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Review Article

Probiotics, Prebiotics and Synbiotics - In Health Improvement by Modulating Gut Microbiota: The Concept Revisited

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A B S T R A C T

Todays consumers are increasingly interested and conscious about their health and the food that they eat need to be healthy or even capable of preventing illness. Studies reveal that the gut health in general is a key sector for maintaining the overall health of people, whose microbiota plays a key role in metabolism and nutrient absorption. Therefore an optimum balance between the host gut and its microbiota is needed. Since their introduction probiotics, prebiotics and synbiotics have attracted much attention for their capacity in health improvement simply by maintaining the gut health. A good number of scientific laboratories now a days are in search for different prebiotics, as well as effective synbiotics preparations (*i.e.* combination of probiotics and prebiotics) to maintain the beneficial microbiota of the human gastrointestinal tract.

Keywords

Probiotics;
Prebiotics;
Synbiotics.

Introduction

The microbiota of the human gastrointestinal tract plays a key role in efficient nutrient absorption and thus maintaining human health. Through the process of fermentation, gut bacteria metabolize various nutritional substrates to end products such as organic acids, vitamins, short-chain fatty acids etc. Therefore, an optimum balance is required between the intestinal microflora and its host. Under certain circumstances such as diet, medication, stress, age and general living conditions, this balance may be altered leading to the colonization of pathogenic species which is manifested as different gut disorders like inflammatory bowel disease, colonic cancer etc. Therefore, knowledge of the gut microflora and its interactions may lead to the development of dietary strategies that serve to sustain or even improve normal gastrointestinal microbiology.

Probiotics, prebiotics and synbiotics are the new concepts that have been developed to modulate the target gastrointestinal microflora balance. Since its introduction, the concept of probiotics, prebiotics and the combination of both *i.e.* synbiotics have attracted much attention and different scientific laboratories have been screening on most efficient synbiotic combination for maintaining sustained human health.

Probiotics

The Greek meaning of the word probiotic is "for life". Which are "viable live microorganisms when administered in adequate amounts confer a health benefit on the host" (Fuller, 1989). Several lactococci, lactobacilli and bifidobacteria are held to be health benefiting bacteria but little is known about the probiotic mechanism of gut microbiota (Gibson and Fuller, 2000). Lactic acid bacteria or LAB constitute an integral part of the healthy gastrointestinal microceology and are involved in the host metabolism (Fernandes *et al.*, 1987). Fermentation has been specified as a mechanism of probiotics (Gibson and Fuller, 2000). Probiotics along with other gut microbiota ferment various substrates like lactose, biogenic amines and allergenic compounds into short chain fatty acids and other organic acids and gases (Gibson and Fuller, 2000; Gorbach, 1990; Jay, 2000). LAB synthesizes enzymes, vitamins, antioxidants and bacteriocins (Fernandes *et al*., 1987; Knorr, 1998). With these properties, intestinal LAB constitutes an important mechanism for the metabolism and detoxification of foreign substances entering the body (Salminen, 1990).

Probiotics - properties and mode of action:

Probiotics have been suggested to have the following properties and functions:-
adherence to host epithelial tissue,
acid resistance and bile tolerance,
elimination of pathogens or reduction in pathogenic adherence,
production of acids, hydrogen peroxide and bacteriocins antagonistic to pathogen growth, safety, non pathogenic and non carcinogenic, and Improvement of intestinal microflora (Kaur *et al*., 2002).

However, the mode of action of probiotics still remains unclear. It has been proposed that probiotics could maintain the healthy intestinal microbiota through competitive exclusion and antagonistic action against pathogenic bacteria in the animal intestine (Fuller, 1989). The ability of lactic acid bacteria to inhibit the growth of various Gram- positive or Gram- negative bacteria is well known. This inhibition may be due to the production of organic acids such as lactic and acetic acid (Gilliland and Speck, 1977), hydrogen peroxide, bacteriocins, bacteriocin like substances and possibly biosurfactants, which are active against certain pathogens. On the other hand, several studies have suggested that adhesive probiotic bacteria could prevent the attachment of pathogens and stimulate their removal from the infected intestinal tract. These antagonistic properties could be very useful in probiotic products. Apart from this, successful probiotic bacteria should be able to survive gastric conditions and colonize the intestine, at least temporarily, by adhering to the intestinal epithelium (Lee and Salminen, 1995). Such probiotic microorganisms appear to be promising candidates for the treatment of intestinal disorders produced by abnormal gut microflora and altered gut mucosal barrier functions (Salminen *et al.*, 1996, 1998). LAB has been found to control intestinal disorders partially due to serum antibodies IgG, and secretory IgA and IgM enhancing immune response (Perdigon *et al*., 2001, Cross, 2002). Certain strains of LAB can intermittently translocate across the intestinal mucosa without causing infection, thus influencing systemic immune events (Cross, 2002). Evidence has been presented that some lactobacilli can directly stimulate the immune system on the gut mucosal surface via localized GI tract lymphoid cell foci (Perdigon *et al*., 2001). Fig. 1 showing some of the proposed mechanism of action of probiotics.

Influencing factors for the functionality of probiotics

Several factors are there, which technically support and influencing the function of probiotics. Among them most important are – strain characteristics, stability, fermentation technology, target prebiotics, viability and non viability, microcncapsulation etc. From Fig. 2, it can be easily assayed how probiotic functionality influenced by different technical factors.

Prebiotics

Prebiotics can be defined as "a non-digestible food ingredient which beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon and thus improving host health". The concept of prebiotics came to light during mid nineties of the twentieth century (Gibson *et al.,* 1995). Prebiotics pass through the digestive system without being broken down by the digestive enzymes *i.e.* reach the large intestine in an intact form. Once these non-digestible carbohydrates pass into the intestines, they serve as a feast for the probiotic bacteria that live there. Prebiotics of proven efficacy are able to modulate the gut microbiota by stimulating indigenous beneficial flora while inhibiting the growth of pathogenic bacteria therein. Preferred target organisms for prebiotics are species belonging to the *Lactobacillus* and *Bifidobacterium* genera. The most efficient prebiotics may also reduce or suppress numbers and activities of organisms seen as pathogenic. For the food ingredient to be classified as a prebiotic, the following three criteria have been defined:

The food ingredient must not be hydrolyzed or absorbed in the stomach or small intestine.

It must be selective for beneficial commensal bacteria in the colon by encouraging the growth/metabolism of the organisms; and

It will alter the microflora to a healthy composition by inducing beneficial luminal/systemic effects within the host.

Any food substrate that enters the colon may be a potential prebiotic, however, selective fermentation is a necessary determinant. Much of the early and present work on prebiotics has been carried out in Japan. The search for bifidobacteria-promoting substances began by screening a range of carbon sources for their ability to increase these organisms in pure culture. For example, Yazawa *et al.* (1978) screened a range of dietary carbohydrates for their ability to promote bifidobacteria in comparison to other intestinal isolates. Further studies used mixed culture, animal models and human trials to determine the efficacy of oligosaccharides to modulate the gut flora composition.

Most of the prebiotics used as food adjuncts, such as inulin, fructo-oligosaccharides, lactulose, dietary fiber and gums, are derived from plants. Two of the best-known prebiotics are inulin and trans-galactooligosaccharides (TOS) occur naturally in foods such as garlic, onions, leeks, shallots, *Asparagus,* spinach, Jerusalem artichokes, chicory, peas, beans, lentils, oats and bananas. The resistance of inulin to digestive processes has been extensively studied by applying both *in vitro* and *in vivo* methodologies. Different *in vitro* data supporting the selective stimulation of bacterial growth by inulin have been generated in numerous studies carried out either in defined pure culture fermentation or by using human feces in both batch and continuous culture. In addition to *in vitro* work *in vivo* studies have also been carried out using animal models that all confirmed the prebiotic effect of inulin. Prebiotic oligosaccharides taking interest amongst food researchers and are getting more global attention than other functional foods because of their multipronged beneficial effects including gut health, higher mineral absorption, lowering of cholesterol, immune system stimulation, pathogen exclusion, etc. Apart from that, natural interventions for the treatment and prevention of hypertension with prebiotics have been proven effective (Rycroft *et al.,* 2001, Roberfroid *et al.,* 2002, Samanta *et al.,* 2007). Therefore, natural prebiotics might serve as potential health beneficial aspects to elevate many human diseases. Finding of new source and types of natural prebiotics might explore new areas of research serving in human endeavor.

Different candidate prebiotics

Prebiotic candidates are taken here for considerations that are generally used as food ingredients. Presently there are only two food ingredients that fulfill these criteria; they are inulin and trans-galactooligosaccharides (TOS).

Inulin

Inulin is a generic term that covers all β (1←2) linear molecules. In any circumstances that justify identification of the oligomers vs. polymers, the terms oligofructose and inulin can be used, respectively. Though inulin hydrolysate and the synthetic compounds slightly different in their DP_{av} (4 and 3.6, respectively), the term oligofructose can be used to identify both of them. Indeed oligofructose and fructooligosaccharides are considered to be synonymous for the mixture of small inulin oligomers with DP_{max} 10.

Trans-Galactooligosaccharides

The TOS or trans-galactooligosaccharides are a mixture of oligosaccharides derived from lactose by enzymatic transglycosylation. The product mixtures depend on the enzymes used and the reaction conditions. They generally consist of oligosaccharides from tri- to pentasaccharide with β (1/6), β (1/3), and β (1/4) linkages.

Other candidates

Glucooligosaccharides, isomaltooligo saccharides, lactosucrose, polydextrose, soybean oligosaccharides, and xylooligosaccharides are oligosaccharides for which preliminary or even promising data already exist. However, the evidence for prebiotic status is still not sufficient, and they cannot presently be classified as prebiotics. The prebiotic potential of several other compounds has also been investigated. However, evidence pointing toward any prebiotic effect is too sparse to justify a detailed review and a classification as prebiotic at the present time. These compounds include germinated barley foodstuffs, oligodextrans, gluconic acid, gentiooligosaccharides, pectic oligosaccharides, mannan oligosaccharides, lactose, glutamine, and hemicellulose-rich substrate, resistant starch and its derivatives, oligosaccharides from melibiose, lactoferrin-derived peptide, and N-acetylchitooligosaccharides.

Methods used for testing prebiotic efficacy

To collect reliable and biologically meaningful informations on different prebiotics, rigorous testing of candidate molecules must be performed using some standardized methodologies. These methodologies must demonstrate resistance to gastric acidity, hydrolysis by mammalian enzymes and gastrointestinal absorption, fermentation by intestinal microflora, and selective stimulation of growth as well as activity of intestinal bacteria**.**

Non-digestibility: testing of prebiotic resistance to gastric acidity, hydrolysis by mammalian enzymes, and gastrointestinal absorption:

Various *in vitro* methods are to be used to determine resistance against acidic conditions and salivary, pancreatic, and small intestinal enzymatic hydrolysis. *In vivo* models are used to measure the recovery in feces of an oral dose given to germ-free rats or to rats pretreated with an antibiotic to suppress the intestinal flora. Other, more invasive methods involve intubations into the gastrointestinal system of living anesthetized rats. Models applicable to humans involve either the direct recovery of undigested molecules in distal ileum and in feces or an indirect assessment that neither glycemia nor insulinemia is significantly increased following oral administration.

Fermentation by intestinal microflora

The most commonly used *in vitro* models to study anaerobic fermentation of carbohydrates (oligomers) by mixed bacterial populations, particularly by fecal bacteria, are batch and continuous culture fermentation. Batch culture vessels are inoculated with either pure cultures of selected species of bacteria or with fecal slurry and the carbohydrate to be studied. Multichamber continuous-culture systems have been developed to reproduce physical, anatomical, and nutritional characteristics of gastrointestinal regions. These models are useful for predicting both the extent and site of prebiotic fermentation. *In vivo* fermentation of nondigestible carbohydrates can be studied in laboratory and companion animals, livestock and humans. In rats the prebiotic under investigation is added to food or drinking water but can also be administered by gavages. Animals are then anesthetized and killed at predetermined time intervals. Lastly fecal samples along with the contents of the gastrointestinal segments are collected for analysis. One interesting model by which to study carbohydrate fermentation in experimental animals is the heteroxenic rat harboring a human fecal flora. To study the fermentation of dietary carbohydrates in humans two major approaches are often used. The first is indirect and collects breath air at regular time intervals to measure the concentration of gases, essentially hydrogen, in volunteers previously given a single oral dose of carbohydrate and the other approach consists of collecting feces after oral feeding and measuring recovery of the tested carbohydrate.

Fig.1 Proposed mechanisms of action of probiotics.

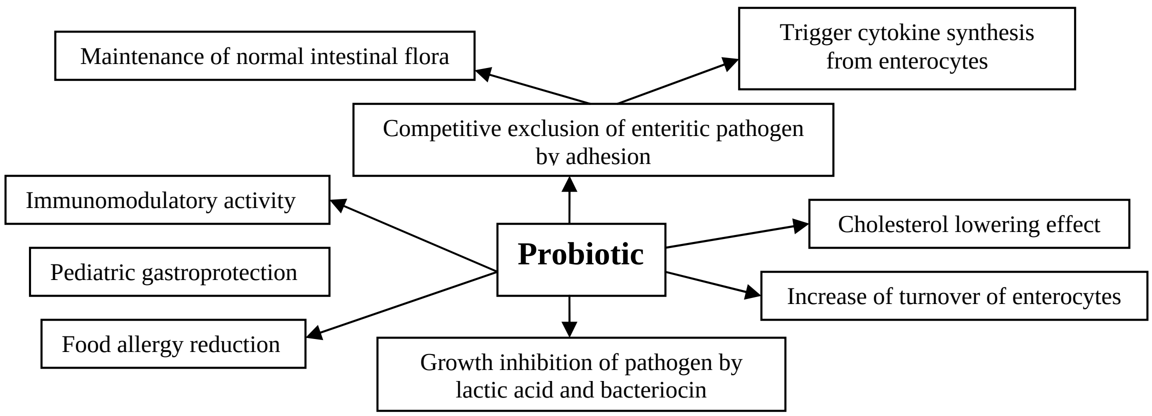
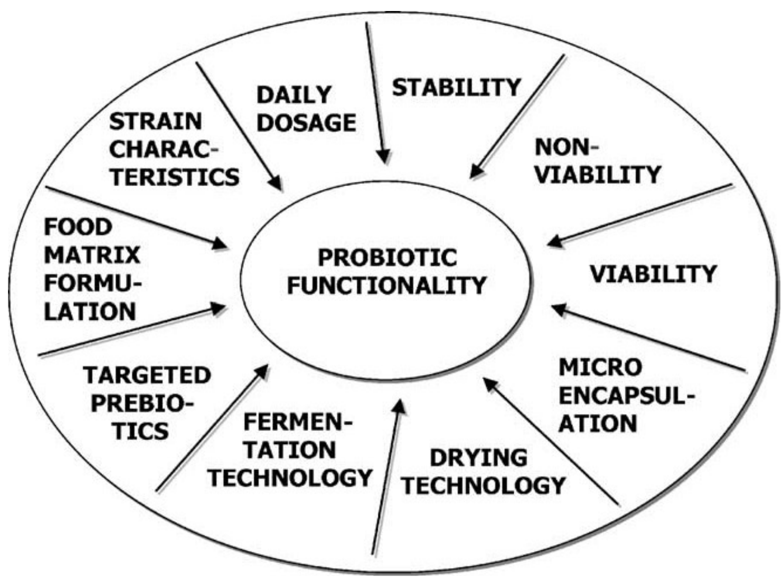


Fig.2 Influencing technological factors for functionality of probiotics.

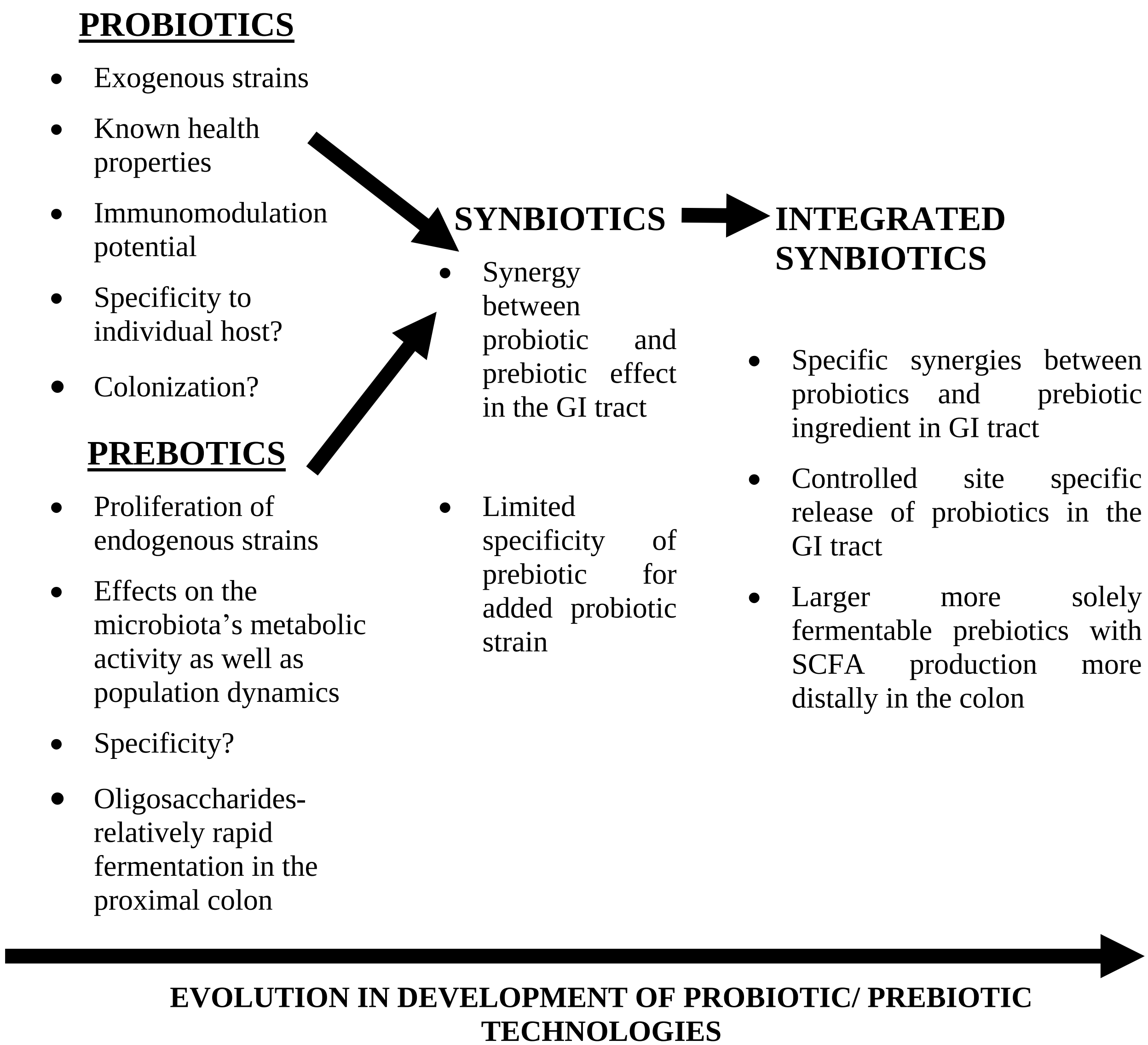


Selective stimulation of growth and activity of intestinal bacteria

As the field of prebiotics has developed one question has raised, how prebiotic selectively stimulate growth and activity of intestinal bacteria (probiotics). Some published literatures have focused on the experimental methodologies based on pure cultures. However, such studies cannot establish that the test carbohydrate is selectively metabolized and should be used for initial screening purposes only.

Study of the changes in populations of selected genera or species in gut established whether or not the fermentation is selective. Fluorescent in situ hybridization (FISH) is often used, which involves the use of group specific oligonucleotide probes that target discrete discriminatory regions of the rRNA molecule sometimes other methodologies like- PCR, direct community analysis and denaturing/ temperature gradient gel electrophoresis are also used.

Fig.3 Evolution in developments of Probiotic/ prebiotic technologies.



Functional prebiotic ingredients-- promoters for probiotics

In addition to the probiotic approach of directly introducing live bacteria to the colon through dietary supplementation, another approach to increase the number of beneficial bacteria in the intestinal microbiota is through the use of prebiotics. Due to the potential synergy between probiotics and prebiotics, foods containing a combination of these ingredients are often referred to as synbiotics (Gibson & Roberfroid, 1995; Collins & Gibson, 1999). Interaction between the probiotic and the prebiotic *in vivo* might be favored by an adaptation of the probiotic to the prebiotic substrate prior to consumption. This might result in a competitive advantage for the probiotic if it is consumed concurrently with the prebiotic. In animal models, the inclusion of resistant starches in the diet has been shown to increase the numbers of bifidobacteria in the intestinal tract (Brown *et al*., 1997; Brown, Wang, Topping, Playne, & Conway 1998; Kleessen *et al*., 1997; *Silvi et al*., 1999; Wang, Brown, Evans, & Conway, 1999). The benefits of using resistant starch extend beyond traditional prebiotics, since resistant starch can be used to ensure the viability of probiotic populations from the food to the large intestine. Resist ant starch offers an ideal surface for adherence of the probiotics to the starch granule during processing, storage and transit through the upper regions of the gastrointestinal tract, providing robustness and resilience to environmental stresses. Bacterial adhesion to starch may also provide advantages in new probiotic technologies to enhance delivery of viable and metabolically active probiotics to the intestinal tract (Crittenden *et al*., 1999). This includes the previously mentioned technology to encapsulate probiotics within starch granules that are then coated with amylose (Myll.arinen *et al*., 2000).

Synbiotics

Recently progressive studies in the field of probiotics and prebiotics introduced a new terminology synbiotics. It is nothing but the synergy between probiotic and prebiotic effect in the GI tract or in other words, synbiotics is the usage of both probiotics and prebiotics in combinations. Indeed synbiotic combinations are considered to have more beneficial effects on human health than probiotics or prebiotics alone. Recent studies established that synbiotics improve the intestinal microbial environment and activate host immune function, leading to prevention of bacterial translocation. Fig. 3 reveals some of the ideas about development of probiotic and prebiotic technologies. Administration of synbiotics as a food supplement is safe, simple, and convenient. Therefore, characterizing a new and novel synbiotic combination would find multifaceted use in disease prophylaxis and management for human use. Now a day the term 'integrated synbiotics' is more preferable than 'synbiotics' alone, when we consider specific synergies between probiotics and prebiotics.

Future perspectives and conclusion

From the above discussion it is clear that prebiotics have great potential as agents to improve or maintain a balanced intestinal microflora to enhance health and wellbeing. They can be incorporated into many foodstuffs. Such thorough comparative studies will allow intelligent choices in incorporating prebiotics into functional foods and that should increase confidence among consumers and regulatory authorities. Similarly, it may be possible to incorporate further biological functionality into the concept, i.e., increasing beneficial bacteria while suppressing pathogens at the same time, perhaps through anti-adhesive approaches. Currently available different scientific studies reveal that most popular targets for prebiotic use are lactobacilli and bifidobacteria and it is largely based on their success in the probiotic area. However, the diversity of the gut flora can be evaluated by using different molecular procedures; the identification and characterization of different bacterial genera, species, and even strains that compose intestinal microflora both qualitatively and quantitatively, changes in that composition and, consequently, to understand how the bacterial cells in the intestine interact and how they contribute to and modulate intestinal more especially to the physiology. Prebiotics will then be a unique tool to create, both in experimental animals and in humans, colonic microflora with ‘‘controlled’’ compositions that will then be correlated with specific physiological conditions.

At the end of the present discussion aimed at revisiting the prebiotic definition, it must be emphasized that only two food carbohydrates, essentially nondigestible oligosaccharides, today fulfill the criteria for prebiotic classification. For other prebiotic candidates, data are promising, but more studies are still required. The prebiotic effect seems to appear rapidly and to last for as long as the prebiotic is ingested. But studies so far performed are limited in time (up to a few months), and it would be of interest to test the effect of much longer administration periods, e.g., up to a few months or even a few years.
One important question as yet basically unanswered is the effect of the prebiotic not on the numbers of bacteria but rather on activities associated with these bacteria. Indeed, the health benefits are directly dependent on what these bacteria do, how they interact with the others, and how they modulate intestinal functions. Published reports have been made by measuring miscellaneous bacterial enzyme activities such as glucuronidase, glucosidases, nitroreductase; metabolites such as SCFAs; and end products of the fermentation of amino acids, mucins, or sterols (especially primary and secondary bile acids) shown to vary (increase or decrease) after ingestion of prebiotics. But the validity of these parameters still remains to be established and well clarified especially in terms of their value as a biomarker of colonic and eventually host health and well-being or disease risk reduction.

The concept of prebiotic is only fifteen years old and has already attracted and stimulated research in many areas of nutrition, dietary and medical sciences. A further desirable attribute for prebiotics is the ability to act in the most distal region of the colon, which is known to be the site of origin of several chronic diseases including colon cancer and ulcerative colitis etc. and thus currently there is much scientific interest in developing prebiotics that target this region of the colon.

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