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Prebiotics: The Concept Revisited[1,2]

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Abstract

A prebiotic is ''a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microflora that confers benefits upon host well-being and health.'' Today, only 2 dietary nondigestible oligosaccharides fulfill all the criteria for prebiotic classification. The daily dose of the prebiotic is not a determinant of the prebiotic effect, which is mainly influenced by the number of bifidobacteria/g in feces before supplementation of the diet with the prebiotic begins. The ingested prebiotic stimulates the whole indigenous population of bifidobacteria to growth, and the larger that population, the larger is the number of new bacterial cells appearing in feces. The ''dose argument'' is thus not supported by the scientific data: it is misleading for consumers and should not be allowed. A prebiotic index is proposed, defined as ''the increase in the absolute number of bifidobacteria expressed divided by the daily dose of prebiotic ingested.'' J. Nutr. 137: 830S–837S, 2007.

A prebiotic was first defined as (1) "a nondigestible food ingredient that 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 improves host health."

Since its introduction, the concept of prebiotics has attracted much attention, stimulating scientific as well as industrial interest. However many food components, especially many food oligosaccharides and polysaccharides (including dietary fiber), have been claimed to have prebiotic activity without due consideration to the criteria required. Not all dietary carbohydrates are prebiotics, and clear criteria need to be established for classifying a food ingredient as a prebiotic. These criteria are (2) *1*) resistance to gastric acidity, to hydrolysis by mammalian enzymes, and to gastrointestinal absorption; *2*) fermentation by intestinal microflora; and *3*) selective stimulation of the growth and/or activity of those intestinal bacteria that contribute to health and well-being.

Resistance, in the first criterion, does not necessarily mean that the prebiotic is completely indigestible, but it should guarantee that a significant amount of the compound is available in the intestine (especially the large bowel) to serve as a fermentation substrate. Although each of these criteria is important, the third is the most difficult to fulfill.

Indeed, simply reporting fermentation in pure cultures of single microbial strains or an increase in a limited number of bacterial genera in complex mixtures of bacteria (e.g., fecal slurries) either in vitro or in vivo cannot be accepted as demonstrating a prebiotic effectsince it does not take bacterial interactions into account. Demonstrating a selective stimulation of growth and/or activity of these intestinal bacteria that contribute to health and well-being requires anaerobic sampling of feces followed by reliable and quantitative microbiological analysis of a wide variety of bacterial genera, e.g., total aerobes/anaerobes, bacteroides, bifidobacteria, clostridia, enterobacteria, eubacteria, and lactobacilli. Molecular-based microbiological methodologies have been developed and should make prebiotic demonstration easier. To monitor the stimulation of bacterial activity, patterns of production of organic acids, gases, and enzymes have been used. However, these have not yet been validated as biomarkers of specific bacterial genera.

As required for all functional food ingredients (3), the final demonstration of a prebiotic effect must be carried out in vivo through appropriate nutritional intervention trials in the targeted species (i.e., humans, livestock, or companion animals), using validated methodologies to produce sound scientific data.

In light of these criteria and the above considerations, this article aims at revisiting the concept of prebiotics 11 y from its first introduction. To do so, it reviews *1*) the methodologies that are relevant to the demonstration of a prebiotic effect; *2*) the candidate prebiotics and the evidence available to support the prebiotic attribute, and *3*) the human data so far available on the prebiotic effect of inulin with the aim of discussing how these data should be analyzed and presented.

It concludes with an update of the prebiotic definition (1).

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Testing methodologies

If reliable and biologically meaningful data are to be collected on different prebiotics, rigorous testing of candidate molecules must be performed using standardized methodologies. For each candidate prebiotic, these methodologies should demonstrate resistance to gastric acidity, hydrolysis by mammalian enzymes and gastrointestinal absorption, fermentation by intestinal microflora, and selective stimulation of growth and/or activity of intestinal bacteria.

Nondigestibility: testing of prebiotic resistance to gastric acidity, hydrolysis by mammalian enzymes, and gastrointestinal absorption. In vitro methods include determining resistance to acidic conditions and enzymatic (salivary, pancreatic, and small intestinal) hydrolysis (4–6).

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 (6). Other, more invasive methods involve intubation into the gastrointestinal system of living anesthetized rats (7). 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 (8). A model that is widely accepted as a valuable alternative to study the small intestinal excretion of nutrients uses individuals who have been subjected to proctocolectomy, ileostomy patients (9–13).

Fermentation by intestinal microflora. The most commonly used in vitro models to study anaerobic fermentation of carbohydrates by mixed bacterial populations, particularly fecal bacteria, are batch- and continuous-culture fermentation systems. Batch-culture vessels are inoculated with either pure cultures of selected species of bacteria or, preferably, 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 (14,15). 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 gavage. Animals are then anesthetized and killed at predetermined time intervals. Fecal samples and 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 (16).

To study the fermentation of dietary carbohydrates in humans, 2 major approaches are 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 the carbohydrate (17). The other approach consists of collecting feces after oral feeding and measuring recovery of the tested carbohydrate.

Selective stimulation of growth and/or activity of intestinal bacteria. As the field of prebiotics has developed, so has the methodology for investigating functionality, in particular flora compositional changes as a response to selective fermentation. Much of the early (and some of the current) literature describes studies performed on pure cultures. However, such studies cannot establish that the test carbohydrate is selectively metabolized and should be used for initial screening purposes only.

A more meaningful in vitro method for studying prebiotic oligosaccharides is the use of a fecal sample, which ensures that a representative range of bacterial species is exposed to the test material. Study of the changes in populations of selected genera or species can then establish whether or not the fermentation is selective. The use of feces probably gives an accurate representation of events in the distal colon. However, more proximal areas will have a more saccharolytic nature, and both the composition and activities of the microbiota indigenous to the colon are variable, dependent on the region sampled. This has been confirmed through studies on sudden death victims, where the colon contents were sampled shortly following death (14,18). The complex gut models, which replicate different anatomical areas, attempt to overcome this and should be used in concert with human trials.

A major problem with the use of fecal samples is identification of the genera and species present. Traditionally, this has been accomplished by culturing on a range of purportedly selective agars followed by morphological and biochemical tests designed to confirm culture identities (19). This approach is adequate to establish that a prebiotic selectively enriches defined "desirable" organisms and depletes "undesirable" organisms but does not give a true picture of the population changes occurring. This is unavoidable using selective culture because it is estimated that only ~50% of the diversity present in the human colon has yet been characterized (20).

A much more reliable approach involves the use of molecular methods of bacterial identification. These have advantages over culture-based technologies in that they have improved reliability and can encompass the full flora diversity. The most often used molecular procedure is fluorescence in situ hybridization (FISH)[3] (21), which involves the use of group (and in some cases species) specific oligonucleotide probes that target discrete discriminatory regions of the rRNA molecule. Groups targeted include *Bacteroides* spp. (22), *Bifidobacterium* spp. (23), *Lactobacillus/Enterococcus* spp. (24), and *Eubacterium* (25). Additionally, FISH provides a means through which hitherto unculturable bacterial species of the gut may be investigated because this is a culture-independent technique and therefore does not require prior often anaerobic growth of an organism on laboratory medium (26). Other more qualitative methodologies are polymerase chain reaction (PCR) (27), direct community analysis (20), and denaturing/temperature gradient gel electrophoresis (28). **Table 1** summarizes the principal techniques used for evaluating bacterial populations in feces, along with some of their advantages and disadvantages.

Review of candidate prebiotics

Only candidates that are used as food ingredients are considered here. For each candidate a brief introduction gives a description of the chemistry followed by an overview of data available to fulfill the criteria for prebiotic classification. Presently there are only 2 food ingredients that fulfill these criteria, i.e., inulin and *trans*-galactooligosaccharides (TOS).

Inulin.

Chemistry and nomenclature of inulin. From a chemical point of view, the linear chain of inulin is either an α-D-glucopyranosyl-[β-D-fructofuranosyl]$_{n-1}$-β-D-fructofuranoside

[3] Abbreviations used: cfu, colony-forming units; DP, degree of polymerization; FISH, fluorescence in situ hybridization; PCR, polymerase chain reaction; TOS, *trans*-galactooligosaccharides.

TABLE 1 Principal methodologies employed to enumerate colonic bacteria

Method	Advantages	Disadvantages
Classical culture and chemical characterization	Straight forward, relatively inexpensive, possibility of performing a large number of replicates.	Subjectivity, limited to culturable bacteria, selectivity of medium is ambiguous, metabolic plasticity of organisms may introduce error.
FISH	Applicable to unculturable as well as culturable bacteria, highly specific.	Availability of probes limited to known bacteria, time consuming.
PCR	Applicable to unculturable as well as culturable bacteria.	Expensive, time consuming.
	High reliability, allows placement of previously unidentified bacteria.	Subject to bias in the PCR process.
Direct community analysis	Culture-independent. Applicable to elucidate the diversity of entire samples.	Subject to bias in the PCR process.
Denaturing/temperature gradient gel electrophoresis (D/TGGE)	Rapid.3 Applicable to both culturable and unculturable bacteria.	Qualitative rather than quantitative.
		Subject to bias in the PCR process.

($G_{py}F_n$) or a β-D-fructopyranosyl-[β-D-fructofuranosyl]$_{n-1}$- β-D-fructofuranoside ($F_{py}F_n$). The fructosyl-glucose linkage is always β (2 ↔ 1) as in sucrose, but the fructosyl-fructose linkages are β-(1←2). Chicory inulin is composed of a mixture of oligo- and polymers in which the degree of polymerization (DP) varies from 2 to ~60 units with a DP_{av} = 12. About 10% of the fructan chains in native chicory inulin have a DP ranging between 2 (F_2) and 5 (GF_4). The partial enzymatic hydrolysis of inulin using an endoinulinase (EC 3.2.1.7) produces oligofructose, which is a mixture of both $G_{py}F_n$ and $F_{py}F_n$ molecules, in which the DP varies from 2 to 7 with a DP_{av} = 4. Oligofructose can also be obtained by enzymatic synthesis (transfructosylation) using the fungal enzyme β-fructosidase (EC 3.2.1.7) from *Aspergillus niger*. In such a synthetic compound, the DP varies from 2 to 4 with DP_{av} = 3.6, and all oligomers are of $G_{py}F_n$ type. By applying specific separation technologies, the food industry also produces a long-chain inulin known as inulin HP (DP 10 to 60) with a DP_{av} = 25. Finally, mixing oligofructose and long-chain inulin has produced specific products known as Oligofructose Synergy. The different industrial products vary in DP_{av}, DP_{max}, and DP distribution, and they have varying properties (29).

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/or inulin can be used, respectively. Even though the inulin hydrolysate and the synthetic compound have a slightly different DP_{av} (4 and 3.6, respectively), the term oligofructose can be used to identify both. Indeed, oligofructose and fructooligosaccharides are considered to be synonymous names for the mixture of small inulin oligomers with $DP_{max} < 10$ (30–33).

Criteria for prebiotic classification.

Resistance to gastric acidity, hydrolysis by mammalian enzymes, and gastrointestinal absorption. The resistance of inulin to digestive processes has been extensively studied by applying all the methods (both in vitro and in vivo) described in the section Testing Methodologies. Inulin is a nondigestible oligosaccharide that, for nutritional labeling, classifies as dietary fiber (34).

Fermentation by intestinal microflora and selective stimulation of the growth and/or activity of intestinal bacteria associated with health and well-being. 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 (35).

In addition to in vitro work, in vivo studies have also been carried out using animal models that all confirmed the bifidogenic effect of inulin-type fructans (36–38).

Human trials with oligofructose and inulin include those with a controlled diet and crossover feeding trials, although the dose, substrate, duration, and volunteers vary (**Table 2**). The efficacy of inulin has also been evaluated with a view to its administration to formula-fed infants (52).

Together the evidence available today from both in vitro and in vivo experiments supports the classification of inulin-type fructans as prebiotic.

trans-*Galactooligosaccharides.*

Chemistry of TOS. The TOS are a mixture of oligosaccharides derived from lactose by enzymatic transglycosylation (53). 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 (54).

Criteria for prebiotic classification.

Resistance to gastric acidity, hydrolysis by mammalian enzymes, and gastrointestinal absorption. The data on nondigestibility do not fully match the criteria. However, there are suggestions that TOS do reach the colon intact (55).

TABLE 2 General information on the published human nutrition studies designed to test for the prebiotic effect of inulin-type fructans

Daily dose, *g*	Duration, *wk*	Volunteers, *n*	Age category of volunteers	Effects of prebiotic on bacteria other than bifidobacteria	Ref.
8	2	23	Elderly	Not significant	39
8	5	6	Adult	Not significant	40
4	2	10	Adult	Not reported	41
8	2	38	Adult	Decreased clostridia	42
15	2	8	Adult	Decreased clostridia, bacteroides, fusobacteria	43
15	2	4	Adult	Not significant	43
20	2	17	Elderly	Not significant	44
5–20	1		Adult	Not reported	45
8	5	8	Adult	Not significant	46
5	3	8	Adult	Decreased clostridia Increased bacteroides	47
6.6	3	31	Adult	Not significant	48
8	4	9	Adult	Not significant	49
9	2	10	Adult	Increased bacteroides	50
8	3	19	Elderly	Decreased *E. rectalis*	51

Fermentation by intestinal microflora and selective stimulation of the growth and/or activity of intestinal bacteria associated with health and well-being. In pure culture studies, all of the bifidobacteria tested, all of the bacteroides, most lactobacilli and enterobacteria, and some streptococci metabolized the TOS, with bifidobacteria displaying the most vigorous growth. The in vitro data presently available do not, however, fully demonstrate a selective stimulation of bacterial growth (55).

In a study by Rowland and Tanaka (56) on gnotobiotic rats inoculated with human fecal flora and fed a TOS-containing diet, analysis of cecal contents on selective agars revealed significant increases in bifidobacteria and lactobacilli and a significant decrease in enterobacteria. This was followed by in vivo human volunteer studies that showed significant increases in fecal bifidobacteria (57,58). Similarly Ito et al. (59) found a significant increase in bifidobacteria and lactobacilli and significant decreases in *Bacteroides* and *Candida*. Infant formula milk supplemented with a mixture of oligosaccharides (90% galactooligosaccharides and 10% inulin) has been shown to increase fecal bifidobacteria in both preterm and term infants (60,61).

Even though the first criterion for prebiotic classification is not totally fulfilled, and because of significant data in human studies, TOS can be classified as prebiotic.

Other candidates. Glucooligosaccharides, isomaltooligosaccharides, 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 (2).

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 (2).

Data analysis: introducing the prebiotic index

In regard to prebiotic evidence, two questions that have attracted attention concern the quantitative aspects of the prebiotic effect. These questions can be formulated as follows: *1*) Are the different inulin-type fructans equally effective? *2*) Can a dose-effect relation be established?

To answer these questions a kind of meta-analysis has been performed based on the results of all studies available including those that have appeared in abstract form only, have been published as part of the proceedings of a conference, or have been given to the author as personal communication. The criteria for including these studies in the analysis were that the available report should have included at least *1*) the daily dose of the prebiotic, *2*) the nature of the prebiotic, i.e., inulin or oligofructose, *3*) the number of volunteers, and *4*) the number of bifidobacteria per gram of feces both at the beginning and at the end of the supplementation period.

These data are presented in **Table 3**, which includes calculations that are usually not performed in discussions of the results of a prebiotic test. Indeed classically (and rightly so) in such studies, the microbiological data are expressed as colony-forming units (cfu) presented as $\log_{10}$ cfu/g of feces, and the prebiotic effect is then expressed as (*D*), the "crude" increase, or "+ *X* $\log_{10}$ cfu/g" of feces (e.g., if the initial and the final

TABLE 3 Summary of the quantitative data on the prebiotic effect of inulin-type fructans resulting from all human intervention studies available

		B	*C*		*E*				
A		Log_{10}	Log_{10}	*D*	cfu/g T_{max}	*F*	Prebiotic index		
Dose, *g/d*	*N*	cfu/g T_0	cfu/g T_{max}	$C - B$	$-$ cfu/g T_0	Log_{10} E	*E/A* $\log_{10}$	*E/A*	Ref.
10	5	8.8	9.5	0.7	25.3×10^8	9.4	2.50×10^8	8.4	62
6	9	8.7	9.8	1.1	55.0×10^8	9.7	9.20×10^8	8.96	63
8	23	8.8	9.7	0.9	44.6×10^8	9.6	5.57×10^8	8.75	39
8	6	9.0	9.5	0.5	22.0×10^8	9.3	2.75×10^8	8.44	40
12.5	20	7.9	9.1	1.2	11.9×10^8	9.1	0.95×10^8	7.98	64
4	10	8.3	9.3	1.0	18.0×10^8	9.25	4.50×10^8	8.65	41
8	38	7.7	9.0	1.3	9.5×10^8	8.98	1.19×10^8	8.07	42
15	8	8.8	9.5	0.7	25.6×10^8	9.41	1.70×10^8	8.23	43
15	4	9.2	10.1	0.9	110×10^8	10.04	7.30×10^8	8.86	43
4	12	9.4	9.86	0.46	47.0×10^8	9.65	11.7×10^8	9.07	65
2.75	11	8.0	9.0	1.0	10.0×10^8	9.0	3.64×10^8	8.56	66
20	17	8.8	9.2	0.4	9.5×10^8	8.96	0.47×10^8	7.67	44
5	8	8.1	9.0	0.9	8.7×10^8	8.94	1.75×10^8	8.24	45
10	8	8.0	9.5	1.5	31.0×10^8	9.49	3.10×10^8	8.5	
20	8	8.2	9.5	1.3	30.4×10^8	9.48	1.52×10^8	8.18	
5	8	8.8	9.8	1.0	57.6×10^8	9.76	11.5×10^8	9.06	47
6.6	31	9.1	9.6	0.5	27.4×10^8	9.44	4.15×10^8	8.62	48
8	9	8.8	9.0	0.2	3.6×10^8	8.55	0.45×10^8	7.65	49
9	10	9.9	10.3	0.4	119×10^8	10.07	13.2×10^8	9.12	50
8	10	5.6	8.4	2.8	2.5×10^8	8.40	0.38×10^8	7.58	51
Total	272								
Mean (SEM)							4.0×10^8	(0.82×10^8)	

numbers of bifidobacteria are, respectively, 8.8 and 9.5 $\log_{10}$ cfu/g, the prebiotic intake has increased the population of bifidobacteria by 0.7 $\log_{10}$ cfu/g). That parameter (*D*) does not correlate with the daily dose (*A*) of the prebiotic ($r = 0.06$; NS). But the real meaning of the "crude" increase (*D*) is generally misinterpreted. Indeed if the initial population of bifidobacteria is 8, 9, or 10 $\log_{10}$ cfu/g, increasing it by 0.7 $\log_{10}$ cfu/g will not have the same meaning in terms of the number of "new" bifidobacteria cells that have appeared because of the prebiotic treatment. As in the example, the prebiotic treatment will have caused the appearance of 5×10^8, 5×10^9, and 5×10^{10} "new" bacterial cells, respectively, or 100 times more cells in the last than in the first case. It is thus necessary to calculate these absolute numbers of "new" bacterial cells and express them as such (*E*) or as $\log_{10}$ values (*F*). But once again, the daily dose (*A*) of the prebiotic does not correlate with these numbers (*E* and *F*) ($r = 0.09$; NS).

The reason is that an important parameter, the initial number of bifidobacteria (*B*), is not taken into account. In the first report of a prebiotic effect, Hidaka et al. (67) have already argued that the initial numbers of bifidobacteria (expressed as $\log_{10}$ cfu/g of feces *B*) influence the prebiotic effect after observing an inverse correlation between these numbers and their "crude" increase after oligofructose feeding. Roberfroid et al. (35), Rao (47), and Rycroft et al. (68) have reached the same conclusion that is also supported by the data in Table 3 ($r = -0.76$; $P < 0.01$). But that correlation holds true only for the "crude" increases, not for the absolute increases in fecal bacteria (*F*) ($r = 0.12$; $P > 0.10$).

To further discuss the prebiotic effect, I propose to introduce a "prebiotic index" defined as "The increase in bifidobacteria expressed as the absolute number (*N*) of 'new' cfu/g of feces (*E*) divided by the daily dose (in grams) of prebiotic ingested (*A*)."

For inulin-type fructans, such a prebiotic index is of the order of a few (average $= 4.00 \pm 0.082$) 10^8 cfu/g, and it directly correlates with the initial number of bifidobacteria ($r = +0.55$; $P < 0.01$). Moreover, the prebiotic indices of the different types of inulin, especially oligofructose and inulin, appear to be similar even if there is a tendency for inulin (average $+5.1 \pm 2.4$) to be more potent than oligofructose (average $+3.7 \pm 0.8$), but data do not allow a final conclusion mostly because oligofructose has been tested more often than inulin.

As suggested by 1 experimental study (38), different types of inulin molecules might affect the bacterial populations that colonize different segments in the gastrointestinal tract differently, especially the different segments of the colon but also different habitats in the colon (e.g., the mucosa or the mucosal layer). But this needs further investigation that requires the development of new methodologies.

Another parameter related to the prebiotic effect that could be of interest is the increase in total daily fecal excretion of bifidobacteria per se and per gram of inulin-type fructan ingested. But unfortunately only 1 of the 22 publications available so far has given the 24-h fecal output of the volunteers (43), and the calculated increases in bifidobacteria (total and per gram, respectively) are $+32 \times 10^{10}$ cfu/24 h or $+2 \times 10^{10}$ cfu/24 h/g oligofructose and $+142 \times 10^{10}$ cfu/24 h or $+9.5 \times 10^{10}$ cfu/24 h/g inulin, respectively.

Future perspectives and conclusion

Prebiotics have great potential as agents to improve or maintain a balanced intestinal microflora to enhance health and well-being. They can be incorporated into many foodstuffs. There are, however, several questions that still need to be answered. For example, this article has based conclusions on prebiotic classification from current evidence. As this continues to accumulate, the picture will become clearer, enabling the classification of certain carbohydrates where evidence is currently sparse or absent. Moreover, as better information on structure-to-function information accrues, as well as individual metabolic profiles of target bacteria are compiled, it may be easier to tailor prebiotics for specific health attributes. Much more information is needed on the fine structure of the changes brought about by regular intake of prebiotics. With the new generation of molecular microbiological techniques now becoming available, it will be possible to gain definitive information on the species rather than genera that are influenced by the test carbohydrate. If comparative information is to be gathered on structure-function relations in prebiotic oligosaccharides, a rigorous approach to the evaluation of these molecules will be required. Such thorough comparative studies will allow intelligent choices in incorporating prebiotics into functional foods and should increase confidence among consumers and regulatory authorities. Similarly, it may be possible to incorporate further biological functionality into the concept, e.g., increasing beneficial bacteria while suppressing pathogens at the same time, perhaps through anti-adhesive approaches (69).

The current most popular targets for prebiotic use are lactobacilli and bifidobacteria. This is largely based on their success in the probiotic area. However, as our knowledge of the gut flora diversity improves (through using the molecular procedures described earlier), it may become apparent that other microorganisms should be fortified through their use. One example may be the *Clostridium coccoides–Eubacterium rectale* cluster that includes bacteria producing butyric acid, a metabolite seen as beneficial for gut functionality and potentially protective against bowel cancer (38). The likelihood of other bacteria (including still unknown genera) also being targets for a prebiotic effect must be put in perspective with our increasing understanding (thanks to new molecular methodologies) of the bacterial diversity in the gut microflora. Indeed, the more we identify and characterize the bacterial genera, species, and even strains that compose the intestinal microflora, the more we will be in a position to describe, both qualitatively and quantitatively, changes in that composition and, consequently, to understand how the myriads of bacterial cells in the intestine interact and how they contribute to and modulate intestinal (especially colonic) physiology. Prebiotics will then become unique tools to create, both in experimental animals and in humans, colonic microflora with "controlled" compositions that will then be correlated with specific physiological conditions. But data are still too preliminary to speculate on these perspectives.

At the end of the present discussion aimed at revisiting the prebiotic definition, it must be emphasized that only 2 food carbohydrates, essentially nondigestible oligosaccharides, today fulfill the criteria for prebiotic classification (**Table 4**). For the other candidates, data are promising, but more studies are still required. In particular, it must be stressed that, with the exception of inulin and oligofructose, data to fulfill criterion 1, i.e., "resistance to gastric acidity, hydrolysis by mammalian enzymes, and gastrointestinal absorption" are lacking. Similarly more in vitro data in mixed culture systems and more in vivo data, especially in reliable human nutrition intervention studies, are 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.

TABLE 4 Summary and conclusion on the prebiotic effect of various oligosaccharides

Carbohydrate	Nondigestibility	Fermentation	Selectivity	Prebiotic status
Inulin and oligofructose	Yes	Yes	Yes	Yes
Galactooligosaccharides	Probable	????	Yes	Yes
Lactulose	Probable	????	Yes	Yes
Isomaltooligosaccharides	Partly	Yes	Promising	No
Lactosucrose	NA	NA	Promising	No
Xylooligosaccharides	NA	NA	Promising	No
Soybean oligosaccharides	NA	NA	NA	No
Glucooligosaccharides	NA	NA	NA	No

NA, data not available; ????, preliminary data, but still need further research.

The daily dose of the prebiotic is not a determinant of the prebiotic effect, even if, in 1 group of volunteers with relatively similar initial counts of fecal bifidobacteria, a limited dose-effect relation can be established (45). The daily dose does not correlate with the "crude" or with the absolute increase in bacterial cells or with the prebiotic index. The major factor that quantitatively controls the prebiotic effect is the number of bifidobacteria per gram of feces the volunteers have before supplementation of the diet with the prebiotic begins. That parameter inversely correlates with the "crude" increase in fecal bifidobacteria, but, more importantly, it directly correlates with the prebiotic index that is otherwise independent of the daily dose. At the population level it is thus the fecal flora composition (especially the number of bifidobacteria) characteristics of each individual that determine the efficacy of a prebiotic but not the dose itself. The ingested prebiotic stimulates the whole indigenous population of bifidobacteria to growth, and the larger that population the greater will be the number of new bacterial cells appearing in feces. The "dose argument" (often used for marketing some prebiotics) is thus not supported by the scientific data; it is misleading for the consumer and should not be permitted.

One important question as yet basically unanswered is the effect of the prebiotic not on the numbers of bacteria, especially bifidobacteria, but rather on activities associated with these bacteria. Indeed, the health benefits for the host are part of the definition, and these benefits are directly dependent on what these bacteria do, how they interact with the others, and how they modulate intestinal functions. 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) have been measured and shown to vary (increase or decrease) after ingestion of prebiotics. But the validity of these parameters still remains to be established, especially in terms of their value as a biomarker of colonic and eventually host health and well-being or disease risk reduction. In that context the effects of inulin-type fructans on these parameters reported so far are contradictory and difficult to interpret (44,65).

The original definition of a prebiotic only considered microbial changes in the colonic ecosystem of humans. However, it may be timely to extrapolate this into other areas that may benefit from a selective targeting of particular microorganisms. As such it has been proposed to refine the original definition to "a prebiotic is a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microflora, that confer benefits upon host well-being and health" (2).

The concept of prebiotic is only 11 y old and has already attracted and stimulated research in many areas of both nutrition and medical sciences. New developments in molecular microbiology will allow more similar studies specifically targeted at answering important but still unsolved questions. In particular, they will help determine health applications and explain mechanisms of effects. 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. There is thus currently much scientific interest in developing prebiotics that target this region of the colon. (69).

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