere to requirements that, in some cases, include pre-market approval of the claim by the regulatory authorities. For example, health claim approval in the US is managed by the Food and Drug Administration (FDA) and in Europe by the European Food Safety Authority (EFSA).

THE PREBIOTIC CONCEPT

Definition and History

The Japanese were the first to recognise the value of fermentable oligosaccharides, initially in feeding piglets and later, during the 1980s, with the identification of human milk oligosaccharides. However, it was not until 1995 that the prebiotic concept for gut microbiota modulation was introduced by Gibson and Roberfroid, who demonstrated a selective increase in faecal bifidobacteria upon consumption of inulin or oligofructose as a substrate. The prebiotic definition continues to evolve, the most recent one being agreed at an ISAPP consensus meeting in 2017:

> *"A prebiotic is a substrate that is selectively utilized by the host microorganisms conferring a health benefit."*

Characterisation of Prebiotic Ingredients

Although not stipulated as a requirement in the definition of a prebiotic, studies so far have mainly focused on carbohydrate compounds as a source of prebiotic activity. Most research has investigated fructans, specifically the polysaccharide inulin or fructo-oligosaccharides (FOS) extracted from crops such as chicory roots, FOS synthesised from sucrose or galacto-oligosaccharides (GOS) produced enzymatically from lactose. Human studies of these ingredients have confirmed selective fermentation and a shift in the microbiota. They have also been linked to health benefits and endorsed by ISAPP. The numerous emerging and candidate prebiotics include specific human milk oligosaccharides (HMOs), lactulose and other oligosaccharides, resistant dextrins, synthetic polysaccharides such as polydextrose, arabinoxylans and resistant starches, polyphenols and polyols such as lactitol and isomalt.

Some prebiotics occur naturally in foods such as chicory and other edible plants such as leek, onions, Jerusalem artichoke, wheat or agave. However, most foods contain only small amounts. So efforts are made to refine the active ingredients from these food crops or produce them by synthesis – for example, enzymatic, chemical or thermal processes – in order to attain the necessary level for foods to have a prebiotic effect.

Many prebiotics and candidate prebiotics today fit the nutritional and regulatory definition of non-digestible carbohydrates and/or dietary fibre* and are categorised as such on nutrient declarations. Like dietary fibre, they are resistant to digestion, and some fibres share their ability to ferment. However, established prebiotics can be distinguished from dietary fibre by the selectivity of their fermentation.

In addition to those prebiotics that are also non-digestible carbohydrates, recent studies suggest that polyphenols – secondary metabolites of plants – may interact with the gut microbiota in a two-way manner: bacteria degrade polyphenols, enhancing their bioavailability, and their metabolites may favour beneficial microbes, culminating in human health benefits. This is an important area of further investigation to comprehend the extent of bioactive compounds' health effects and enable the development of functional foods.

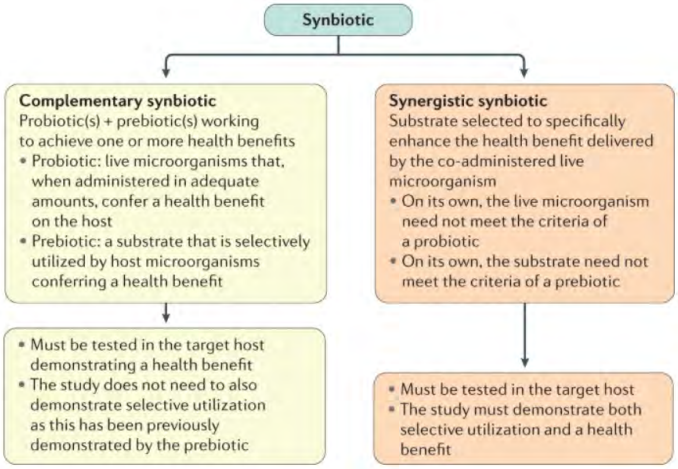
* Mono- and disaccharides (DP1, and DP2) are typically not considered as dietary fibre according to the EU and CODEX definitions of dietary fibre.

Criteria for Prebiotic Selection

The prebiotic concept is based on the selective utilization of a substrate by the host microorganisms, which may be one or a few types, which then promotes a desired health benefit. Thus, prebiotics have an action complementary to, but distinct from, probiotics.

It is essential to measure the effect of a candidate prebiotic on bacterial growth *in vivo*; it is not enough simply to know, for example, that fermentation of a substrate has taken place *in vitro*. Although *in vitro* tests can be used to screen potential candidates, the increase in target microbes following consumption of acceptable amounts must be quantified in human trials in order to establish the selective effect on the microbes. Such an effect should be demonstrated using good microbiological practices and, preferably, using modern molecular technologies, especially for the gut microbiota in order to take the complete microbial community into account. Equally important, human intervention studies are essential to demonstrate a health benefit of the potential prebiotic.

FIGURE 7. Synbiotics can be formulated using two approaches.



A complementary synbiotic comprises a probiotic plus a prebiotic, working independently to achieve one or more health benefits. A synergistic synbiotic is composed of a live microorganism and a selectively utilized substrate but neither needs to meet the minimum criteria for probiotics and prebiotics. Instead, these components are designed to work together, with the substrate being selectively utilized by the co-administered microorganism. Reprinted by permission from Springer Nature Customer Service Centre GmbH: Springer Nature, Nature Reviews Gastroenterology & Hepatology "The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of synbiotics" Swanson KS et al., Copyright ©2020, Aug; 17, 687–701, doi: 10.1038/s41575-020-0344-2 Epub 2020 Aug 21

The main site of action for established prebiotics is the colon. Such prebiotics must be able to resist the effects of gastric acidity and digestive enzymes in order to reach the colon intact. Once there, prebiotics confer their purported benefits through the selective growth stimulation of specific microbes. The foremost target genera for prebiotic action are bifidobacteria and lactobacilli, although this may change as knowledge of the microbial diversity and functionality expands.

Application of Prebiotics in Food

Some prebiotics or candidate prebiotics are naturally occurring and widely consumed at low levels in the normal diet, such as inulin-type fructans in wheat and onions. The commercial prebiotic ingredients GOS and inulin-type fructans are used in infant foods when their safety and efficacy have been demonstrated – in some countries, this may require premarket approval. In foods for general consumption, the target intake level of prebiotics like chicory inulin, FOS or GOS may range from 3g to 20g per day in multiple servings, depending on the specific prebiotic and dose required for the desired or approved health effect. These amounts can readily be incorporated into a variety of foods, such as cereals, bread, confectionery, biscuits, yoghurts, table spreads, sauces and drinks. Similar to probiotics, the health benefits of prebiotics need to be demonstrated in high quality clinical trials. A prebiotic health claim or reference to the bifidogenic effect of specific prebiotics on the gut microbiota must follow country-specific food regulations or follow an application supported by human and mechanistic studies.

Synbiotics

Prebiotics and probiotics may be combined into so-called synbiotics, defined by ISAPP in 2020 as *"a mixture comprising live microorganisms and substrate(s) selectively utilized by host microorganisms that confers a health benefit on the host"*. Criteria for synbiotics are delineated in Figure 7, including subcategories of complementary or synergistic synbiotics.

HEALTH EFFECTS OF PREBIOTICS AND PROBIOTICS

Research Approaches

In order to demonstrate that probiotic and prebiotic foods have beneficial effects on human health, evidence should be available from good quality intervention studies in human subjects, i.e. randomised, well-controlled, blinded trials. Supportive evidence may be gathered from *in vitro* laboratory models and, if essential, in vivo feeding studies in animals. *Ex vivo* laboratory studies, which examine blood or tissue samples taken from humans or animals, and *in vitro* studies, which examine isolated cells cultured in the laboratory and subject to various experimental conditions, may provide further documentation. While non-human studies can provide insights into various research questions, for example on mechanisms of action, they are not suitable to substantiate a human health benefit.

The lack of generally accepted biomarkers of GI health and immune function was among the factors that previously hampered research progress on the health impact of functional foods and gaining regulatory acceptance for making probiotic and prebiotic health claims. Biomarkers are surrogate markers of health endpoints. For example, the cholesterol level in blood is an accepted biomarker, which indicates risk for cardiovascular disease. Biomarkers of GI function, including stool frequency, consistency, bulk and transit time of the whole GI tract, can be used to demonstrate the benefit of prebiotics and probiotics and are currently accepted by EFSA, for example. While there are numerous biomarkers used in relation to the immune system, knowledge is lacking about the role of single biomarkers of function, such as immune cell function or cytokine levels, in the health of the immune system overall. Nowadays ratios of regulatory and pro-inflammatory cytokines as well as the generation of regulatory cells are used as measures for the impact of bioactive food components on immune function. The absence of validated biomarkers means that clinical endpoints, such as reduced susceptibility to infection, reduction in the duration of validated symptoms and improved antibody responses to vaccines during intervention with bioactive food components, are still more widely accepted as evidence of an immune benefit than changes in a single biomarker.

Another challenge common to all research in humans is inter-individual variation in response to any diet or intervention. This refers to the variability of responses observed to a specific endpoint among different subjects. Inter-individual variability depends on a wide range of factors, including host genetics, diet, microbiota, age, nutritional status and other lifestyle factors. Researchers try to account for these differences by including a sufficient number of subjects in a study and randomizing the subjects so such factors are evenly distributed among intervention and placebo groups.

When assessing the impact of a dietary ingredient on health, the effects may be more evident in people at high risk of, or diagnosed with, a disease than in healthy subjects. To observe effects in healthy people, often substantially larger study populations are required. Alternatively, in some cases, a healthy population with

mild symptoms may be used, for example subjects with occasional constipation.

When considering studies on prebiotics, it should be remembered that ISAPP and regulatory authorities currently only recognise a few prebiotics as established. Similarly, a limited number of microbes have been documented as probiotics. In general, prebiotics and probiotics should be consumed regularly for a certain period of time in order to confer a benefit

Impact on Human Health of Prebiotics and Probiotics

Gut Microbiota

Historical literature often reported higher proportion of bifidobacteria and lactobacilli as a sort of biomarker for a 'healthier' intestinal microbial composition. This was partly based on evidence from infants, where a bifidogenic effect was related to improved infant health, as discussed later in this section and in the section on mechanisms. Bifidobacteria ferment carbohydrates, produce lactate and acetate, are not toxigenic and have been extensively studied for physiological health effects in human clinical trials. However, this way of thinking may be an oversimplification of the actual situation. The composition or functions of a "healthy microbiota" have not been defined, and changes in specific members of the intestinal microbial community, with the exception of reducing specific, known pathogens, may not be clearly causally linked to health. Yet research continues on species such as *Akkermansia muciniphila*, *Faecalibacterium prausnitzii* and butyrate-producing species, in addition to bifidobacterial and lactobacilli species, in an effort to identify microbes important for health.

Studies of human subjects, including infants, as well as animal and *in vitro* studies have provided ample evidence that established prebiotics, particularly fructans and GOS, selectively increase the level of bifidobacteria and, sometimes, lactobacilli in the gut microbiota. The growth and metabolism of numerous other microbes may also be affected due to cross-feeding of substrates and the organic acids/SCFAs produced. Concomitantly, health benefits associated with prebiotic administration have been measured. Both selective utilization of the prebiotic by the resident microbiota – including those beyond lactobacilli and bifidobacteria - and a health benefit must be demonstrated in the same study to meet the criteria of a prebiotic. This requirement is substantial and may play a role in the limited number of recognised prebiotics so far.

In the case of probiotics, the consumption of adequate doses of *Bifidobacterium*, lactobacilli and strains from closely related genera often results in a measurable increase of these specific microbes in the faeces, while there may be a decrease in unfavourable organisms such as staphylococci. For pre-term infants, who usually harbour reduced numbers of bifidobacteria, there is good evidence that the ingestion of bifidobacteria not only increases their number but also reduces the number of clostridia. In practice, the effect of prebiotics and probiotics on the microbiota is somewhat variable and difficult to generalise.

The factors behind this are discussed in Techniques to Explore the GI Microbiota on page 10.

In addition to considering an increase in the number or proportion of certain microbes, it is also important to consider their metabolic capacity, which may be changed by prebiotic or probiotic consumption with no alteration in microbial levels. Recent human data on probiotics, obtained with new techniques, have enabled measurements of components which reflect the genes that are actively expressed at any given time. The link between gene expression and health outcomes will no doubt be the subject of future research.

Mimicking the Effect of Human Milk for Infant Formula

Human milk provides all essential nutrients for new born infants, and its composition adapts to the growing babies' evolving requirements. It contains a wide range of proteins, lipids and carbohydrates, including oligosaccharides. The human milk oligosaccharides (HMOs) in breast milk, with their fucosyl, galactosyl and sialyl structures, have been shown to be largely responsible for a bifidogenic effect and may be considered natural prebiotics. Indeed, breastfeeding contributes to the maturation of microbiota by providing necessary components to feed specific bacteria and ensure an enrichment of key members of the human microbiota. Some HMOs have shown a stimulating impact on the growth of bifidobacteria, in particular *Bifidobacterium longum ssp. infantis* and *Bifidobacterium breve*. Human milk and formula milk supplemented with specific HMOs and certain *Bifidobacterium* species in various human studies have been associated with reduced risk of atopic diseases, the development of the gut barrier, brain and cognitive functions and the maturation of the immune system.

The strong bifidogenic effect of human milk has been historically associated with better infant health. Consequently, over the past decade, prebiotics with a bifidogenic effect have increasingly been added to infant formulae. Numerous intervention studies show that infant formula supplemented with GOS, (long-chain) inulin and FOS, alone or in combination, help stimulate the growth of the bifidobacteria characteristic of breast-fed infants in a dose dependent manner. Further, infants fed formula with these oligosaccharides have a gut microbiota, stool pH and SCFA pattern similar to that of breast-fed infants. The stool consistency and frequency of prebiotic-fed infants (softer and more frequent) is also closer to that of breast-fed infants than infants fed standard formula. Infant studies further showed a link between some of these prebiotic mixes and a reduced risk of atopy and improved resistance to infection.

The use of specific levels of GOS, inulin and FOS prebiotics in infant formula is widespread and accepted as safe. Based on the growing capacity to synthesise individual HMOs and the clinical evidence of safety and physiological health effects in infants, it can be expected that HMOs will be increasingly used as new prebiotic supplements in infant formulae.

Stool Frequency, Consistency and Bulking

There is strong evidence that prebiotics and probiotics

can influence gut function. The effect of prebiotics is thought to be due to their fermentation in the colon, resulting in increased bacterial mass and the production of SCFAs, which are used as fuel by cells in the gut wall and as regulators of immune responses. The increased bacterial mass and SCFAs are thought to stimulate salt and water absorption, increasing the moisture level of the colonic contents through osmotic pressure. This contribution to increased stool weight and moisture may lead to softer stools and increased stool frequency. There is also some evidence that SCFAs, especially butyrate which is a key energy source for colon epithelial cells, have a positive effect on the intestinal mucosa function and peristalsis, which improves transit. Due to the inverse link between stool mass and transit time, prebiotics may also decrease transit time.

In some studies, prebiotics are reported to reduce symptoms of intestinal discomfort, such as bloating, abdominal pain and flatulence. Some prebiotics have also been shown to block proinflammatory receptors and signals during an inflammatory event in the small intestine and stomach, thereby improving gut function. In Europe, chicory-derived inulin has received an approved health claim in relation to supporting bowel function. As with dietary fibre in general, rapid high intakes of certain prebiotics can lead to issues such as flatulence, although such side effects generally subside if consumption is reduced or when habituation occurs.

Studies of certain probiotic strains have demonstrated an impact on gut function in terms of normalisation of transit time and stool frequency – a reduction in self-reported, minor digestive discomfort symptoms may also be associated with this. Improved stool frequency and transit time may reduce putrefactive activity, as indicated by studies that have found reduced levels of proteolytic fermentation products, such as cresol and indoles.

These stool-regulating effects are considered beneficial to gut health as they reduce the risk of constipation. An improvement of stool function is likely to be important to the general population since dietary fibre intake is almost universally lower than recommended in developed countries. Further, the number of people reporting digestive problems is extremely high – in some surveys accounting for more than 80% of women.

Improved Lactose Digestion with Probiotics

As discussed in the section on Bacterial Fermentation and Metabolism (page 7), many microorganisms ferment lactose, the sugar present in milk and many milk-based products. Although infants rely on lactose, which contributes 30% to 40% of the energy in breast milk, many populations around the world have a high proportion of adults who are unable to digest the sugar. The expression of the lactase enzyme is down-regulated in most humans in adulthood, with the exception of Caucasians and certain population groups in East and West Africa. Lactose intolerance is a condition where the colonic fermentation of undigested lactose results in abdominal pain, bloating, rumbling or laxation. There is evidence that live yoghurt bacteria and some probiotics may compensate for the lack of endogenous lactase in the human gut by metabolising lactose in the small intestine. The typical measure of improved lactose diges-

tion is a reduction in breath hydrogen excretion (breath hydrogen is usually raised when undigested carbohydrate reaches the colon and is fermented). This improved digestibility reduces the symptoms related to lactose intolerance in some lactose maldigesters.

Irritable Bowel Syndrome

Irritable bowel syndrome (IBS) is a distressing condition characterised by an array of symptoms, such as abdominal pain, bloating and altered bowel habits associated with constipation and/or diarrhoea. As similar symptoms occur from time to time in the general population, a specific set of criteria, known as the Rome criteria, was developed to support the consistent diagnosis of IBS. In industrialised countries, IBS may affect between 5% and 20% of the adult population, with rates higher in women and older people. Recently, there has been interest in the role of inflammatory processes as a potential cause of IBS. In a certain subset of subjects, it appears that previous gut infections play a role in IBS onset (post-infectious IBS). Furthermore, in some studies, lower levels of bifidobacteria have been observed in subjects with IBS than healthy subjects.

Because of the lack of good therapy for IBS and the identification of abnormal microbiota in IBS subjects, both probiotics and prebiotics have been investigated for their ability to help subjects manage the condition. A number of probiotic preparations have been shown to reduce the global symptom score (the sum of scores for individual symptoms) and abdominal pain. However, no change in diarrhoea, constipation or bloating was observed. In other studies, some strains had no effect or resulted in a worsening of symptoms. Although few studies have investigated the effect of prebiotics on IBS symptoms, some have shown that low doses led to an improvement in the condition, while a higher load led to an exacerbation of the perceived symptoms. Thus, additional research is necessary to determine whether prebiotics and probiotics provide any consistent benefits for those with IBS.

Mineral Absorption

Animal and human studies have demonstrated that some prebiotics contribute to improved mineral absorption. A wealth of data shows increased calcium absorption, growth and skeletal mass in rats, with some studies showing enhanced absorption of magnesium and iron. Further evidence of improved mineral absorption is also available from studies of pigs, considered a better model than rodents for extrapolation to humans. Numerous human intervention studies for specific prebiotics consistently show an increase in calcium absorption. One long-term human intervention study in adolescents has assessed the effects on bone health from a combination of oligofructose and long-chain inulin (50:50). After a year, bone mineral density and mineral content were significantly higher at certain bone sites in the supplemented group. Whether this effect is common to all prebiotics or unique to the studied formulation requires further clinical study. Several underlying mechanisms have been implicated. These include SCFA effects, which reduce luminal pH, thereby increasing calcium solubility and enhancing absorption. Other proposed mechanisms are the enlargement of the absorptive area, and interaction with tight junctions of intestinal epithelium.

Metabolic health, Weight Management and Food Intake

Diet and lifestyle are not alone in influencing the risk of obesity. The composition of the intestinal microbiota may also play a role. Consequently, it is no surprise that prebiotics and probiotics have been investigated in relation to metabolic health and obesity. Diabetes is intimately linked to obesity rates since a high body mass index (BMI) is the most critical risk factor. Numerous rodent studies of specific prebiotic fibres, mainly fructans, have shown consistent effects, reducing food intake and decreasing fat mass, though not necessarily body weight. In several studies, this effect has been associated with an impact of SCFAs in the distal colon on the activation of energy expenditure in brown adipose tissue. However, the overall evidence gathered from an increasing number of human studies, again mainly with fructans, is inconsistent, even though daily prebiotic consumption has promising effects on reducing appetite and maintaining or lowering body weight or fat mass. Mechanisms implicated include modulation of the microbiota that reduce circulating lipopolysaccharide (LPS), which can contribute to a reduction in local and systemic inflammatory processes. In addition, increased levels of SCFAs or a change in bile acid profile may induce the production of satiety hormones by enteroendocrine cells that strengthen intestinal permeability. Particularly acetate, produced by the fermentation of some prebiotics, is strongly implicated in improved insulin sensitivity and glucose homeostasis in human studies. Some, but not all, of these studies examined the composition of the gut microbiota, where shifts in the microbiota were confirmed.

Some probiotics may be beneficial in weight management. Although their consumption does not lead to weight loss, they may contribute to weight maintenance. Similar to prebiotics, this effect may be mediated through an influence on satiety and hunger hormones.

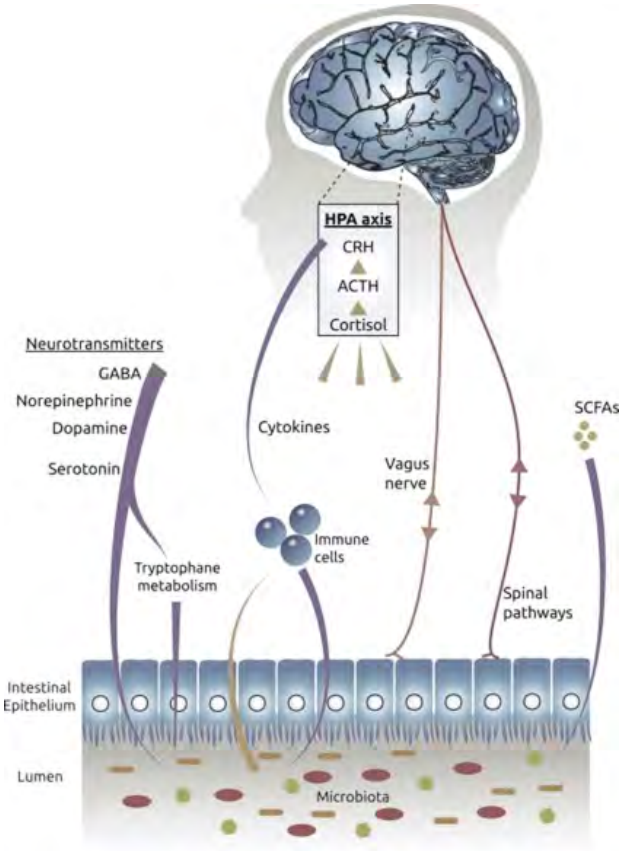
Mental Health Gut-Brain axis

Substantial evidence is emerging that the intestinal microbiota influences behaviour through the gut-brain axis (Figure 8). The enteric nervous system in the gut is the second largest nervous network after the brain. Especially in animal models, strong evidence indicates that manipulation of the gut microbiota and specific bacterial metabolites, such as indoles and SCFAs, can enhance the expression of important neurotransmitters, influence stress and anxiety and help cognitive functions. Recent studies have also observed the attenuating effect of bacterial metabolites on brain inflammation, which improved mental health. Evidence suggests that probiotics and prebiotics may positively affect responses to stress and anxiety among human subjects in experimental settings. However, more research is required to confirm these preliminary findings.

Gastrointestinal Infection

The small intestine is the main target of many GI infections, caused by rotavirus, *Salmonella* species and some E. coli types. As early as 1916, it was reported that *S. enterica subsp. enterica serovar Typhimurium* was cleared from the GI tract of healthy carriers when strains of the normal gut microbiota were introduced. Probiotics have long been associated with a purported ability to counteract pathogenic bacteria through so-

FIGURE 8. Routes of communication between gut microbes and brain, including vagus nerve, SCFAs, cytokines, and tryptophan. ACTH, adrenocorticotropic hormone; CRH, corticotropin releasing hormone.



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called competitive exclusion. This exclusion process refers to the competition for space and nutrients or the production of SCFAs, bacteriocins or hydrogen peroxide. Recent controlled studies have tested several potentially beneficial strains for their ability to reduce levels of pathogenic bacteria.

The first line of treatment for the symptoms of diarrhoea is oral rehydration – and no other dietary treatment should be substituted for this, especially in infants. However, some probiotics can be used as an adjunct under medical supervision where appropriate. Certain probiotics seem to be most effective in improving symptoms when the diarrhoea is the result of a viral (rather than bacterial) infection, and they are used in sufficient amounts early in the course of the infection. In terms of reduced susceptibility to infection, some studies have found a decreased risk of infection in infants, mainly in developing countries, and in institutionalised or hospitalised elderly. Efficacy is clearly strain related, hence some strains are effective and others not.

Some antibiotics can significantly disrupt commensal bacteria, resulting in side effects that include antibiotic-associated diarrhoea (AAD). The estimated incidence of AAD is as high as 25% for some antibiotics and may lead to patients failing to complete the course of treatment. There is evidence that specific probiotics can reduce the risk of AAD. Indeed, several meta-analyses conclude that the risk of AAD may even be halved in adults or the elderly, while the effect is less consistent in children. The observed effects relate to a limited number of specific probiotic strains. With regard

to prebiotics, it has been shown that the administration of FOS following an antibiotic treatment reduced the re-occurrence of AAD from more than 30% in the control group to less than 10% in the prebiotic group. As this was not associated with a decrease in subjects testing positive for C. *difficile,* this could suggest that the prebiotic had a stabilising effect on the microbiota, supporting the return of eubiosis.

C. difficile infection is a frequent cause of diarrhoea in institutionalised populations, for example in hospitals and long-term care homes. It is often associated with antibiotic use but can also be linked to other risk factors, such as age greater than 65 years or a compromised immune system owing to illness, medication or GI surgery. Research indicates that probiotics can reduce the risk of *C. difficile* infection or reduce the severity or duration of symptoms in adults.

A bacterium known as *Helicobacter pylori* is present in the stomach of a small proportion of young adults but in as many as 50% of those aged 60 years and over. It colonises the mucous layer next to the gastric epithelium and may cause acute gastritis (i.e. pain, bloating, nausea and vomiting), which can lead to chronic gastritis and peptic ulcers. Treatment involves long-term administration of strong antibiotics. Although probiotics do not speed up the eradication of *H. pylori*, several studies have shown that they reduce the side effects of treatment, thereby improving treatment compliance. Further, probiotics may contribute to less disturbance of the microbiota during *H. pylori* eradication therapy.

The microbiota of pre-term infants is less diverse and differs in composition from that of healthy, full-term infants. Potentially beneficial bifidobacteria, in particular, are not well established in the pre-term neonatal gut. The microbiota is further challenged by bacteria from the hospital environment, and the common use of antibiotics in pre-term infants puts them at increased risk of necrotising enterocolitis (NEC). Several hospitals have implemented the use of probiotics in their clinical practice, as many trials have shown that various probiotic strains and strain combinations can reduce the risk of NEC. Although the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) and the American Gastroenterological Association have conditionally recommended certain probiotics to reduce NEC rates, additional studies are required to optimise probiotic strains and doses. Furthermore, the use of live microbes in such a susceptible population makes confirmation of safety and quality a prime objective.

Impact on Immune Function, Inflammation and Response to Infections

Germ-free animals have, as mentioned, an underdeveloped immune system and GI epithelium, resulting in reduced resistance to infection compared with conventional animals. It is, thus, accepted that commensal organisms are vital for the maturation of the immune system and the gut barrier function. Moreover, there is increasing evidence that SCFAs and indoles produced by the microbiota have a positive effect on immunity, inflammation and response to infection. The potential for

probiotics and prebiotics to impact immune responses and reduce the risk of infections has been the subject of a number of human studies. Their results, combined with evidence from mechanistic studies showing changes in certain immune parameters, support the notion that the effect of probiotics and prebiotics on the immune system can translate into measurable health benefits.

The impact of prebiotics or probiotics on vaccination efficacy is a useful model for testing if they support immunity. It is possible that documented evidence may be acceptable to substantiate a health claim on food by EFSA. In addition, improved response to a vaccine may be a benefit in itself, as poor vaccine responses are an issue, especially in today's aging society. Several studies have shown that specific prebiotics could improve the antibody titres for various vaccines, such as hepatitis B, influenza and measles vaccines, in both humans and animals. In young adults, oral supplementation with long-chain inulin was shown to enhance the efficacy of a hepatitis B vaccine. Compared to other vaccines, hepatitis B vaccination is less efficacious and requires multiple shots to build an appropriate response. This makes the vaccine an excellent model for demonstrating the enhanced immunity effects of prebiotics, as studies can be conducted with fewer volunteers and over shorter study periods.

Animal studies have convincingly demonstrated that certain probiotic strains can both enhance the immune response to a vaccine and reduce the risk of subsequent infection. Human studies are much fewer, but an increasing number of well-controlled trials have been conducted. A number of studies reported that the response to vaccines against influenza, tetanus, cholera or childhood diseases could be enhanced by selected probiotics, measured by the number of subjects who responded to the vaccine, an increase in the level of serum immunoglobulins or higher responses of lymphocytes. The effects are strain-specific in terms of probiotic efficacy and, in the case of influenza, also specific to the pathogen strains. Several interventions have shown that specific prebiotics could improve antibody levels for various vaccines, such as hepatitis B, influenza and measles vaccines in humans. An animal study also showed a promising effect of a synbiotic in improving antibody responses to a vaccine as well as reducing oral Salmonella infection symptoms.

A number of studies of various age groups have investigated the potential for probiotics to impact susceptibility to upper respiratory tract infection (URTI), its duration and symptoms. Studies were conducted with a range of strains, some reporting reduced incidence or shorter duration and most reporting effects on symptoms. The evidence is convincing but requires probiotic consumption to commence well before the start and throughout the URTI season. Interestingly, studies have shown that, in addition to an improved quality of life, the consumption of probiotics also leads to reduced health care costs associated with URTIs.

Evidence is similarly increasing for specific prebiotics such as fructans and HMOs in supplemented formulae for infants and prebiotic supplements for children and the elderly. This shows a reduced sus-

ceptibility to URTI and associated fever or reduced sinusitis when supplemented groups are compared to control groups. Such improvement may be related to the production of SCFAs and effects on GI epithelial cells.

There has also been interest in the use of probiotics in urogenital medicine. Certain probiotic strains have been shown to improve recovery from bacterial vaginosis during antibiotic treatment. Potential mechanisms for the effect include antimicrobial antagonism, restoration of balanced lactobacilli-dominated microbiota or an enhanced immune response.

Allergic Conditions

An allergy can be defined in simple terms as an inappropriate immune reaction or over-reaction to an otherwise harmless foreign antigen (mostly proteins or peptides). In medical terms, it is described as a hypersensitivity reaction mediated by specific antibodies (IgE) or cell-based mechanisms. Common allergies include reactions to certain food proteins (e.g. milk, eggs, peanuts, tree nuts, soy, wheat/cereals, fish, shellfish and shrimps) or environmental allergens such as pollen (hay fever), house dust mite and pet hair. Food allergies are more common in infants and children than adults. The most serious form of allergy, resulting in anaphylaxis (which can be fatal when the throat and respiratory tract swell and restrict breathing) is rare, albeit a lifelong concern. Less severe symptoms of allergies are more common – about 2% for food allergies and up to 30% for respiratory allergies – and can substantially reduce the quality of life for allergic subjects.

The prevalence of allergy has increased in modern societies. There is growing evidence that the nature of microbiota acquired by the infant in the postnatal period has an important bearing on the maturation of the immune system. Some evidence indicates that atopic children tend to have a degree of dysbiosis, with more clostridia and fewer *Bifidobacterium* at genus and species level than non-atopic children. In addition, it seems that breast-fed infants are less prone to allergic conditions. On this basis, it has been suggested that prebiotics may help reduce the risk of developing atopy or reduce the associated symptoms of atopic eczema or allergic rhinitis. A follow-up of one intervention has produced promising evidence that prebiotic-supplemented infant formula may not only reduce susceptibility to atopy, but that the benefits also persist up to 2 years of age. Furthermore, studies have found reduced levels of IgE and some IgG fractions in infants at high risk of allergy, who were fed supplemented formulae for 6 months.

There have been several studies of the impact of probiotics on the development of allergic symptoms in high-risk infants. From these studies, it is clear that the mother must commence consumption of the probiotic prior to birth. After birth, the infant should continue to consume the probiotic for 6 months to 2 years. Results have shown a decreased risk of eczema at 2 years of age and beyond and generally indicate a strain-specific effect. Past and ongoing studies have also targeted the management or reduction of allergic symptoms. Regarding the use of probiotics in the treatment of atopic eczema symptoms, the results have not revealed a convincing health benefit. Symptoms of allergic rhinitis,

however, appear to be positively influenced by probiotic consumption. The difference in outcome between these two allergic conditions probably reflects the complexity of the allergic disease spectrum and the fact that a range of clinical designs was used. With respect to prebiotics, infants who received a formula supplemented with a prebiotic mix (GOS, inulin and pectic-derived acidic oligosaccharides) showed bifidogenic shifts and a lower risk of atopic eczema, the latter appearing to persist for 5 years. Moreover, a synbiotic combination of a probiotic and prebiotic mix of chicory FOS and long-chain inulin has been seen to improve atopy in newborn infants with cow milk allergy.

Chronic Inflammatory Gut Conditions

The inflammatory bowel diseases (IBD) are serious conditions, often with an unclear cause. They include Crohn's disease (CD), which can affect both the small and large intestine, and ulcerative colitis (UC), which is restricted to the large bowel. IBD is associated with a breakdown of the normal barrier function provided by the gut epithelial lining and its associated mucus. Whether the inflammation causes the breakdown of the barrier or a breakdown of the barrier allows inflammation to develop is not clear. It is known from studies that, compared to normal animals, germ-free animals are not susceptible to experimental IBD, and the presence of commensal bacteria can initiate and/or exacerbate inflammatory bowel conditions. Consequently, CD and UC may result from an inappropriate mucosal immune response to the GI microbiota in genetically susceptible individuals. There is also some evidence from clinical studies that the balance of different groups of commensal bacteria may be altered in IBD patients.

Numerous animal studies with probiotics and prebiotics have shown a positive impact on the risk and management of IBD. However, their effect in patients depends on the type of IBD. While clinical evidence shows that they are not effective in prolonging CD remission, other promising data indicates that some probiotics are useful in extending remission in UC. In another inflammatory bowel condition known as pouchitis, which can occur after surgery to treat UC, a specific mixture of probiotic strains appear to be effective in helping maintain remission. The potential for prebiotics and synbiotics to support IBD management, mainly through the reduction of inflammatory markers, has been seen in several small studies with fructans, However, as yet, it is not yet possible to draw any final conclusions about the effect of prebiotics or probiotics on IBD. Importantly, none of the trials conducted so far have raised concerns about their safety in patients with IBD at the tested doses.

Colon Cancer

Colon cancer has been linked to diets low in dietary fibre in epidemiological studies. So, the potential for prebiotics to reduce colon cancer risk has naturally also been investigated, mainly using *in vitro* techniques and animal models. Results from animal studies, with endpoints such as DNA damage, aberrant crypt foci and colon tumours, suggest that prebiotics may reduce the risk of colon cancer. This is supported by ample in vitro evidence. Further, some probiotics have been reported to reduce the expression of carcinogen-activating microbial enzymes and faecal carcinogen levels in humans.

Synbiotics were investigated in a few animal studies and were found to be more effective than prebiotics and probiotics alone. One synbiotic study in humans found a reduction in DNA damage and cell proliferation in colon biopsies. Potential mechanisms for a prebiotic effect on colon cancer risk have been identified in animal studies and include changes in gut bacterial enzyme activities, which modify the fermentation products, and up-regulation of apoptosis (programmed cell death – in this case of pre-cancerous cells). SCFAs resulting from the fermentation of dietary fibres, including prebiotics, are also known to modulate the immune system, which may have some inhibitory role in cancer development. Evidence that probiotics or prebiotics may reduce the risk of colon cancer in human subjects is lacking and requires robust, multi-centre, prospective human trials.

PROBIOTICS AND PREBIOTICS: MECHANISMS OF ACTION

Overall Mechanism

Probiotics, prebiotics and synbiotics are thought to work largely through direct or indirect effects on host functions and/or on the gut microbiota and environment, respectively. In the case of probiotics, live microorganisms are consumed in a range of dosages spanning from ~10^8 to 10^{12} cells/day, depending on the product. This large number of microbes has the potential to have a greater impact in the upper GI tract, where microorganisms are present in lower densities, but the impact is believed to extend to the colon. Prebiotic products enhance the growth of specific endogenous native microbiota and their metabolic products. In addition, prebiotics may stimulate the growth of specific probiotics when combined in synbiotics. Thus, probiotics and prebiotics share many common mechanisms of action, mediated through an impact on the microbes that inhabit the gut of the host. The mechanisms behind health effects that relate to prebiotics or probiotics (Figure 9A and 9B) alone have either been described or suggested in the section above on health effects.

Via their stimulation of commensal organisms, probiotics and prebiotics act on and interact with the host by two main modes of action or a combination of actions:

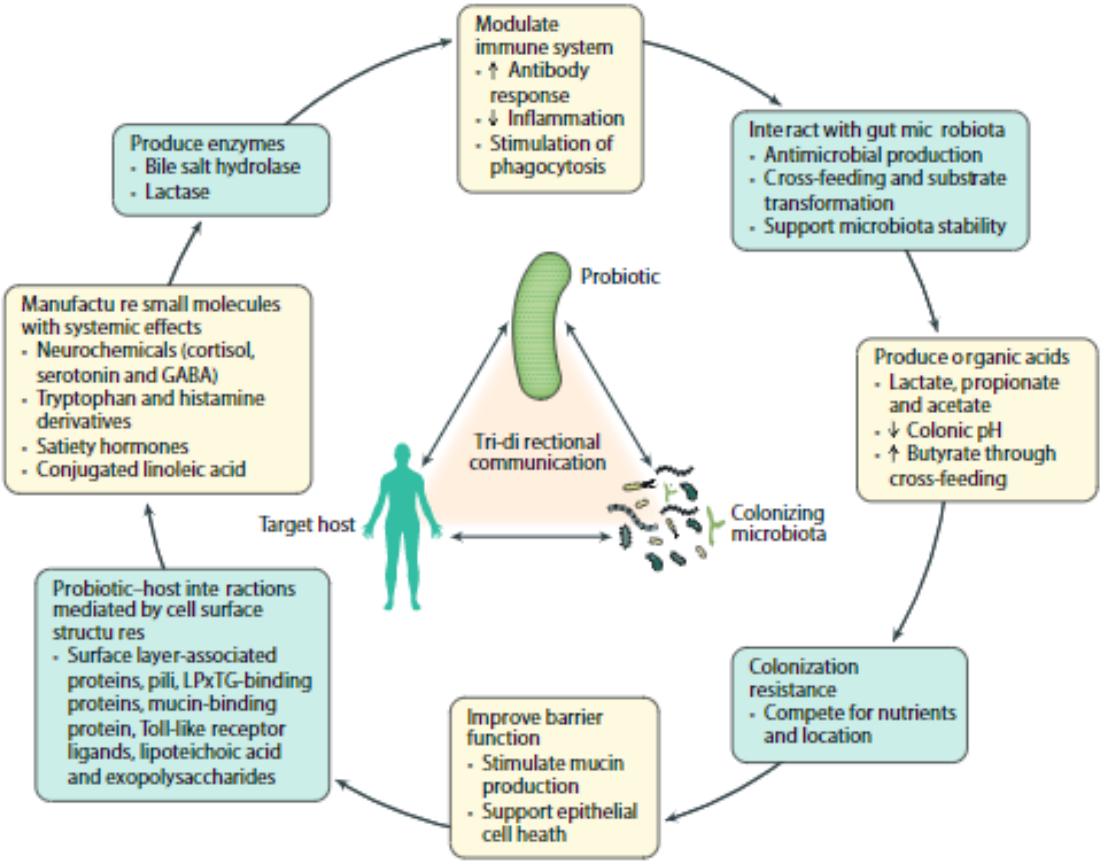
- Impact of microorganisms or their metabolites/enzymes on the host's GI tract and its microbiota (Figure 10A)
- Cross-talk with the host (Figure 10B)
  - Interaction with the host's cells and immune system
  - Impact of microbial metabolites on the host's metabolic homeostasis
  - Impact of metabolites on bone health
  - Modulation of brain function and health

GI Tract and its Microbiota

Most microbes, including the bifidobacteria and lactobacilli in the colon, preferentially ferment non-digestible carbohydrates that escape digestion in the upper GI tract, resulting in the production of SCFAs and a reduced pH in the colon. Bifidobacteria ferment fructans via their ß-fructofuranosidase enzyme, which either lack or have lower activity in other bacteria. This gives bifidobacteria a competitive advantage when exposed to fructans in the human gut. Some species of *Bifidobacterium* are able to ferment HMOs, as they can express the fucosidases and sialydases required for their fermentation. Similarly, the presence of β-galactosidase in lactobacilli or streptococci provides a competitive advantage in GOS and acacia gum fermentation. The metabolism of prebiotic fructans by bifidobacteria primarily yields the acidic compounds acetate and lactate. Cross-feeding of these fermentation products to other species gives rise to the SCFAs, butyrate and propionate, which are also formed directly from the fermentation of other dietary carbohydrates. A lower pH in the colon supports the multiplication and survival of commensal microorganisms that prefer acidic conditions and generally inhibits the ability of some pathogens to adhere, grow, translocate across the epithelium or colonise the GI tract.

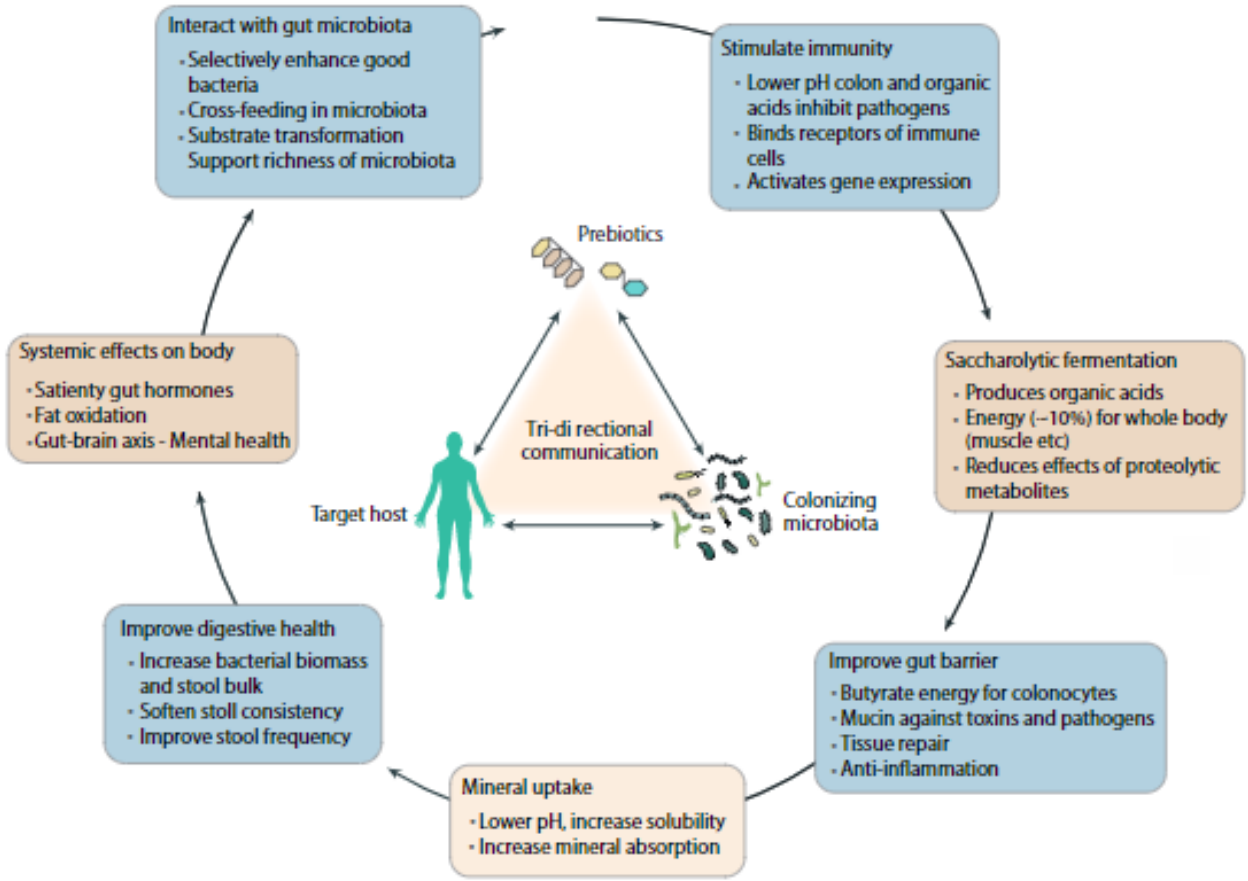
FIGURE 9. A

Probiotics and prebiotic mechanisms of action.



Probiotics. Diverse mechanisms are likely to drive probiotics benefits to host health. In some case, microbial products and cross-feeding other resident microorganisms, these mechanisms are driven directly by interactions with the resident microbiota. In other cases, such as direct interaction with immune cells, their effects might be direct via interaction with host cells. Overall, clinical benefits delivered by probiotics could result from the combined action of several mechanisms. GABA, gamma-aminobutyric acid.

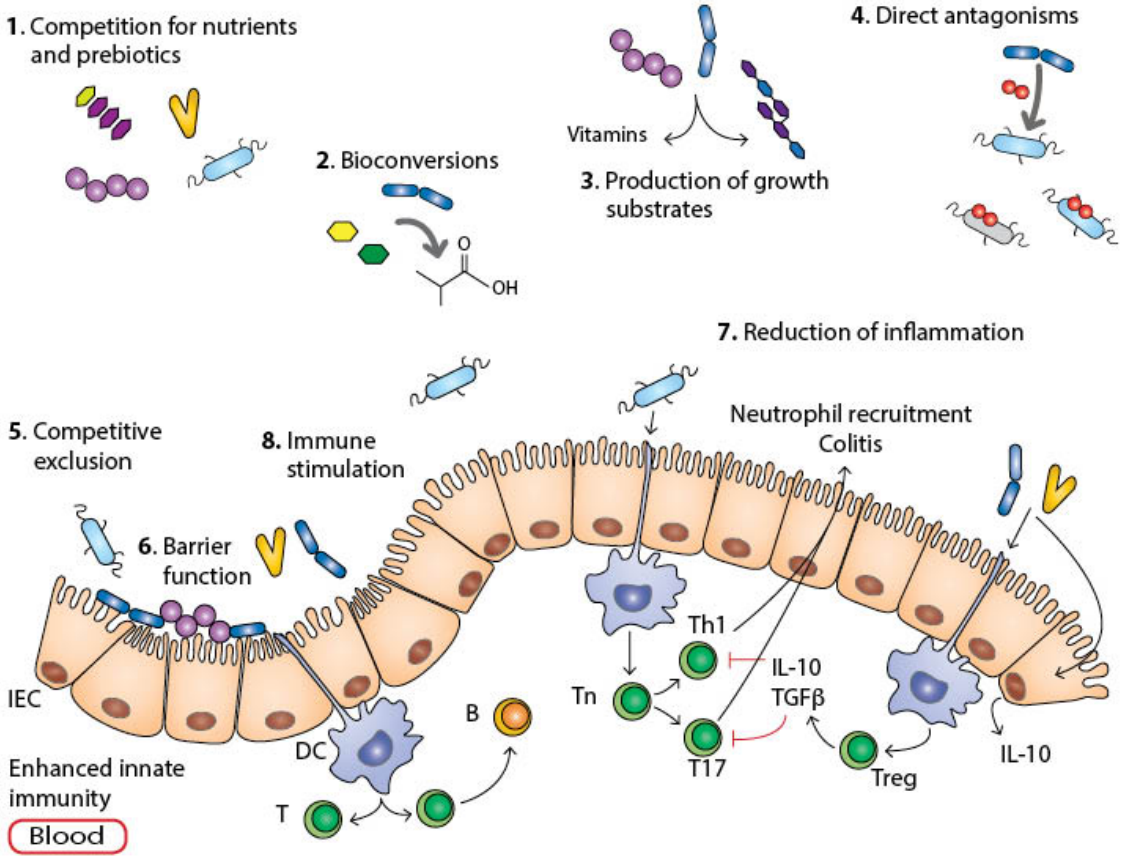
B



Prebiotics. Diverse mechanisms are implicated in prebiotic benefits to host health. Key is the selective stimulation of beneficial microbiota such as bifidobacteria and production of metabolites such as organic or short chain fatty acids which interact with the body. Such mechanisms together promote benefits such as enhanced digestive health, immunity, mineral uptake, lipid oxidation and brain health.

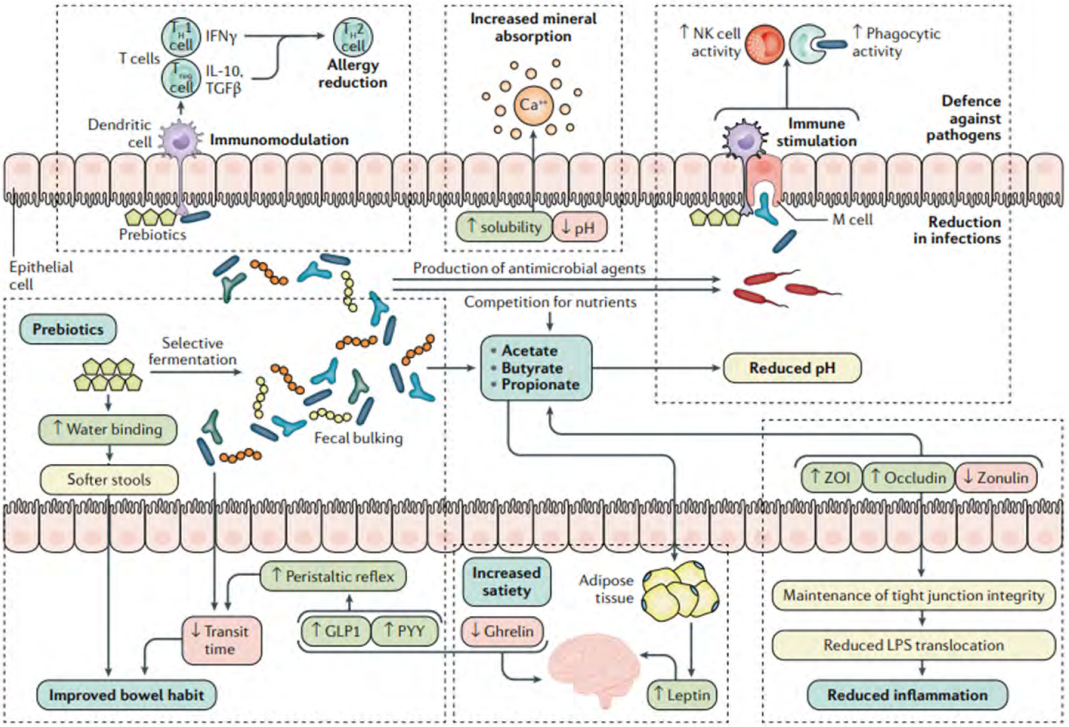
FIGURE 10. A

Mechanisms of actions of probiotics and prebiotics.



Schematic diagram illustrating potential or known mechanisms whereby probiotic bacteria might impact on the microbiota.

B



Mechanisms of actions of prebiotics. The premise is that prebiotics enter in the gut are selectively utilized. This step increases bacterial growth and functionality of specific genera or species. As a result of either of both effects, health benefits can accrue. Fecal bulking and improved bowel habits occur due to microbial growth. Immune regulation can be influenced by increased biomass and cell wall components of the bacteria. Metabolic products include organic acids, which lower intestinal pH and have concomitant effects upon microbial pathogens and mineral absorption. Metabolic products can also influence epithelial integrity and hormonal regulation. Bacteria that respond to prebiotics intake can influence the microbiota composition through elaboration of antimicrobial agents (for example, peptides) and competitive interactions, possibly reducing infections and bacteria containing lipopolysaccharide (LPS). GLP1, glucagon like petide1; M cell, microfold cell; NK cell, natural killer cell; PYY, peptide YY; TGFβ, transforming growth factor β; TH1 cell, type 1 helper cell; TH2 cell, type 2 helper cell; Treg cell, regulatory T cell; ZO1, zonula occludens 1.

The intestinal barrier plays an important role in protecting against the entry of harmful substances. A dysfunctional intestinal barrier, or leaky gut, is associated with various diseases and disorders, such as infections caused by intestinal pathogens, inflammatory bowel disease, irritable bowel syndrome, obesity, celiac disease, non-celiac gluten sensitivity, food allergies and even autoimmune diseases such as type 1 diabetes. *In vitro* studies suggest that probiotics and prebiotics may enhance the barrier function of the intestinal epithelium by increasing the resistance of tight junctions, possibly by influencing the production of tight junction proteins (e.g. occludins and claudins) that regulate the passage of small molecules and ions through the space between epithelial cells. Increased expression of genes that code for tight junction proteins has been shown in a human study where subjects received the probiotic *L. plantarum* WCFS1. Some *in vivo* studies with prebiotics point to an improvement in gut barrier function. Additionally, the increase in mucin production and consequent thickness of the mucus layer is beneficial to the intestinal barrier. This helps protect the epithelial cells from potential pathogen translocation and may enhance the clearance of pathogens from the GI tract. Cell culture evidence shows that an increase in the production of mucins may result from an enhancement of gene expression in mucus-producing goblet cells lining the GI tract. Some prebiotics have also been shown to have direct effects on bacterial pathogens by serving as decoys or by changing the sugar decoration that serves as an anchor for bacteria on gut epithelial cells, preventing pathogens from binding to them.

Many bacteria produce antimicrobial peptides/proteins such as bacteriocins which reduce the survival of competing microorganisms by inhibiting their pore formation or cell wall synthesis. In vitro studies have shown that bacteriocins produced by probiotic bacteria, such as lactobacilli and *Bifidobacterium* strains, reduce the ability of pathogens, such as *E. coli* O157:H7, to adhere to and invade cultured intestinal cells. Bacteriocin production following prebiotic administration has also been reported. This may be one of the mechanisms by which probiotics and prebiotics decrease the infection rate in humans and animals and increase the survival rate of mice treated with a lethal challenge by a pathogen. Additional supporting evidence for this mechanism comes from studies using probiotic bacteria, which have been modified in such a way that they can no longer produce bacteriocins. In in vitro studies, such microorganisms have been seen to lose their ability to prevent the adherence and translocation of pathogens and/or to reduce the infection/survival rates of infected animals. In addition, probiotics have been shown *in vitro* to alter the gene expression of certain pathogens, thereby reducing their virulence.

Probiotics and prebiotics may also enhance the ability of specialised Paneth cells in the intestine to produce the antibacterial peptides known as defensins, including β- and β-defensins and cathelicidins. They are active against bacteria, fungi and viruses and stabilise the gut barrier function. This putative effect is supported by *in vitro* intestinal epithelial (e.g. Caco-2) cell culture studies, showing that certain probiotics and prebiotics can stimulate human β-defensin mRNA expression and peptide secretion.

Animal and *in vitro* studies have shown that some probiotics and prebiotics can compete with pathogens for receptor sites on epithelial cells or in the mucous layer, thereby preventing pathogens from adhering or translocating. In contrast, other probiotics may directly bind to the pathogen, thus reducing its ability to colonise the intestine. There is good evidence from animal studies that feeding certain probiotic strains and prebiotics can greatly reduce the ability of pathogens such as *S. enterica subsp. enterica serovar Typhimurium* and pathogenic *E. coli* to translocate and invade the liver and spleen. Additionally, *in vitro* evidence shows that the same probiotic strains have the ability of pathogens to adhere to cells. Influence on pathogen translocation in infected animal models has also been shown for some prebiotics.

Saccharolytic fermentation of carbohydrate prebiotics concomitantly reduces the potentially adverse effects of protein fermentation and other processes, which give rise to nitrogen and sulphur-containing compounds, such as ammonia, N-nitroso, azo and sulphides. Many of these products, especially hydrogen sulfide and nitroso compounds, are toxic to intestinal cells and are implicated in the etiology of colorectal cancer. Similarly, saccharolytic fermentation modulates bile acid metabolism after consumption of dietary fats. Bacteria deconjugate and dehydroxylate the host-secreted primary bile acids to the secondary bile acids, such as deoxycholic acid and lithocholic acid, altering their affinity to the receptors and, thus, their impact on host metabolism – for example, insulin sensitivity, lipid metabolism and energy expenditure – and immunity. While the exact underlying mechanisms are not yet perfectly clear, it is known that bacterial metabolism is involved in the formation of TMA from choline and carnitine. Prebiotics have been shown to reduce the formation of trimethylamine (TMA) and its hepatic metabolite trimethylamine oxide (TMAO). TMAO has been linked with atherosclerosis. A human study has reported that prebiotic arabinoxylan oligosaccharides (AXOS) reduced serum TMAO.

Cross-Talk with the Host

The most complex of the mechanisms by which probiotics and stimulated endogenous microbes may act is the interaction with the GI immune cells and lymphoid tissue to modulate the immune and inflammatory responses of the host. This provides potential for an impact beyond the gut (Figure 9).

The mammalian immune system consists of two major arms: the innate (or non-specific immediate) and the acquired (or specific adaptive) immune systems. Both parts of the immune system are extremely complex and involve cells (cellular immunity) and other components secreted into the blood, such as antibodies and cytokines. The two arms work together to protect the host from pathogens (bacteria, viruses, fungi) and other foreign materials (antigens), as well as tumour cells arising in the host. For more information, see the ILSI Europe Concise Monograph on Nutrition and Immunity in Man.

Through so-called bacterial-epithelial cell 'cross-talk', it seems that ingested and endogenous microbes can impact both the innate and adaptive responses of the host immune system. The interaction between microbial

cells (commensal, probiotic or pathogen) and host cells is mediated by the interaction with specific receptors, such as the toll-like receptors (TLR) associated with cells lining the mammalian GI tract. The activation of these receptors initiates a cascade of concerted immune signals, leading to different responses. For example, the response can ensure balanced populations of mature T cells (Th1 vs. Th2) and T-regulatory cells, which allows an appropriate response to potential pathogens and food antigens. In the absence of sufficient T-regulatory cell action, an inappropriate T-cell response is thought to be one of the features of allergic conditions. Further, activation of the immune pathways may also result in B-cell differentiation and the production of protective antibodies, such as IgA, which are secreted into the intestinal lumen. Along the same lines, the ingestion of specific probiotic strains or prebiotics in human and animal studies has been shown to increase anti-inflammatory cytokines, such as IL-10 and TGF-ß, and decrease pro-inflammatory cytokines, such as TNF-α and IFN-γ. It has been proposed that these changes in cytokine balance could be a mechanism by which prebiotics and probiotics might be able to mitigate IBD as well as autoimmune disease, such as type 1 diabetes.

Various probiotics and some prebiotics or synbiotics modulate the activity of phagocytic cells (neutrophils and macrophages) and natural killer (NK) cells (non-T non-B lymphocytes) in animals and humans. TLRs, also the so-called G-protein receptors in certain white blood cells, can be acted upon by both probiotics and prebiotics. Additionally, prebiotics may impact the immune system indirectly, as the SCFAs resulting from microbial metabolism may interact with several membrane receptors in the gut and the blood (TLR and GPCRs) (Toll-like receptors and G protein-coupled receptors).
Although studies in humans have found changes in biomarkers, such as cytokine levels, and in the number and activity of immune cells, it is still of prime importance that human studies measure clinical outcomes. Clinical measures, such as a reduced incidence of infection or enhanced immune response to a vaccine, can then be linked to measures of humoral or cellular immune biomarkers. Even though results from animal studies cannot necessarily be extrapolated to humans, animal models represent a valuable means to understanding the complex signalling cascade that underlies a protective immune response.

Bacterially-derived metabolites in the gut may have an impact on health by modulating the physiology of distant organs, such as the brain and liver, skeletal muscle and bone. It is possible that SCFAs, particularly butyrate and propionate, mediate the production and action of the hunger and satiety hormones, for example increasing peptide YY (PYY) and oxyntomodulin and decreasing ghrelin production by endocrine-type cells in the intestine. SCFAs can also induce the expression of the glucagon-like peptide 1 (GLP-1). This, in turn, initiates other signal transduction pathways in peripheral tissues, for example increasing insulin secretion and glucose utilisation and reducing cholesterol and lipid syntheses in the liver, both of which have great implications for metabolic health. In weaned pigs, oral administration of SCFAs is known to attenuate fat deposition by reducing lipogenesis and enhancing lipolysis

of different tissues – further evidence that SCFAs are a mediator of metabolic health.

Prebiotics are known to promote mineral and trace element absorption, including calcium, resulting in increased whole-body bone mineral content and bone mass density. The exact mechanisms contributing to calcium absorption may involve the acidification of the lumen content by SCFAs to increase calcium solubility, the trophic effect of SCFAs on the size of the mucosal absorption surface, and interaction with tight junctions of intestinal epithelium. In addition, SCFAs can influence bone remodelling through the inhibition of bone resorption by blunting osteoclast differentiation.

The gut microbiota may modulate brain development, structure and function and influence emotions and behaviour. Gut microbes can communicate with the neural system through a variety of routes, including the vagus nerve by producing neurotransmitters (Figure 8). Probiotics, prebiotics and synbiotics regulate the production of gamma-aminobutyric acid (GABA), serotonin, glutamate, and brain-derived neurotrophic factor (BDNF), which all play important roles in controlling the neural excitatory-inhibitory balance, mood, cognitive functions and learning and memory processes. For example, a mice study showed that ingestion of *L. rhamnosus* JB-1 regulated emotional behaviour and central GABA receptor expression via the vagus nerve. Certain bacteria have been found to regulate the production of neurotransmitters. For example, lactobacilli and *Bifidobacterium* spp produce GABA; *Escherichia*, *Bacillus* and *Saccharomyces* spp produce *noradrenaline*; *Candida*, *Streptococcus*, *Escherichia* and *Enterococcus* spp can produce serotonin; *Bacillus* produce dopamine; and l*actobacilli* produce acetylcholine. Cytokines such as interleukin (IL)-1 and IL-6, produced from the cross-talk between microbes and immune cells, can travel via the bloodstream to the brain to modulate the hypothalamic-pituitary-adrenal (HPA) axis and the release of cortisol, which is the most potent activator of the stress system. The human studies to date support the view that the gut microbiota is altered during a major depression and that prebiotics and probiotics can have an impact on anxiety and depression symptoms.

OVERALL CONCLUSIONS

The science around the concept of probiotics and prebiotics continues to expand. Current global research efforts have greatly contributed to the understanding of the role of GI commensal microorganisms in their extraordinary symbiotic relationship with humans. Continued research into the gut microbiota will no doubt lead to improved insights into the impact of probiotics and prebiotics on human health.

Probiotics can compensate for, substitute for or add to the gut microbiota, and, thereby, impact the host directly or indirectly through 'cross-talk' with the gut microbiota and/or the host. Probiotics may also act independently of the microbiota. Prebiotics are designed to improve the intrinsic microbiota by selectively stimulating those groups considered important for eubiosis. The effects of prebiotics and probiotics may be local in the GI tract or systemic, providing health benefits for the host.

The past decades of research have demonstrated the potential health benefits of probiotics and prebiotics and contributed to our understanding of the mechanisms by which these effects are obtained. The most commonly reported impact of probiotics and prebiotics is on supporting intestinal function, including stool frequency and consistency, and reducing incidence of AAD and infectious diarrhoea. Evidence continues to emerge that probiotics and prebiotics have an influence on the immune system, indicating that they may enhance resistance to infections, particularly those of the GI or respiratory tract, and help mitigate allergies, particularly in infants and young children. Further evidence highlights the potential for probiotics and prebiotics to impact other conditions of the GI tract, such as UC, pouchitis, and IBS. In the case of prebiotics, a well-established role in enhancing calcium absorption remains to be documented as a proven benefit for bone health. The emerging role of prebiotics and probiotics in appetite control and weight management could also be very important. Another expanding area of interest for both prebiotics and probiotics is their potential anti-inflammatory role beyond the gut, indicating benefits for cardiovascular health, obesity management, metabolic syndrome prevention and mental health.

One critically important fact to bear in mind is that reported benefits of probiotics should be considered strain-specific unless otherwise demonstrated. Prebiotics, depending on the type and structure, will also have substance-specific effects. It is vital that future human studies take this into account. Apart from establishing the effects of each ingredient, such studies should also aim to improve our understanding of the mechanisms of action and possibly lay the groundwork for validated biological markers.

This monograph is an attempt to summarise the science and principles behind the prebiotics and probiotics used in foods today. It is noteworthy that these ingredients can readily be incorporated into a balanced diet and that there is a growing body of evidence for established and potential new health benefits.

ABBREVIATIONS

AAD	Antibiotic-associated diarrhoea
APCs	Antigen presenting cells
AXOS	Arabino xylo-oligo saccharides
BCFA	Branched chain fatty acids
BDNF	Brain-derived neurotrophic factor
CD	Crohn's disease
CFU	Colony forming units
DP	Degree of polymerisation i.e. the number of monomers in a molecule
EFSA	European Food Safety Authority
FDA	Food and Drug Administration
FOS	Fructo-oligosaccharides – typically applied to mixtures of DP3-DP9
GABA	Gamma-aminobutyric acid
GALT	Gut-associated lymphoid tissue
GI	Gastrointestinal
GLP-1	Glucagon-like peptide 1
GOS	Galacto-oligosaccharides – typically applied to mixtures of DP3-DP9
GRAS	Generally recognized as safe
HMO	Human milk oligosaccharide
IBS	Irritable bowel syndrome
IBD	Inflammatory bowel disease
IL	Interleukin
IPA	International Probiotics Association
ISAPP	International Scientific Association for Probiotics and Prebiotics
NEC	Necrotising enterocolitis
PYY	Peptide YY
QPS	Qualified presumption of safety
SCFA	Short chain fatty acids
TLR	Toll like receptors
TMAO	Trimethylamine oxide
UC	Ulcerative colitis
URTI	Upper respiratory tract infection

GLOSSARY

Antibody

A specific protein produced in the blood or tissues as part of the immune response to a foreign antigen, such as a bacterium or toxin, or a food protein that interacts with the antigen thereby inactivating it, thus forming the basis of immunity.

Antigen

A substance, most often a peptide or protein, that the body recognises as foreign and that can evoke an immune response (e.g. bacterial antigen, food antigen or toxin).

Commensal

From the Latin – «common table». It means two organisms living together in a way that is beneficial to both or, at least, not harmful to either. Hence, commensal bacteria live in the human gut and may be neutral or beneficial.

Cytokines

Low-molecular-weight proteins (other than antibodies) produced by various cell types and involved in cell-to-cell communication and control of the inflammatory and immune response. Cytokines include interferons, interleukins and lymphokines.

Dysbiosis

The condition of the gut microbiota where one or a few potentially harmful microorganisms are present in high numbers, thus creating a disease-prone situation or otherwise noticeable disturbances, such as liquid stools, gastrointestinal infections or inflammations.

Eubiosis

Formally referred to as "normobiosis" this characterises the composition of a stable or balanced gut microbiota in a healthy individual. There is incomplete understanding of what constitutes eubiosis and, hence, no general definition in terms of bacterial composition or function.

Fermentation

The anaerobic oxidation of organic compounds to generate metabolic energy in the absence of oxygen as an electron sink. Reduction equivalents are released as hydrogen, ammonia, hydrogen sulphide, methane or alcohols. For example, the oxidation of carbohydrates may produce short chain fatty acids, ethanol, lactic acid and/or gases that produce energy in the form of ATP.

Microbe/microorganism

Small, often single cell organisms, including bacteria, archaea, yeast, mould, algae, plankton and fungi (fungi may also be multicellular). Although definitions vary, we have taken the view that microbes do not include viruses.

Microbiota

All the microbes that are found in a particular region or habitat – hence gut microbiota describes the whole microbial population found in the gut or gastrointestinal tract. The term 'microflora' is no longer used.

Oligosaccharide

A carbohydrate that consists of 3-9 monosaccharide units, joined by glycosidic linkages. Some are prebiotics

Prebiotic

A substrate that is selectively utilised by the host microorganisms conferring a health benefit

Probiotic

Live microorganisms that, when administered in adequate amounts, confer a health benefit on the host

Polysaccharide

A carbohydrate comprising 10 or more monosaccharide units. Some are prebiotics.

Synbiotic

A mixture comprising live microorganisms and substrate(s) selectively utilised by host microorganisms that confers a health benefit on the host

Taxonomy

The science of identifying species and arranging them into a classification

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