Critical Review

The Bifidogenic Nature of Chicory Inulin and Its Hydrolysis Products

Marcel B. Roberfroid,*[1] Jan A. E. Van Loo† and Glenn R. Gibson**

*Unité de Biochimie Toxicologique et Cancérologique, Department of Pharmaceutical Sciences, Université Catholique de Louvain, B1200 Brussels, Belgium; †ORAFTI, B3300 Tienen, Belgium; and **Microbiology Department, Institute of Food Research, Reading Laboratory, Earley Gate, Reading, U.K.

ABSTRACT Research data on the bifidogenic effect of β(2-1)fructans, which at present are commercialized in the U.S., Japan and Europe as food ingredients, are presented. These food ingredients originate from two different sources. Short-chain fructo-oligosaccharides are synthesized from sucrose and are composed of GF_n [n β(2-1) linked fructose moieties bound to a glucose molecule; $2 \leq n \leq 4$]. The longer chain length molecule inulin is extracted with hot water from chicory roots (*Cichorium intybus*) and is also composed of GF_n molecules (with $2 < n < 60$). Oligofructose is a partial hydrolysate of inulin and is composed of GF_n and F_m molecules (n and m indicate the number of fructose moieties with $2 \leq n, m \leq 7$). All types of β(2-1)fructans are well fermented by intestinal bacteria. For a given chain length, there is no difference in fermentation rate between GF_n- and F_m-type β-fructans. In vitro fermentation of inulin revealed that molecules with a chain length (degree of polymerization or DP) > 10 are fermented on average half as quickly as molecules with a DP < 10. All $\beta(2-1)$fructans are bifidogenic and classified as biobiotics. J. Nutr. 128: 11–19, 1998.

KEY WORDS: • *inulin* • *oligofructose* • *bifidobacteria* • *bifidogenicity* • *dose effect* • *prebiotic*

Dietary carbohydrates that have escaped digestion in the upper gastrointestinal tract form the predominant substrates for bacterial growth in the colon. There is also a (lesser) contribution from proteins and amino acids, as well as endogenously produced carbohydrates and glycoproteins. Resistant starch is not hydrolyzed by pancreatic amylases but can be metabolized by bacterially produced enzymes, e.g., from saccharolytic clostridia, bacteroides and bifidobacteria. Non-starch polysaccharides, such as celluloses, hemicelluloses, pectins and gums, may also be fermented in the large gut. Other sugars such as lactose, raffinose and stachyose and certain sugar alcohols, like sorbitol and xylitol, also contribute to the fermentable carbohydrate load (Cummings and Englyst 1995). The metabolism of these carbohydrates produces a variety of products such as short-chain fatty acids (e.g., acetate, propionate and butyrate), gases (e.g., H_2, H_2S, CO_2 and CH_4), and organic acids (e.g., lactate, succinate and pyruvate). These products may have varying effects on host health. However, another form of dietary carbohydrate, oligosaccharides, is attracting increasing interest from the human health perspective (Cummings and Roberfroid 1997).

An oligosaccharide is characterized by number, type and sequence of its monosaccharide moieties. On average, up to 10 monomeric units are contained in the chain, which may be either linear or branched. Oligosaccharides that are not hydrolyzed by digestive enzymes in the upper gastrointestinal tract are non-digestible and therefore reach the colon intact. Non-digestible oligosaccharides include those that contain fructose, glucose, xylose and galactose (Delzenne and Roberfroid 1994).

Among these, fructans extracted from the root of *Cichorium intybus* (chicory) have been authorized as food ingredients in all European countries as well as in the U.S., Canada and Japan. Similar products obtained by an enzymatic synthesis via transfer of fructosyl units from sucrose molecules are widely used in the Japanese food industry where they have been ascribed various functional properties (Bornet 1994).

The nutritional and biological properties of these food ingredients include dietary fiber effects, selective stimulation of the growth of bifidobacteria in the colon, systemic modulation of lipid metabolism and future potential as low energy sugar or fat substitutes (Gibson et al. 1994, Roberfroid et al. 1993).

Because of the potential health-promoting properties of certain food components, we have recently introduced the concept of prebiotics (Gibson and Roberfroid 1995). A prebiotic is "a non-digestible 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, that can improve the host health." For a food ingredient to be classified as a prebiotic, it must meet the following criteria: *1*) not be hydrolyzed or absorbed in the upper part of the gastrointestinal tract; *2*) be a selective substrate for one or a limited number of potentially beneficial bacteria commensal to the colon, e.g., bifidobacteria and lactobacilli, which are stimulated to grow, and *3*) be able, as a consequence, to alter the colonic microflora toward a potentially more healthy composition and/or activity.

Any food ingredient that enters the large intestine is a candidate prebiotic. However, to be effective, selective fermentation by the colonic microbiota is crucial. This has been

[1] To whom correspondence should be addressed.

[2] Abbreviations used: BFM, *Bifidobacterium* sp. fermented milk; DP, degree of polymerization; F, fructose; G, glucose; n, number of monosaccharides in oligosaccharides.

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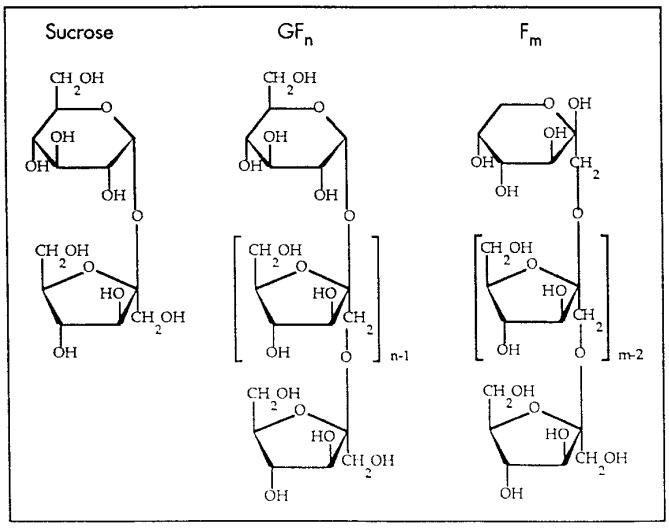

FIGURE 1 Chemical structure of the various fructo oligosaccharides. G, *glucose*; F, *fructose*; n or m indicate the number of fructose moieties in the molecules.

demonstrated with non-digestible oligosaccharides (especially those that contain fructose). Bifidobacteria have been identified as preferred target microorganisms for prebiotics (Gibson et al. 1995). This is because bifidobacteria may exert a variety of effects that may contribute towards host health and comprise one of the dominant bacterial populations in the human large intestine.

Chicory-derived fructans, i.e., inulin and its hydrolysate, oligofructose, are prebiotics that may also be classified as functional food ingredients. This classification requires a strict scientific approach, whose strategy implies the identification as well as an understanding of the mechanisms of interactions between the food ingredient and particular physiologic functions. This should also be followed by a demonstration of any such interaction. Although the difference between functionality and health promotion is large and should be distinguished, the approach may also lead to certain health claims (Roberfroid 1995 and 1996).

In this review, available data that confirm the ability of chicory inulin and oligofructose to specifically stimulate the growth of bifidobacteria in the human colonic microbiota will be summarized and compared. Throughout, the term chicory fructo-oligosaccharides will be used to refer to both inulin and the products of its partial enzymatic hydrolysis, whereas the term synthetic fructo-oligosaccharides will be used for those products obtained by enzymatic synthesis from sucrose.

THE CHEMICAL COMPOSITION OF CHICORY INULIN AND ITS HYDROLYSIS PRODUCTS

Chicory inulin and its enzymatic hydrolysis products are mixtures of $\beta(2\text{-}1)$ linked fructans of the GFn and Fm types (**Fig. 1**). G refers to the glucosyl moiety, F to the fructosyl moiety and n or m indicates the number of fructosyl moieties

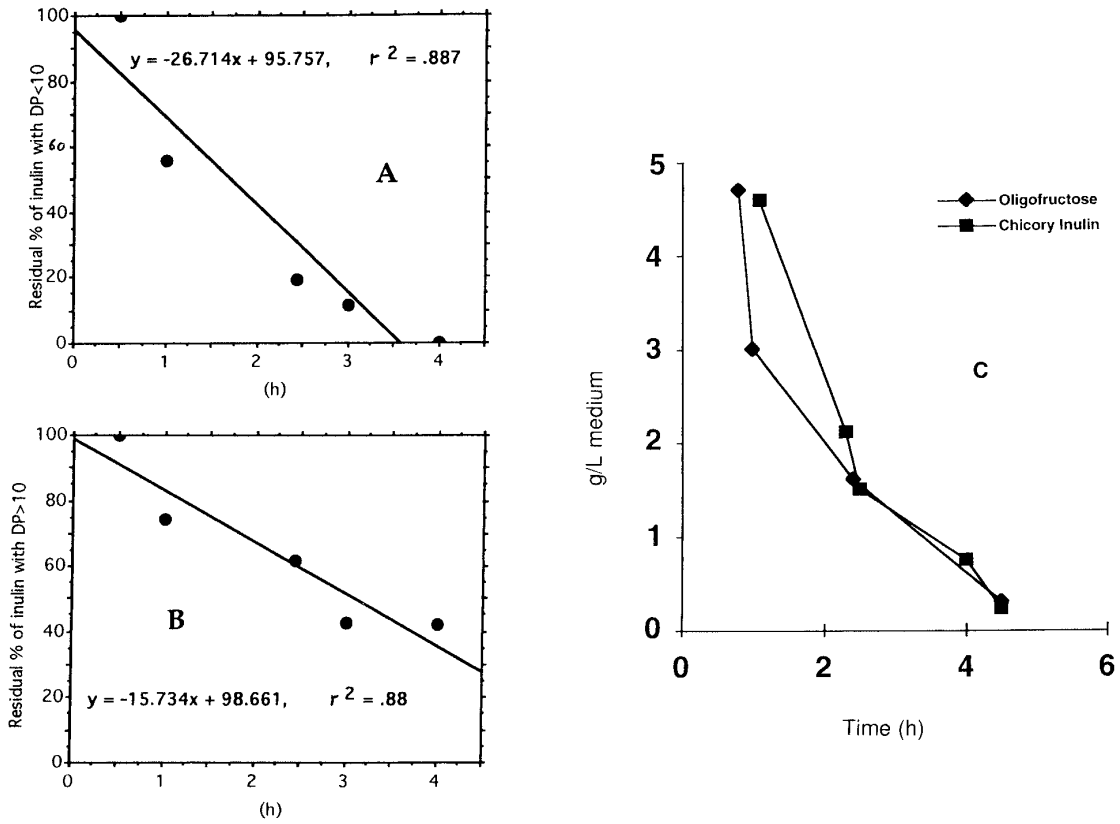

FIGURE 2 Time course of disappearance of 7 g/L chicory fructo-oligosaccharides from an anaerobic in vitro culture inoculated with mixed human fecal bacteria (100 g/L). Chromatographic analysis of the fructo-oligosaccharides was performed as previously reported (Van Loo et al. 1995). An aliquot of 5 g of medium was extracted with 2 mL hexane to remove fatty compounds. The sugars subsequently were silylated and analyzed by means of gas chromatography (Van Loo et al. 1995). *Panels A* and *B* represent the time course of disappearance of molecules with a degree of polymerization <10 and >10, respectively. Each point is the mean of 2–3 measurements.

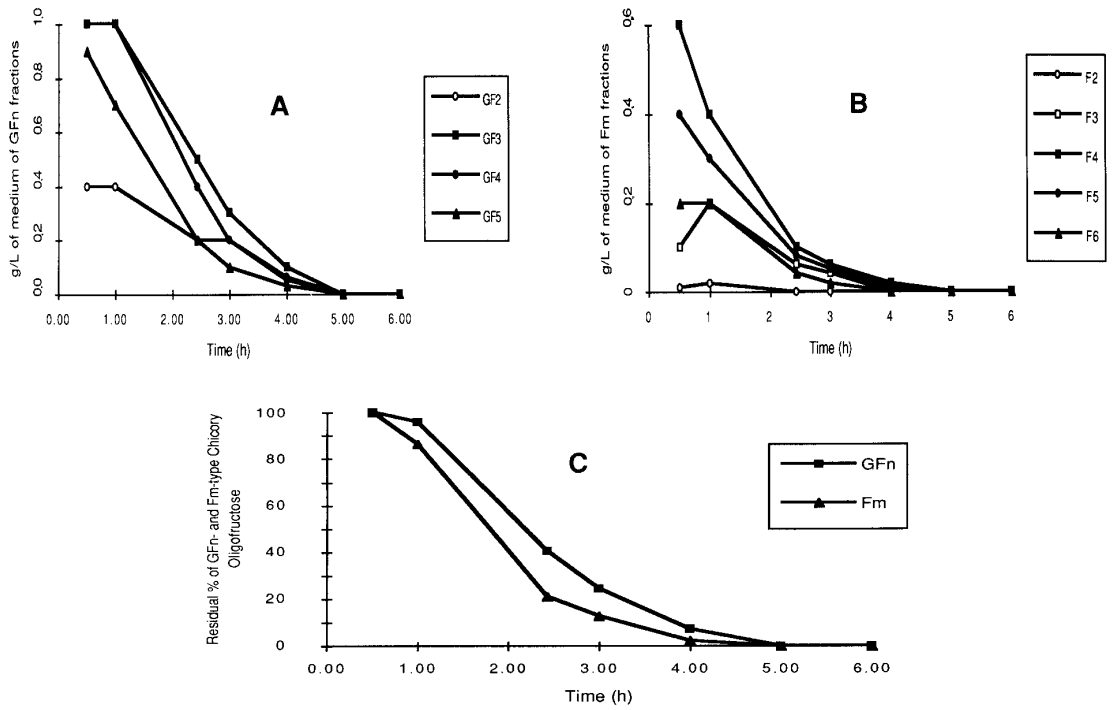

FIGURE 3 Relative rate of degradation of total and individual Fructosyl$_n$-Glucose (GF_n) (*panel A*) and Fructosyl$_{m-1}$Fructose (F_m) (*panel B*) molecules in inulin hydrolysate (7 g/L) anaerobically incubated with human fecal slurry (100 g/L). There was no preference for either GF_n or F_m as demonstrated by the relative overall rate of disappearance (*panel C*). Chromatographic analysis as described in Figure 2.

TABLE 1

Drop in culture pH due to fermentation of selected fructo-oligosaccharides by different bacterial species[1,2]

Group of bacteria (*n*)[3]	Synthetic fructo-oligo-saccharides (ΔpH)[4]	Chicory oligofructose (ΔpH)	Chicory inulin (ΔpH)	Glucose (ΔpH)
Bacteroides spp. (16)	−0.8[a]	−0.9[a]	−0.6[a]	−1.1[b]
Clostridium spp. (26)	−0.4[b]	−0.4[b]	−0.2[a]	−1.4[c]
Enterococcus fecalis (3)	−0.4[a]	−0.6[b]	−0.4[a]	−1.9[c]
Klebsiella spp. (2) (insufficient data for adequate statistical analysis)	−1.3	−1.3	−0.4	−1.9
Lactobacillus spp. (9)	−0.8[b]	−1.0[b]	−0.5[a]	−2.2[c]
Proteus spp. (2)	−1.2[b]	−1.2[b]	−0.3[a]	−2.0[c]
Staphylococcus spp. (3)	−0.4[a]	−0.7[a]	−0.3[a]	−1.9[b]

[1] Pure cultures of various strains of the bacterial genera were prepared in test tubes after incubation on modified GAN broth medium (Calpis Food Industry, Tokyo, Japan) and 24-h anaerobic incubation at 37°C. To measure the drop in pH due to fermentation of the selected fructo-oligosaccharides, pure cultures were added as 100 g/L inoculum to a medium composed of tryptone, 10 g/L; yeast extract, 10 g/L; pepsin, 0.2 g/L; $NaHCO_3$, 0.4 g/L; K_2HPO_4 and KH_2PO_4, 0.04 g/L; $CaCl_2$ and $MgSO_4$, 0.008 g/L; L-cysteine·HCl, 0.5 g/L; and agar, 1.5 g/L. After adding 5 g/L of the selected substrates, incubation was performed in an anaerobic cabinet with an atmosphere composed of $H_2/CO_2/N_2$ in the ratio 10:10:80.

[2] The initial pH of all cultures was 7, and changes were measured directly in culture tube.

[3] (*n*) indicates the number of strains tested. Within a row, values with different superscripts are significantly different ($P < 0.05$; one-factor ANOVA, repeated measures).

[4] The drop in pH induced by fructo-oligosaccharide fermentations is expressed as pH (averaged value) at the end of fermentation in the presence of test carbohydrate minus pH at the end of the reference fermentations in which no test carbohydrate was added (adapted from Wada 1990).

(n ranges between 2 and 60 for chicory inulin and between 2 and 7 for chicory oligofructose). Inulin that is manufactured by ORAFTI (Tienen, Belgium) as Raftiline® ST (chicory inulin as it is present in the root) is obtained industrially by hot water extraction of fresh chicory roots (Gibson et al. 1994). It contains 92% fructo-oligosaccharides, almost exclusively (>98%) of the GF_n type, with an average degree of polymerization (DP) of 10 hexose units. About 10% of the fructan chains have a DP ranging between 2 (F_2) and 5 (GF_4). Oligofructose (manufactured as Raftilose) is produced by partial enzymatic hydrolysis of native inulin and is a mixture of GF_n- and F_m-type oligomers with an average DP of 4–5. It is available in different well-defined qualities and contains up to 90% F_m-type molecules. In the various preparations of oligofructose, oligomers with a DP from 2 (F_2) up to 5 represent about 70% of the total fructo-oligosaccharides (Van Loo et al. 1995). The synthetic fructo-oligosaccharides (commercialized as Actilight, BMI, Paris, France) contain fructo-oligosaccharides of the GF_n type, with an average DP of 3.7.

FERMENTATION OF CHICORY FRUCTO-OLIGOSACCHARIDES BY HUMAN FAECAL BACTERIA: CHEMICAL EVIDENCE

In vitro experiments on the comparative fermentation of chicory inulin and oligofructose in anaerobic batch culture fermenters inoculated with 100 g/L mixed human fecal bacteria and 7 g/L of the substrate have been conducted (Gibson and Wang 1994a, Wang and Gibson 1993). Residual fructo-oligosaccharides were analyzed by means of gas chromatography (De Leenheer and Hoebregs 1994, Van Loo et al. 1995) at various time intervals after inoculation. Data reported in **Figure 2** show that both inulin and oligofructose were rapidly and completely metabolized by the microbial flora in these fermenters. Moreover, the relative rate of fermentation was

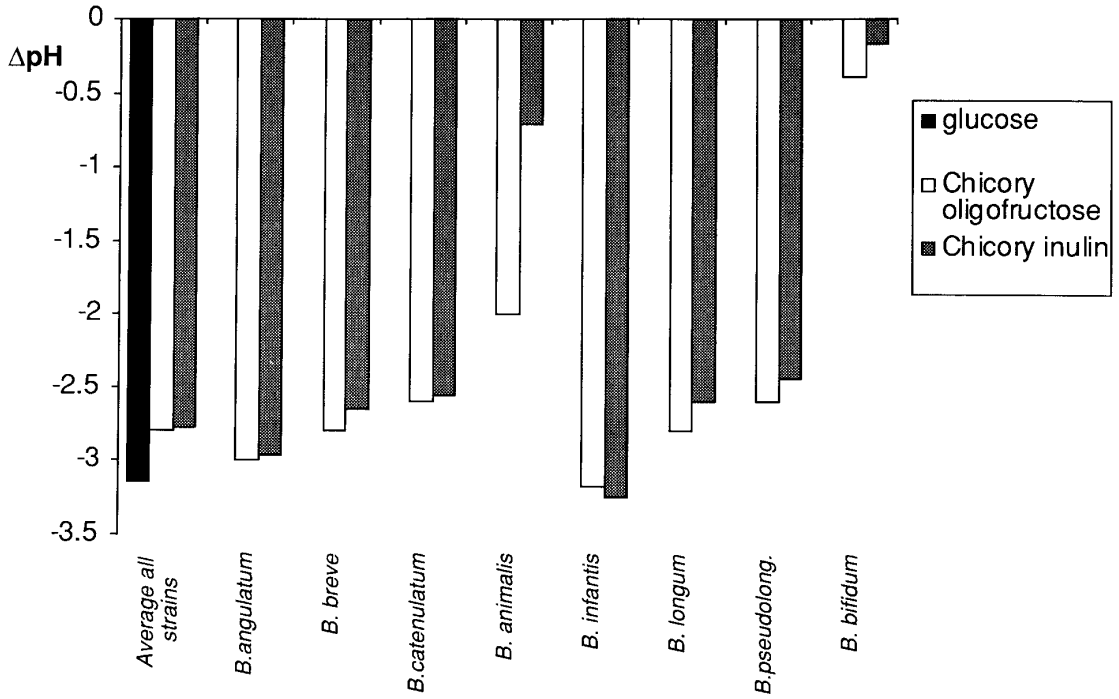

FIGURE 4 Drop in pH of the batch culture media inoculated with selected strains of bifidobacteria and incubated anaerobically for 24 h in the presence of 7 g/L glucose, oligofructose or inulin. Values are means of 3 determinations (SD ≤ 1%) and initial culture pH was 7.0 (adapted from Wang 1993).

similar for both substrates. A more detailed analysis, however, revealed that the rate of degradation of oligomers with a DP <10 (Fig. 2*a*) was approximately twice that of molecules with a higher DP (Fig. 2*b*). The data presented in **Figure 3** (*a*, *b*, *c*) demonstrate, moreover, that the GF_n- and F_m-type components of inulin hydrolyzates disappeared from the culture media at a similar rate.

FERMENTATION OF CHICORY FRUCTO-OLIGOSACCHARIDES BY PURE CULTURES OF COLONIC BACTERIA AND MIXED POPULATIONS FROM FECAL SLURRIES: EFFECT OF FERMENTATION ON CULTURE MEDIUM pH

In vitro fermentations of chicory fructo-oligosaccharides by human colonic bacteria, mainly bifidobacteria, produce lactate and short-chain carboxylic acids (mostly acetate). Consequently, the bacterial metabolism of these substrates causes a marked decrease in the culture medium pH. Batch culture experiments have been conducted, using pure cultures of different bacterial species, incubated for 96 h in the presence of 5 g/L substrate (Wada 1990; **Table 1**). Data indicate that the utilization of oligofructose by *Bacteroides* spp., clostridia, enterococci, *Klebsiellae*, lactobacilli, *Proteus* and *Staphylococcus* was comparable to that of synthetic fructo-oligosaccharides but for both oligosaccharides, it was lower than that of glucose ($P < 0.05$). The utilization of inulin by the same bacterial species was also lower than that of glucose ($P < 0.05$), but it was also lower than that of the two other fructo-oligosaccharides except for *Bacteroides* spp., enterococci and staphylococci, incubated for 24 h in the presence of 7 g/L substrate (**Fig. 4**). When incubation was performed in the presence of various strains of bifidobacteria, data from Wang (1993) showed that the utilization of oligofructose was comparable to that of glucose and that inulin was also well fermented by all strains of bifidobacteria tested. Neither oligofructose nor inulin were to a significant extent fermented by *Bifidobacterium bifidum*. The only strain for which inulin appeared to be a less efficient substrate (−0.7 pH units) than the lower DP oligomers (−2.0 pH units) was *Bifidobacterium animalis*. When incubations were performed using human fecal bacteria as inoculum, both inulin and oligofructose were well fermented (**Fig. 5**). However, pH

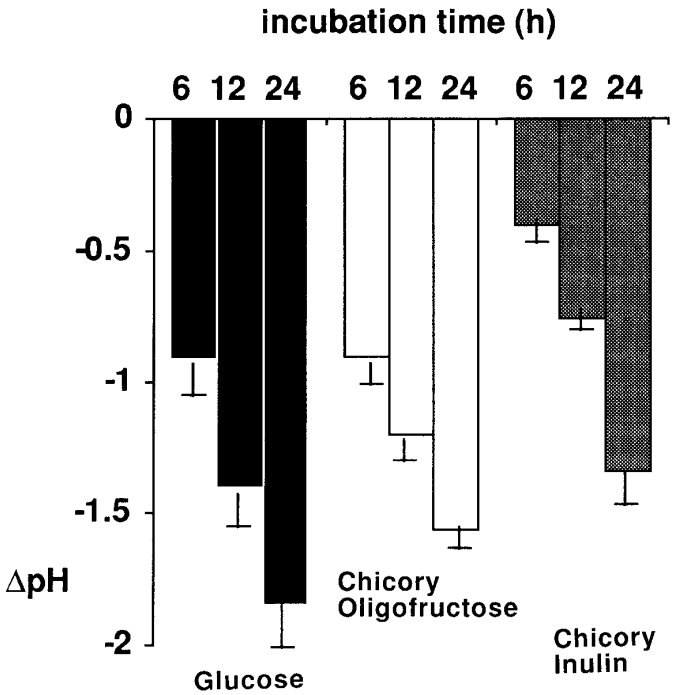

FIGURE 5 Drop in pH as a function of time of the batch culture media inoculated with 100 g/L human fecal slurry and incubated anaerobically for 24 h in the presence of 7 g/L glucose, oligofructose or inulin at 37°C. Values are means ± SD from duplicate determinations from 6 different volunteers. Initial culture pH was 7.0 (adapted from Wang 1993).

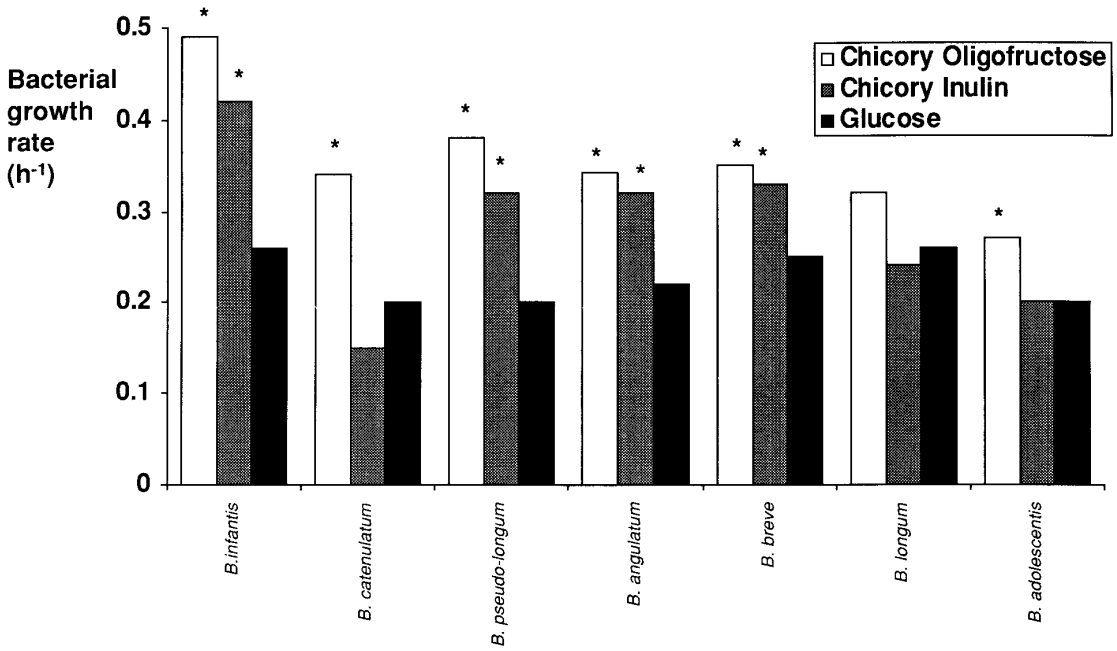

FIGURE 6 Growth rate of various strains of bifidobacteria incubated anaerobically at 37°C in the presence of 10 g/L glucose, oligofructose or inulin. (*Significantly ($P < 0.05$) higher growth rate than rate on glucose). Results are means of 3 determinations and SD was always ≤10% (adapted from Wang 1993).

measurements after 6 and 12 h showed that the rate of inulin metabolism might be somewhat lower than that of oligofructose.

Fructo-oligosaccharides are thus efficient substrates for most strains of bifidobacteria. This is due to the intracellular 2,1 β-D fructan-fructanohydrolase (EC 3.2.1.7) activity of the latter. Although pH change is a relatively poor indicator of bacterial growth, data reported in Table 1 indicate that bacteria other than bifidobacteria also are able to ferment fructo-oligosaccharides. These include *Klebsiella pneumoniae*, *Staphylococcus aureus* and *epidermidis*, *Enterococcus faecalis* and *faecium*, *Bacteroides vulgatus*, *thetaiotaomicron*, *ovatus* and *fragilis*, *Lactobacillus acidophilus* and *Clostridium* spp. (mainly *Cl. butyricum*).

IN VITRO FERMENTATION OF CHICORY FRUCTO-OLIGOSACCHARIDES: CONFIRMING THE BIFIDOGENIC EFFECT

The in vitro profiles of bacterial fermentative activities on certain growth substrates give the potential for such events in

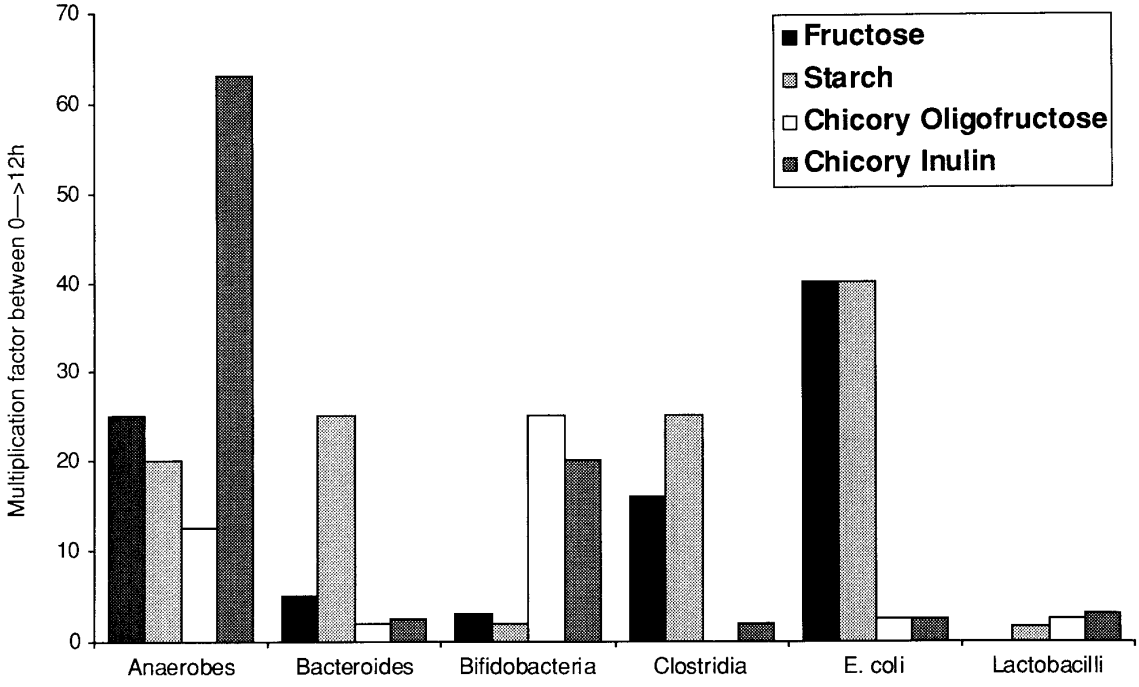

FIGURE 7 Changes in the numbers, expressed as multiplication factors (ratio $CFU_{t=12\ h}/CFU_{t=0\ h}$) of human fecal bacteria after the in vitro incubation for 12 h in the presence of 7 g/L fructose, starch, oligofructose or inulin (calculated values from Wang and Gibson 1993).

TABLE 2

Composition of the microflora of human fecal slurries (100 g/L) after six turnovers in single-stage continuous culture fermenters containing glucose, oligofructose or inulin as the growth substrate (10 g/L)[1]

	Glucose	Chicory oligofructose	Chicory inulin
	log_{10} of viable bacteria per L medium		
Anaerobes	13.4	12.6	12.1
Bacteroides	12.0	9.4	9.3
Bifidobacteria	10.0	12.7	12.1
Clostridia	9.9	8.4	9.6
Coliforms	5.3	5.5	5.3
Lactobacilli	10.5	7.3	<8
Difference log_{10} Bifidobacteria − log_{10} Bacteroides	−2.0	+3.3	+2.8

[1] The fermenters were operated anaerobically at pH 6.8 with a dilution rate of 0.3 per hour (adapted from Gibson and Wang 1994b and Wang 1993).

vivo. For example, a preferred fermentation by bifidobacteria may be of some benefit to the host if the purported beneficial aspects associated with these microorganisms are reflected in a natural habitat, such as the human large intestine. The in vivo potential requires confirmation, however.

In vitro data on this selectivity for the chicory fructo-oligosaccharides is shown in **Figure 6**, which gives specific growth rates (per hour) of various pure cultures of bifidobacteria cultivated on either inulin, oligofructose or glucose as the fermentable growth substrate. Bifidobacteria always grew better on oligofructose than on glucose. Their growth rate on inulin was reduced; it was comparable to the growth rate on glucose for 3 out of 7 tested strains and was higher than on glucose for 4 of the 7 strains tested.

Using human fecal slurries incubated in batch anaerobic cultures, Wang and Gibson (1993) reported that, after 12 h of incubation in the presence of 7 g/L fructose, starch, inulin or oligofructose, both of the chicory fructo-oligosaccharides had a relatively specific effect on growth of the bifidobacteria (**Fig. 7**).

Using continuous chemostat cultures inoculated with 160 g/L fecal slurries, Gibson and Wang (1994b) showed that after six turnovers, chicory fructo-oligosaccharides, but not glucose, were able to selectively stimulate bifidobacterial growth; the number of these bacteria was almost three orders of magnitude higher than bacteroides. With glucose as substrate, bacteroides were two orders of magnitude higher than bifidobacteria (**Table 2**).

COLONIC FERMENTATION AND BIFIDOGENIC EFFECT OF CHICORY FRUCTO-OLIGOSACCHARIDES: HUMAN IN VIVO STUDIES.

Both chicory inulin and its hydrolysis product, oligofructose, have been used in human volunteer studies. In the studies reported by Gibson et al. (1995), the subjects were maintained on strictly controlled diets to which the chicory fructo-oligosaccharides were added as supplements (15 g/d for 15 d, with sucrose as a control placebo). These studies showed that the intake of the chicory fructo-oligosaccharides significantly modified the composition of the fecal microbiota. The most marked effect was an increase in the number of bifidobacteria to the same extent and with the same statistical significance for both oligofructose and inulin. More particularly, in the oligofructose experiment, a significant ($P < 0.05$) reduction in bacteroides, fusobacteria and clostridia was observed (**Figs. 8** and **9**). The total number of bifidobacteria excreted in 24 h increased by five and eight times after feeding of oligofructose and inulin, respectively. In terms of the composition of the colonic microbiota, there was a major shift toward bifidobacteria, the latter becoming by far the most numerically predominant bacterial group during the chicory fructo-oligosaccharides feeding.

The in vivo bifidogenicity of inulin has also been supported by Kleessen et al. (1994 and 1997) who showed that, in the elderly, the feeding of 20 and 40 g/d of inulin caused a significant ($P < 0.01$) increase in bifidobacterial counts in feces (from $10^{7.9}$ to $10^{8.8}$ and $10^{9.2}$, respectively).

Bouhnik et al. (1996) assessed the effects of prolonged ingestion in healthy humans of *Bifidobacterium* sp. fermented milk (BFM) with or without inulin (18 g/d) on fecal Bifidobacteria. They concluded that BFM substantially increased the proportion of bifidobacteria in the colonic flora, but the concurrent administration of inulin did not enhance the effect. More in-depth analysis of the data, however, revealed that 2 wk after stopping the consumption of the dairy products, the volunteers ($n = 6$) who

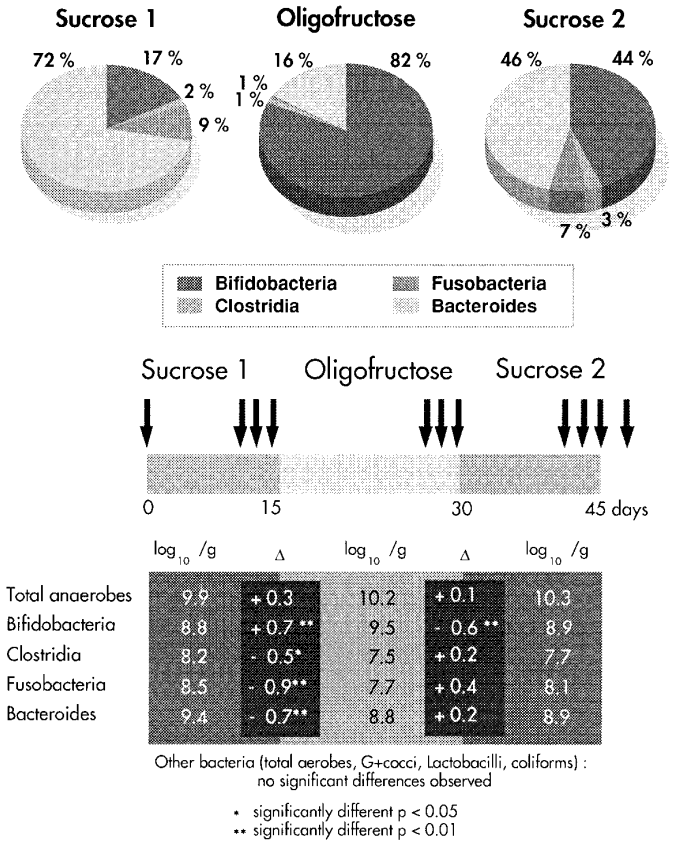

FIGURE 8 Changes in the predominant fecal bacterial groups of eight volunteers fed a strictly controlled diet for 45 d. The diets were supplemented with sucrose (d 0–15), oligofructose (d 16–30) and sucrose (d 31–45) (adapted from Gibson et al. 1995). The pies represent the relative percentage of the four major bacterial groups, with 100% as the sum of their individual counts. Δ represents the differences in bacterial counts at the end of each feeding period.

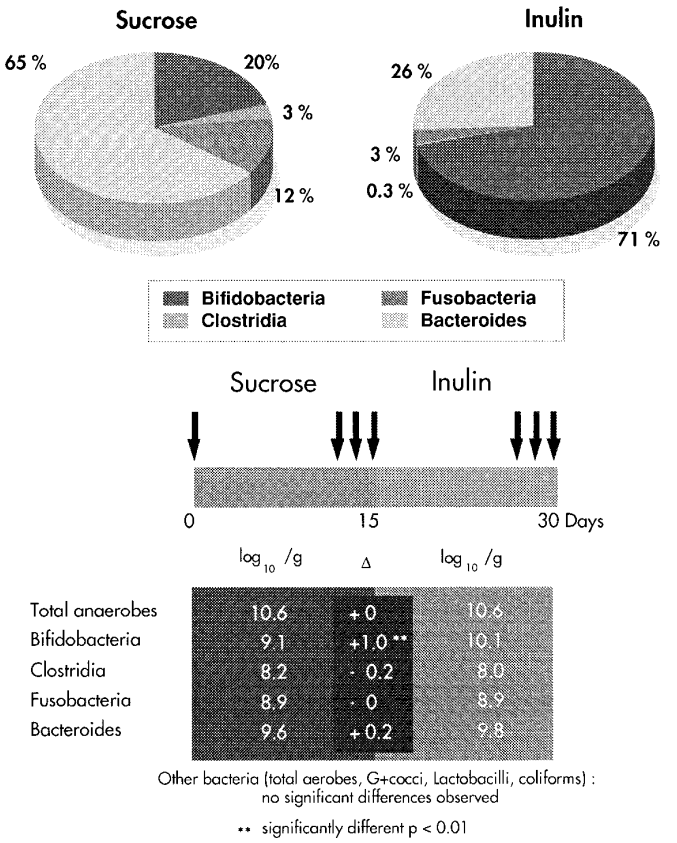

FIGURE 9 Changes in the predominant fecal bacterial groups of four volunteers fed a strictly controlled diet for 30 d. The diets were supplemented with sucrose (d 0–15) and inulin (d 16–30) (adapted from Gibson et al. 1995). The pies represent the relative percentage of the four major bacterial groups, with 100% as the sum of their individual counts. Δ represents the differences in bacterial counts at the end of each feeding period.

received the inulin supplement had a significantly ($P < 0.01$) higher number of bifidobacteria compared with those receiving BFM ($n = 6$) only. However, the study took in consideration only the counts of bifidobacteria, and not those of other bacterial genera; as such, a selective bifidogenic effect cannot be confirmed. In addition, the bifidobacteria counts with BFM alone were very high, not leaving room for additional increase by inulin. The hypothesis tested in this study concerns a "synbiotic" (Gibson and Roberfroid 1995) rather than a prebiotic effect.

Roberfroid (1997) reported a study in which eight healthy volunteers were administered a controlled diet for 2 wk followed by 3 wk of a free diet. Both diets were supplemented with 8 g/d of chicory oligofructose containing 90% F_m molecules. At the end of the feeding periods, a significant increase in fecal bifidobacterial counts with a concomitant reduction in *Bacteroides* spp. was observed in comparison with the placebo period. This demonstrated that the F_m-type fructo-oligosaccharides have a bifidogenic potential similar to that of the GF_n-type molecules.

Comparable human studies in European, Japanese and North American populations have also been reported for synthetic fructo-oligosaccharides (**Table 3**). Volunteers fed a diet supplemented with 4, 8 or 12.5 g chicory fructo-oligosaccharides per day showed a significant increase in bifidobacteria in feces. However, most of the studies referred to in Table 3 did not give information on counts of other bacterial genera, thus leaving open to question the specificity of the effect.

Another important question with regard to the effect of fructo-oligosaccharides on human fecal bifidobacterial counts is the dose-effect relationship. By combining all data reported for chicory oligofructose and synthetic fructo-oligosaccharides, it can be concluded that log increases in these counts do not necessarily correlate with the daily doses administered (**Fig. 10***a*). One variable that might correlate with these increases is the initial number of bifidobacteria in feces, independently of the dose of the fructo-oligosaccharides. Indeed, on the basis of this meta-analysis of different human studies, it appears that the lower this initial number, the greater is the increase whatever the daily dose, within a range of 4–20 or more grams (Fig. 10*b*). However, this does not exclude the possibility that, a dose-effect relationship might be observed if it were to be measured in the same group of volunteers with similar initial counts of bifidobacteria (e.g., 10^8). But within the general population, in which fecal counts of bifidobacteria vary considerably (from 10^7 to 10^9), such a dose-effect relationship would be difficult to observe. Consuming a few grams (4 or more) of any of these fructo-oligosaccharides daily could thus be sufficient to cause a significant increase in colonic bifidobacteria.

CONCLUSIONS

From the information given in this review, the following facts can be emphasized: *1*) Chicory fructo-oligosaccharides are a group of at least two food ingredients, i.e., oligofructose, which is composed of GF_n and F_m oligomers with a DP ranging from 2 to 7, and an average DP of approximately 4 to 5, and inulin, which is composed almost exclusively of GF_n molecules with a DP ranging between 2 and 60, and an average DP of 10. *2*) All chicory fructo-oligosaccharides are rapidly and completely metabolized when incubated in the presence of anaerobic batch cultures of human fecal bacteria. The GF_n and F_m oligomers are metabolized at similar rates. The fermentation, however, is slower

TABLE 3

Summary of the published data showing an in vivo increase in the counts of bifidobacteria in human feces after consumption of various doses of synthetic fructo-oligosaccharides added to the usual diet

Reference	Dose	Start of trial	End of trial	Statistical significance
	g/d	*log_{10} viable bifidobacteria/g fecal matter*		*P<*
Rochat et al. (1994)[1]	8	$10^{7.7}$	10^9	0.001
Bouhnik et al. (1994)[1]	12.5	10^8	$10^{9.2}$	0.01
Buddington et al. (1996)[1]	4	$10^{8.8}$	$10^{9.6}$	0.03
Mitsuoka et al. (1987)[2]	8	$10^{8.8}$	$10^{9.7}$	0.005

[1] Mean of 2 groups.
[2] Data as mentioned in paper.

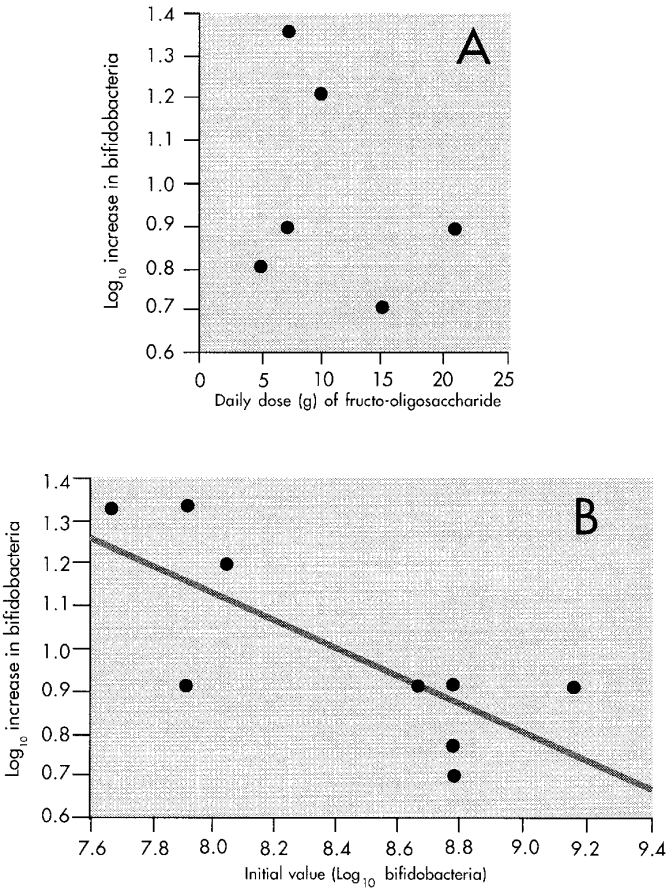

FIGURE 10 Correlation of the daily dose of β(2-1) fructan initial bifidobacteria counts with the following respective subsequent increases in number of bifidobacteria: *1*) The increase in counts of bifidobacteria ($\log_{10}$ of the ratio of the counts after and before the feeding period) in human feces is not correlated with the ingested dose (g/d) of chicory oligofructose or inulin and synthetic fructo-oligosaccharides. *2*) The increase in counts of bifidobacteria ($\log_{10}$ of the ratio of the counts after and before the feeding period) in human feces is correlated with the initial number (log units) of these bacteria before the trial. Adapted from the human studies published by Gibson et al. (1995), Buddington (1994), Kleessen (1997) and Roberfroid (1997).

for DP >10. *3*) The metabolism of chicory fructo-oligosaccharides in human fecal slurries is accompanied by a progressive fall in the pH of the culture medium (~1.5 pH unit after 12 h), which may indicate fermentation by bifidobacteria. Growth of pure cultures of various bifidobacterial species is well promoted by both inulin and oligofructose. When other bacterial genera are considered, it appears that only a small proportion are able to grow on fructo-oligosaccharides. *4*) Bifidobacteria grow faster than other intestinal bacteria on chicory fructo-oligosaccharide, and this is confirmed by in vivo experiments. In vivo human studies also suggest that the log increase in the number of bifidobacteria depends more on the initial number of bifidobacteria, irrespective of the dose of the fructo-oligosaccharides.

FINAL REMARKS

Chicory inulin and its partial enzymatic hydrolysate oligofructose selectively promote the growth of bifidobacteria in the human gut. The results presented here indicate that both GF_n- and F_m-type molecules have a bifidogenic effect.

Further important research areas also have arisen from the above findings. First, improved monitoring and detection of the human gut microbiota are required. The present methodologies are laborious, possibly inaccurate and do not estimate the full diversity of bacteria involved. A molecular approach is suggested for increased precision and accuracy in the monitoring of the colonic microflora in response to dietary modulation, in particular prebiotics ingestion. Furthermore, it should be determined whether dietary modulation of the composition of gut microbiota does indeed have any health advantage. More particularly, the meaning of an increase of bifidobacteria by 1 log unit remains difficult to assess. The absolute increase in the number of bifidobacteria probably is less important than the statistical significance of the increase. It can be stated that the observation of 1 log–fold increase in bifidobacteria is a clear indication of a modification of the intestinal flora, and that systematically at the same time a decrease in potential pathogens is observed. There are indications of other potential beneficial effects such as immunostimulation and increased barrier effect (colonisation resistance). This indicates the need for more functional assays of gut microflora activity involving volunteer trials. However, as long as the full composition of the flora is not known, such advances will be limited.

It is certainly worthwhile exploiting this research because the possible health advantages and optimal nutrition of the general population could be vastly improved. There is probably also a role for prebiosis in the prophylactic prevention and possible treatments of gut disease, especially that induced by the resident microbiota.

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