Prebiotics: preferential substrates for specific germs?[1–3]

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ABSTRACT A prebiotic is "a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or the activity of one or a limited number of bacteria in the colon." The premise is based on the hypothesis that the large gut in humans contains bacteria that are beneficial or detrimental to health. Although this generalization probably gives too simplistic a view of gut microbiology, it is a feasible working concept. Currently, food components that seem to exert the best prebiotic effects are inulin-type fructans. In pure culture, most species of bifidobacteria are adapted to the utilization of these nondigestible oligosaccharides but many other bacteria are also capable of metabolizing them. Clearly, these studies of pure bacteria are of limited use unless their results are supported by the results of studies using mixed cultures. Indeed, as many components of the gut microbiota as possible should be measured to indicate a true prebiotic effect. Simple stimulation of bifidobacteria is insufficient to demonstrate an effect; the effects on other gut microorganisms in vivo with human volunteers is necessary. Adjustment of the composition and activities of the colonic microflora so that health-promoting activities are optimized remains key in functional food development. New methods are being applied extensively to human gut microbiology and promise the degree of reliability required to detect subtle changes in colonic microflora composition and to correlate such changes with health benefits. This is a review of the present state of knowledge concerning prebiotics, with emphasis on the criteria used for classification, mechanisms of selective growth stimulation, and physiologic effects. *Am J Clin Nutr* 2001;73(suppl):406S–9S.

KEY WORDS Prebiotics, inulin, probiotics, bifidobacteria, functional foods, substrate, review

INTRODUCTION

The human large gut contains a large variety of bacterial genera, species, and strains, which are either beneficial (eg, *Bifidobacterium, Eubacterium,* and *Lactobacillus*) or detrimental (eg, *Clostridium, Shigella,* and *Veillonella*) to the host's health. Although this generalization probably gives too simplistic a view of gut microbiology, it is a feasible working concept for the development of functional food components to modulate the composition of the colonic microbiota (1). It is in that context that a prebiotic has been defined as "a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or the activity of one or a limited number of bacteria in the colon" (2). Compared with a probiotic, which introduces exogenous bacteria into the colonic microbiota, a prebiotic aims at stimulating the growth of one or a limited number of the potentially health-promoting indigenous microorganisms, thus modulating the composition of the natural ecosystem. Of the currently available food ingredients, the nondigestible oligosaccharides (ie, oligosaccharides that resist hydrolysis and digestion in the upper gastrointestinal tract but are hydrolyzed and fermented in the large bowel) (3) are the only known components for which convincing evidence has been reported in favor of a prebiotic effect. Moreover, of the various nondigestible oligosaccharides available for food applications, the inulin-type fructans are the prebiotics that have been investigated most extensively for their nutritional properties (4, 5).

Inulin-type fructans are composed of β-D-fructofuranoses attached by β-2–1 linkages. The first monomer of the chain is either a β-D-glucopyranosyl or β-D-fructopyranosyl residue. They constitute a group of oligosaccharides derived from sucrose that are isolated from natural vegetable sources. A product with a degree of polymerization (DP) from 2 to ≥60 is extracted from chicory roots and it is labeled as inulin (Raftiline; Orafti, Tienen, Belgium). Oligofructose, which is produced by partial enzymatic hydrolysis of inulin, has a DP < 10 (Raftilose; Orafti) and the inulin from which the small-molecular-weight oligomers have been eliminated is called high-performance inulin (Raftiline HP; Orafti). With the use of sucrose as a substrate and a 1,2-β fructan 1^F-fructosyltransferase–catalyzed reaction, a synthetic low-molecular-weight fructan is produced that has a DP < 4 (Neosugar or Actilight; Beghin-Meji Industries, Paris).

The aim of this review was to present the current state of knowledge concerning prebiotics, with emphasis on the criteria used for classification, mechanisms of selective growth stimulation, and physiologic effects. The data reviewed was published up to 1998, the year of the symposium.

PREBIOTICS: CRITERIA AND A HYPOTHESIS FOR MECHANISMS

The criteria used for classification of a food component as a prebiotic are as follows: resistance to digestion, hydrolysis and fermentation by colonic microflora, and most importantly, selective

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TABLE 1
Inulin-type fructans and the basic criteria for classification as prebiotics

Criteria	Model	Reference
Resistance to digestion	Ileostomy patients	6, 8
Hydrolysis and fermentation by colonic microflora	Chemical analysis and pH drop	7
Selective stimulation of growth of bacteria in the colon	In vitro fecal slurries, in vivo fecal analysis	9–12

stimulation of growth of one or a limited number of bacteria in the feces (in vivo in humans). Resistance to digestion should, ultimately, be shown in vivo; the most adequate model probably is ileostomy patients (6). To show and quantify hydrolysis and fermentation by colonic microflora, human fecal slurries are a valuable surrogate for colonic content, even though both quantitative and qualitative differences may exist in the microbiota colonizing the different segments of the large bowel.

Chemical analysis, a decrease in the pH of the incubation mixture (as an indirect measurement of acid production and fermentation), and an increase in bacterial biomass are all indicators that prove the disappearance of the prebiotic from the fecal slurry. To demonstrate that this fermentation leads ultimately to selective stimulation of the growth of one or a limited number of bacteria, preferentially one of the potentially health-promoting ones, requires both in vitro and in vivo experiments showing a selective change in the composition of the complex fecal microbiota. Indeed, it is the selective stimulation of growth in such a complex microbial environment that is the only valid criterion. This implies that the demonstration of a prebiotic effect cannot be made by measuring only one single population of bacteria in fecal samples. The selectivity of the stimulation can only be proven if, at a minimum, the major populations are quantitatively analyzed by using methods that have been validated for each of these bacteria. As long as such criteria are met, in vitro experiments with human fecal samples that show selective growth stimulation—in combination with convincing data that show resistance to digestion and analytic evidence of quantitative hydrolysis and metabolization—indicate a strong prebiotic effect.

Experimental data reviewed recently (7) showed a prebiotic effect of inulin-type fructans (**Table 1**). However, results of experiments with pure cultures—in which various strains of *Bifidobacterium, Bacteroides, Clostridium, Enterococcus, and Lactobacillus* were incubated in the presence of inulin-type fructans (including the synthetic, low-molecular-weight fructooligosaccharides)—did not show a strictly selective fermentation pattern (**Table 2**). Indeed, all the bacteria tested had the capacity to ferment these substrates, but there were differences in efficacy among strains (as indicated by different decreases in pH); bifidobacteria was the most efficient species. These results also suggest that the shorter the chain length of the fructans, the less the specificity of the fermentation process might be. On the basis of the change in pH (which was directly proportional to the amount of acids produced in the medium and, thus, indirectly related to the degree of fermentation), the efficiency of fermentation of inulin-type fructans by bacteria other than bifidobacteria was on average 40% less than that of the synthetic low-molecular-weight fructooligosaccharides.

Concerning the mechanism of the prebiotic effect, these data seem to rule out the hypothesis that this food component acts exclusively as a selective substrate for one species of bacteria—bifidobacteria. The change in the composition of the fecal microflora these bacteria induce both in vitro and in vivo cannot fully be explained by substrate specificity even if certain substrates preferentiality play a role. A prebiotic effect in a complex ecosystem such as the colonic-fecal microbiota also involves other processes, among which a change in pH, in metabolic complementarity, or in antibiotic activities likely plays a role. A fermentative advantage cannot solely explain the prebiotic effect. It is a more complex effect that, most probably, involves the interactions between different populations of bacteria. Another argument in support of the hypothesis of such an ecologic effect is the observation that, at least in vivo, the dose-response effect of prebiotics on the growth stimulation of bifidobacteria in the complex human fecal microbiota does not appear to be straightforward (7). The human studies reviewed thus far showed no dose-response effect on the increase in the log number of bifidobacteria at the end of the prebiotic feeding period. A major factor determining that increase was the number of bifidobacteria in the feces at the start of the feeding regimen. This observation is paramount for the recognition of prebiotics as functional foods. Reference to an "effective prebiotic (or bifidogenic) dose" would be misleading and scientifically incorrect for the general population because of the known large variability in the composition of fecal microflora between individuals. The demonstration of a dose-response effect in a particular group of volunteers cannot be generalized to the whole population.

HEALTH BENEFITS OF PREBIOTICS

It was not the aim of this article to extensively review the scientific base in support of the health benefits of prebiotics; other articles in this issue do that (13–16). However, as concluded in our recent reviews (4, 5), in which we critically assessed the data, the major nutritional and physiologic effects of a model prebiotic, namely inulin-type fructans (**Table 3**), concern the composition of the colonic flora, the bowel functions, calcium absorption, and possibly, lipid metabolism and reduction of the risk of colon cancer. By reference to the working definition that says that "a food can be regarded as functional if it is satisfactorily demonstrated to affect beneficially one or more target functions in the body, beyond adequate nutritional effects, in a way which is relevant to either the state of well-being and health or the reduction of the risk of a disease" (17), prebiotics, especially inulin-type fructans, are thus functional foods. The selective growth stimulation of bifidobacteria in the colonic microbiota by

TABLE 2
Comparative fermentation of inulin and synthetic low-molecular-weight fructooligosaccharides as measured by a decrease in pH in the culture medium after 96 h of incubation in the presence of various strains of bifidobacteria, clostridia, enterococci, and lactobacilli[1]

	Inulin	Synthetic fructooligosaccharides
Bifidobacteria [20]	−1.70	−1.90
Bacteroides [16]	−0.30	−0.90
Clostridia [26]	−0.20	−0.45
Enterococci [5]	−0.45	−1.15
Lactobacilli [10]	−0.40	−0.85

[1] The concentration of substrates was 0.5% (by wt). The values in parentheses are the number of different strains tested.

TABLE 3
Inulin-type fructans as functional foods: summary of evidence (for review *see* references 4 and 5)

Functional effects	Classification of evidences
Hypolipidemia	Promising
Colonic flora	Strong
Bowel function	Strong
Ca bioavailability	Promising/strong

inulin-type fructans was observed in both in vitro and human studies. The scientific evidence for a bifidogenic effect of inulin and oligofructose is strong and supports the claim that these prebiotics effectively modify the composition of the colonic flora. However, important questions remain to be answered.

1) How long does the bifidogenic effect persist during consumption of a fructan-rich diet and when such consumption stops?
2) What are the functional effects of the so-called synbiotic approach, which combines fructans as a prebiotic and a probiotic strain (2)?
3) Most importantly, what are the health benefits of having a colonic flora in which bifidobacteria predominate?

An indirect consequence of the stimulation of bifidobacterial growth is fecal bulking, for which strong evidence was published previously (for a review, *see* references 4 and 5). A second effect worth considering when discussing potential functional effects of prebiotics, like inulin-type fructans, is the increased bioavailability of minerals (16). Although data on the balance of magnesium, iron, or zinc are too preliminary to be considered, scientific evidence does exist to support the effects of inulin-type fructans on calcium absorption in both experimental animals and in humans and this evidence is considered strong. Such evidence might have health implications if it is shown that prebiotics may contribute to a reduction in the risk of osteoporosis. The effects of prebiotics on lipid metabolism are also discussed in this issue (15). Experimental data support the hypothesis that one prebiotic, ie, oligofructose, inhibits hepatic lipogenesis in rats and consequently induces a significant hypotriglyceridemic effect. The potential mechanisms of this effect include metabolic or genetic effects of short-chain carboxylic acids, low glycemia and insulinemia, or both. Thus, there is preliminary evidence of a hypotriglyceridemic effect of inulin-type fructans; a recent study of slightly hypertriglyceridemic volunteers confirmed this effect (18).

A last area for further research of inulin-type fructans is cancer. Experimental data published recently showed that the incidence of the so-called aberrant crypt foci induced by colon carcinogens like azoxymethane or dimethylhydrazine was reduced significantly in rats fed inulin-type fructans (19, 20). For this particular effect, a synbiotic approach combining inulin and bifidobacteria was shown to be more effective than either the probiotic or the prebiotic alone (20). Furthermore, Taper et al (21) reported that the growth rate of implanted tumors was slower in mice supplemented with inulin-type fructans than in control mice. Fontaine et al (22) reported that inulin stimulated the production of sulfomucin and a reduction in sialomucin in heteroxenic rats harboring human colonic flora, 2 effects known to be associated with a reduced risk of colon cancer (23, 24). In regard to functional food development, these cancer-inhibitory effects of prebiotics in experimental animals indicate the need for careful evaluation of the potential health implications of prebiotics in humans.

CONCLUSION

Adjustments in the composition and activities of the colonic microflora in such a way that health-promoting activities are optimized remains key in functional food development. The prebiotic effect is an ecologic effect and needs to be treated as such. The demonstration of this effect requires an extensive qualitative and quantitative analysis of the colonic flora and its modulation by the prebiotic treatment. New methods are being extensively applied to human gut microbiology and promise the degree of reliability required to detect subtle changes in colonic microflora composition and to correlate them with health benefits that are likely not to be limited to gastrointestinal physiology. Systemic effects of prebiotics have been identified and deserve further investigation. The potential applications of inulin-type fructans for reducing the risk of colon carcinogenesis as well as for improving calcium bioavailability and lipid homeostasis are of particular interest. The development of functional foods is a unique opportunity to improve the quality of the food available to consumers to benefit their health and well-being. Thus, prebiotics, especially inulin-type fructans, are natural products that may receive classification as functional food ingredients with valid health claims. However, only a rigorous scientific approach producing sound data will justify such a classification, which remains a challenge for both the scientific community and for the food industry.

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