J_ID: Z7S A_ID: 22755 Art: FST_100005 Date: 7-MAY-10 Stage: I

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Food Science and Technology | Bulletin

Dietary prebiotics: current status and new definition

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Food Science and Technology Bulletin: Functional Foods **7** (1) 1–19
DOI: 10.1616/1476-2137.15880. Accepted 27 January 2010
ISSN 1476-2137

J_ID: Z7S A_ID: 22755 Art: FST_100005 Date: 7-MAY-10 Stage: I

Abstract

In November 2008, a group of scientists met at the 6th Meeting of the International Scientific Association of Probiotics and Prebiotics (ISAPP) in London, Ontario, Canada, to discuss the functionality of prebiotics. As a result of this, it was concluded that the prebiotic field is currently dominated by gastrointestinal events. However, in the future, it may be the case that other mixed microbial ecosystems may be modulated by a prebiotic approach, such as the oral cavity, skin and the urogenital tract. Therefore, a decision was taken to build upon the current prebiotic status and define a niche for 'dietary prebiotics'. This review is co-authored by the working group of ISAPP scientists and sets the background for defining a dietary prebiotic as "a selectively fermented ingredient that results in specific changes in the composition and/or activity of the gastrointestinal microbiota, thus conferring benefit(s) upon host health".

Keywords: prebiotics, gut microbiology, oligosaccharides, microbial fermentation

1. Introduction

A complex, resident gut microbiota is present in humans, with the number and composition of bacterial communities varying throughout the gut (Gibson and Collins 1999). This variability is largely due to different physicochemical conditions (pH, transit time, nutrient availability) in the various gut regions (Lambert and Hull 1996). The vast majority of bacteria in the human body resides in the large intestine, where the slow transit time, availability of nutrients, anaerobic conditions and pH are favourable for microbial growth.

Colonic microorganisms have ample opportunity to degrade available substrates, which may be derived from either the diet or by endogenous secretions (Cummings and Macfarlane 1991). Major substrates available for colonic fermentation are starches that, for various reasons, are resistant to the action of pancreatic amylases and can be degraded by bacterial enzymes, as well as dietary fibres (typically polysaccharides, degree of polymerisation (DP) $\geq$ 10) such as pectins and xylans. Other carbohydrate sources available for fermentation in lower concentrations include oligosaccharides (DP3–DP9) and portions of non-absorbable sugars and sugar alcohols. In addition, proteins and amino acids can be effective growth substrates for colonic bacteria, whilst bacterial secretions, lysis products, sloughed epithelial cells and mucins may also make a contribution. These materials are degraded by a wide range of bacterial polysaccharidases, glycosidases, proteases and aminopeptidases to smaller oligomers and their component sugars and amino acids. Intestinal bacteria are then able to ferment these intermediates to organic acids, H_2, CO_2 and other neutral, acidic and basic end products (Gibson and Macfarlane 1995). Fermentation by gut bacteria consists of a series of energy-yielding reactions that do not use oxygen in respiratory chains. The electron acceptors may be organic (e.g. some products of the fermentation) or inorganic (e.g. sulphate and nitrate).

The stomach is home to a relatively small number of microorganisms due to acidic conditions (pH 1–3), with numbers typically around 10^3 colony-forming units (cfu) per millilitre contents (Holzapfel *et al*. 1998). In the small intestine, although the pH is higher and more favourable for bacterial growth, bacterial numbers and diversity are limited by a rapid transit time and digestive secretions such as bile acids and pancreatic juices (approximately 10^4–10^6 cfu per millilitre contents). Main inhabitants of the small intestine are streptococci, staphylococci and lactobacilli, with bacterial numbers showing a progressive increase (Salminen *et al*. 1998). The human large intestine is one of the most diversely colonised and metabolically active organs in the human body (Eckburg *et al*. 2005). Here, microbial populations comprise approximately 10^{11}–10^{12} cfu per gram of contents. The colonic environment is favourable for bacterial growth, since it has a slow transit time, readily available nutrients and a favourable pH (Cummings and Macfarlane 1991). The majority of microbes in the large intestine are strict anaerobes (Moore and Holdeman 1974). Commonly encountered genera include bacteroides, eubacteria, fusobacteria, bifidobacteria, peptostreptococci, clostridia, lactobacilli and streptococci (Salminen *et al*. 1998). The composition and activity of the microbiota has a marked influence on health and disease through its involvement in pathogenesis and immune function of the host (Gibson and Roberfroid 1995).

In terms of end products, a variety of different metabolites arise. Predominant amongst these are the short-chain fatty acids (SCFA) such as acetate, propionate and butyrate (Cummings 1995). The majority are absorbed into the blood stream and can be further metabolised systemically. Transport to and further metabolism of SCFA in the liver, muscle or other peripheral tissues is thought to contribute about 7–8% of host daily energy requirements. Other products include electron-sink metabolites such as ethanol, pyruvate and lactate, which are mostly further converted to SCFA and therefore do not accumulate to any significant level in the large bowel (Macfarlane *et al*. 1992).

The end products from a saccharolytic gut fermentation may be considered as benign or even positive. Acetate is metabolised systemically (brain, muscle tissues), whereas propionate is cleared by the liver (Salminen *et al*. 1998).

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Propionate function is still not completely clear; however, it may lower the hepatic production of cholesterol by interfering with its synthesis. Recent data suggest an inhibitory role of propionate in lipogenesis. Butyric acid is a good fuel for healthy colonocyte function and has also been suggested as a stimulator of intestinal apoptosis (Barcenilla *et al*. 2000). On the other hand, metabolites from protein and/or amino acid fermentation such as amines, ammonia and some phenolic compounds (Smith and Macfarlane 1996) can be detrimental towards host welfare. Such metabolites may impact on certain disease states and promote gut disorders (Mykkanen *et al*. 1998). Vitamins and proteins are also synthesised by certain intestinal bacteria and are partly absorbed and used by the host (Conly *et al*. 1994).

Colonic bacteria can be categorised as being either beneficial or potentially harmful due to their metabolic activities and fermentation end products. Generally, bacteria having an almost exclusive saccharolytic metabolism (e.g. no proteolytic activity) can be considered as potentially beneficial. Such a metabolic profile is typical for lactobacilli and bifidobacteria. Genera with a peptolytic or mixed saccharolytic/peptolytic metabolism, however, are either less beneficial or even harmful, in particular when they are able to form toxins out of gut contents or are themselves pathogens or opportunistic pathogens. Further active health-promoting effects of the microflora may include immunostimulation, improved digestion and absorption, vitamin synthesis, inhibition of the growth of potential pathogens, cholesterol reduction and lowering of gas distension (Gibson and Roberfroid 1995, 1999; Macfarlane and McBain 1999; van der Waaij 1999). The equilibrium between species of resident bacteria provides a gut microbiota that directly influences gastrointestinal (GI) health (Guarner and Malagelada 2003). Harmful effects include carcinogen production, intestinal putrefaction, toxin production, diarrhoea/constipation, liver damage and intestinal infections. Examples of bacteria with such confirmed metabolites with and without saccharolytic fermentation are proteolytic clostridia. Bifidobacteria and lactobacilli are considered to be examples of health-promoting constituents of the microflora. Lactobacilli may aid digestion of lactose in lactose-intolerant individuals, reduce constipation and infantile diarrhoea, help resist infections caused by salmonellae, prevent traveller's diarrhoea and help in irritable bowel syndrome (IBS; Salminen *et al*. 1998). Bifidobacteria are thought to stimulate the immune system, produce B vitamins, inhibit pathogen growth, reduce blood ammonia and blood cholesterol levels and help to restore the normal flora after antibiotic therapy, among other things (Gibson and Roberfroid 1995; Guarner 2006). A number of factors influence the composition of the microbiota. These may be related to changes in physiological conditions of the host (age, health status, stress, etc.), composition of the diet and environmental circumstances (e.g. use of pharmaceutical compounds such as antibiotics; Steer *et al*. 2001).

Recognition of the health-promoting properties of certain gut microorganisms has encouraged dietary-based modulation of the human intestinal microflora towards a more beneficial composition and metabolism (Gibson and McCartney 1998). The main focus of discussion here is prebiotics. However, as prebiotics owe their development largely to probiotics, these will also be included.

2. Dietary modulation of the gut microbiota: probiotics

One well-used approach for modulation of the gut microbiota composition is through the use of live microbial dietary additions, such as probiotics. The word probiotic is translated from the Greek, meaning "for life". An early definition was given by Parker (1974): "organisms and substances which contribute to intestinal microbial balance". However, this was subsequently refined by Fuller (1989) as: "a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance". This definition removed the reference to particles and a probiotic would therefore incorporate living microorganisms, seen as beneficial for gut health, into the diet. A further definition of probiotics was given as "a live microbial feed supplement that is beneficial to health" (Salminen *et al*. 1998). A WHO/FAO working party defined probiotics as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" (United Nations Food and Agriculture Organization of the United Nations 2001, 2002).

Early records of bacterial drinks being taken by humans are more than 2000 years old. At the beginning of this century, the approach was first put onto a scientific basis by the work of Metchnikoff (1907) at the Pasteur Institute in Paris. He hypothesised that the normal gut microflora could exert adverse effects on the host and that consumption of certain bacteria could reverse this effect. Metchnikoff refined the treatment by using pure cultures of what is now called *Lactobacillus delbrueckii* subsp. *bulgaricus*, which, with *Streptococcus salivarius* subsp. *thermophilus*, is used to ferment milk in the production of traditional yoghurt.

Subsequent research has been directed towards the use of intestinal isolates of bacteria as probiotics. Over the years, many types of microorganismshave been used. They mainly consist of lactic acid-producing bacteria (lactobacilli, streptococci, enterococci, lactococci, bifidobacteria) but also *Bacillus* spp. and fungi/yeasts such as *Saccharomyces* spp. and *Aspergillus* spp.

Main positive effects associated with probiotics include protection against gastroenteritis, improved lactose tolerance, and stimulation of the immune system through non-pathogenic means (Fuller 1997; Fuller and Gibson 1997). This also has implications for disorders thought to be mediated by gut bacteria. To this end, IBS, inflammatory bowel diseases and colorectal cancer have all been

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researched. For systemic benefits, reduced cholesterol and/or triglyceride levels, protection from atopic reactions and better absorption of minerals are suggested. These health aspects have been summarised in the literature (Marteau *et al*. 2001, 2002; Fuller 1992, 1997; Naidu *et al*. 1999; Tannock 1999; Nobaek *et al*. 2000; de Vrese *et al*. 2001; D'Sousa *et al*. 2002; Wullt *et al*. 2003; Yamano *et al*. 2006; Sanders *et al*. 2007).

Desired characteristics of a good probiotic are as follows:

- Exerts a beneficial effect when consumed
- Non-pathogenic and non-toxic
- Contains a large number of viable cells
- Has the capacity to survive and metabolise in the gut
- Retains its viability during storage and use
- If incorporated into a food, it should have good sensory qualities (Goldin 1998; Guarner and Schaafsama 1998; Bezkorovainy 2001; Dunne *et al*. 2001).

3. Dietary modulation of the gut microbiota: prebiotics

An alternative approach for microflora management through diet is the use of prebiotics, which are directed (at present) towards genus level changes in the gut microbiota composition. Here, the selective growth of indigenous gut bacteria is required. These were first developed in order to induce beneficial changes in the gut microbiota and to overcome some of the survivability issues that can occur with probiotics (in the product and after ingestion).

In terms of microbiota modulation, the term prebiotic was first coined in the mid-1990s (Gibson and Roberfroid 1995), and the first definition was 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 already resident in the colon". Thus, the prebiotic approach advocates administration of non-viable entities. The prebiotic concept considers that many health-promoting microorganisms, such as bifidobacteria and lactobacilli, are already present in the human colon. This definition was updated in 2004, and prebiotics are now defined as "selectively fermented ingredients that allow specific changes, both in the composition and/or activity in the GI microflora that confer benefits upon host wellbeing and health" (Gibson *et al*. 2004). The latter definition not only considers the microflora changes in the colonic ecosystem of humans but also, in the whole GI tract, and as such extrapolates the definition into other areas that may benefit from a selective targeting of particular microorganisms. As previously mentioned, the target genera are lactobacilli and bifidobacteria; however, prebiotic success has predominantly been with the latter. This is probably because there are usually more bifidobacteria in the human colon than lactobacilli, and they exhibit a preference for oligosaccharides. There is ongoing debate on extending the range of target microorganisms or introducing multiple functionalities, including anti-adhesive properties, etc. However, since prebiotics were introduced, it is the bifidobacteria that have been the focus. This ought to be the case for the foreseeable future, as they are viewed as a health positive genus with a history of use as probiotics (see earlier). This has led to the derivation of dietary prebiotics, defined as "selectively fermented ingredients that result in specific changes, in the composition and/or activity of the gastrointestinal microbiota, thus conferring benefit(s) upon host health".

Any dietary material that is non-digestible and enters the large intestine is a candidate prebiotic. This includes polysaccharide-type carbohydrates such as resistant starch and dietary fibre, as well as proteins and lipids. However, current prebiotics are confined to non-digestible oligosaccharides, many of which seem to confer the degree of fermentation selectivity that is required (towards bifidobacteria). Oligosaccharides are carbohydrates consisting of three and nine saccharide units, while polysaccharides consist of 10 or more saccharide units. Some prebiotics occur naturally in several foods such as leeks, asparagus, chicory, Jerusalem artichokes, garlic, onions, wheat and oats as well as soybeans. However, the overall intake from these sources within a normal, in particular Western-type diet is small. An effective route to achieving a health-promoting intake is the fortification of more frequently eaten foodstuffs with prebiotic ingredients. Prebiotics are thus a sub-category of functional food ingredients and can be added to many foods including yoghurts, cereals, breads, biscuits, milk desserts, ice creams, spreads, drinks as well as an animal feeds and supplements. Polysaccharides are usually obtained by extraction from crops, e.g. inulin from chicory or agave. Oligosaccharides can be commercially produced through the hydrolysis of polysaccharides (e.g. oligofructose (OF) from inulin), or through catabolic enzymatic reactions from lower molecular weight sugars, e.g. short-chain fructooligosaccharides (scFOS) from sucrose, or *trans*-galactooligosaccharides (TOS) from lactose. The review by Crittenden and Playne (1996) gives an overview of various aspects of the production and properties of food-grade oligosaccharides.

The three criteria required for a prebiotic effect are as follows (Gibson *et al*. 2004):

- Resistant to gastric acidity and hydrolysis by mammalian enzymes and GI absorption
- Can be fermented by intestinal microflora
- Selectively stimulates the growth and/or activity of intestinal bacteria associated with health and wellbeing.

For the purposes of this text, these three criteria were used to determine food ingredients that may be classified as prebiotics (Table 1). However, there are other materials

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Table 1. Relevant studies with oligosaccharides attempting to confirm a prebiotic effect

Subjects	Substrate	Dose	Duration	Results	Reference
Fructans					
23 senile adults	scFOS	8 g/day	14 days	Significantly increased bifidobacteria.	Hidaka *et al.* 1986
2 adults	scFOS	8 g/day	2 months	Increase in bifidobacteria, reduction in SCFA and putrefaction.	Hidaka *et al.* 1986
23 adults	scFOS	8 g/day	2 weeks	Increase in faecal bifidobacteria by about 10 times and decrease in stool pH.	Mitsouka *et al.* 1987
10 adults	N scFOS	4 g/day	14 days	Increased bifidobacteria and lactobacilli.	Williams *et al.* 1994
20 adults, 10 per group	scFOS	12.5 g/day	12 days	Significant increase in bifidobacteria by about 10 times was demonstrated on selective agars.	Bouhnik *et al.* 1996
8 adults	OF and inulin	15 g/day (in controlled diet)	15 days	Both test substrates significantly increased bifidobacteria. OF significantly reduced bacteroides, clostridia and fusobacteria.	Gibson *et al.* 1995
12 adults	scFOS	4 g/day (in controlled diet)	25 days	Significant increase in aerobes, enterobacteria and bifidobacteria. Only the latter decreased after feeding stopped.	Buddington *et al.* 1996
10 senile adults	Inulin	20 g/day, then 40 g/day	8 days, then 11 days	Significant increase in bifidobacteria. For 40 g/day, significant reduction in enterococci, bacteroides and enterobacteria.	Kleessen *et al.* 1997
40 adults	scFOS	2.5, 5, 10 and 20 g/day	7 days	Selective agars showed that bifidobacteria were most increased by 10 and 20 g doses of OF when compared with 2.5 g and that the optimum dose of OF was found to be 10 g/day.	Bouhnik *et al.* 1999
8 persons	Inulin	34 g/day	2 months	FISH revealed an increase in bifidobacteria from 9.8 to 11.0 $\log_{10}$ cells per gram of dry faeces. The effect lasted for the whole 2 month period when the volunteers received the prebiotic.	Kruse *et al.* 1999
8 persons	60% liquid OF	8 g/day (= 5 g of OF per day)	2 weeks	Selective agars showed an increase in faecal bifidobacteria.	Menne *et al.* 2000
8 young volunteers	OF	5 g/day	3 weeks	By means of selective agars, an increase in faecal bifidobacteria was observed.	Rao 2001
10 adults	HP-inulin	8 g/day	2 weeks	FISH revealed significant increase in bifidobacteria.	Tuohy *et al.* 2001a
31 adults	Biscuits containing OF	8 g/day	3 weeks	FISH revealed a specific increase in faecal bifidobacteria.	Tuohy *et al.* 2001b
19 elderly persons	scFOS	8 g/day	3 weeks	Increase in faecal bifidobacteria of approximately 2.8 log cfu per gram of faeces.	Guigoz *et al.* 2002
14 adult volunteers	Inulin	9 g/day	2 weeks	Quantifications of all bacteria, bifidobacteria, the *Eubacterium rectale – Clostridium coccoides* group (Erec group), *Bacteroides* and eubacteria were counted with FISH probes. A significant increase in bifidobacteria and a significant decrease in Erec group were observed.	Harmsen *et al.* 2002
12 elderly persons	scFOS	8 g/day	4 weeks	Bifidobacterial counts were significantly increased.	Bouhnik *et al.* 2007
45 adult volunteers	Jerusalem artichoke or chicory inulin in snack bars	7.7 g of first week, then 15.4 g/day	21 days	Significant increase in bifidobacteria and reduced *Bacteroides*/*Prevotella* in number and the *Clostridium histolyticum*/*C. lituseburense* group in frequency.	Kleessen *et al.* 2007

(Continued)

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Table 1. ***(Continued)***

Subjects	Substrate	Dose	Duration	Results	Reference
30 adults	Inulin	5 and 8 g/day	2 weeks	Bifidobacterial levels significantly increased upon ingestion of both the low and high inulin dose when compared with placebo.	Kolida *et al*. 2007
19 adults	OF/inulin (50/50)	10 g/day	4 weeks	A significant correlation was seen between baseline bifidobacteria counts and the effect of prebiotic intake.	de Preter *et al*. 2008
Trans-galactooligosaccharides					
5 men	TOS	3 g/day, then 10 g/day	1 week, then 1 week	3 g/day – little effect; 10 g/day – significant increase in bifidobacteria and lactobacilli and significant decrease in bacteroides.	Tanaka *et al*. 1983
12 men	TOS (oligomate)	0, 2.5, 5, then 10 g/day	1 week for each dose	Bifidobacteria increased with dose. For 10 g/day, significant increase in bifidobacteria and lactobacilli.	Ito *et al*. 1990
12 men	TOS	15 g/day	6 days	Significant increase in bifidobacteria and lactobacilli, significant decrease in bacteroides.	Ito *et al*. 1993
8 adults	TOS	10 g/day	21 days	Significantly increased bifidobacteria.	Bouhnik *et al*. 1997
6 adults	TOS	15 g/day	14 days	Significant increase in total count on media for lactic acid bacteria. No change in bifidobacteria.	Teuri *et al*. 1998
30 adults	TOS (Bimuno)	7.5 and 15 g/day	7 days	Significant selective increase in bifidobacteria.	Depeint *et al*. 2008
Lactulose					
12 humans	Lactulose	2 × 10 g/day	4 weeks	Bifidobacteria, streptococci and lactobacilli significantly increased whilst bacteroides, clostridia, coliforms and eubacteria decreased.	Ballongue *et al*. 1997
8 humans	Lactulose	3 g/day	14 days	Bifidobacteria significantly increased while *Clostridium perfringens*, bacteroides, streptococci and Enterobacteriaceae decreased.	Terada *et al*. 1992
Rats	Lactulose	10%		Significantly increased bifidobacteria.	Suzuki *et al*. 1985
30 adults	Lactulose	10 g/day	3 weeks	Significantly increased bifidobacteria.	Tuohy *et al*. 2002
65 volunteers	Lactulose	20 g/day	7 days	An increase in faecal bifidobacteria counts and β-galactosidase activity was observed.	Bouhnik *et al*. 2004a
16 healthy volunteers	Lactulose	5 g/day	6 weeks	Lactulose ingestion led to a significant increase in faecal bifidobacteria counts.	Bouhnik *et al*. 2004b

FOS, fructooligosaccharides; scFOS, short-chain fructooligosaccharides; OF, oligofructose; FISH, fluorescent *in situ* hybridisation; SCFA, short-chain fatty acids; TOS, *trans*-galactooligosaccharide.

that may partially fulfil these criteria (or remain to be proven). These will be described later.

4. Examples of prebiotics

Given the aforementioned criteria, Table 1 shows oligosaccharides that are confirmed prebiotics – namely some fructans, galactans and lactulose (a sugar currently mainly used as laxative). Most research has hitherto been carried out with fructans, in particular fructooligosaccharides (FOS). Note that in some cases, both *in vitro* and animal data are cited. However, several independent human trials from different researchers are needed for the definitive assessment of a prebiotic effect. Therefore, it is evident that some candidate prebiotics are lacking on human output data when compared with the accepted forms (see later).

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4.1 Lactulose

Lactulose is a synthetic disaccharide in the form of Gal β1-4 Fru. It has been used as a laxative as it is not hydrolysed or absorbed in the small intestine. At sub-laxative doses, lactulose has received attention as a bifidogenic factor and has been administered as such. *In vitro*, lactulose increased lactobacilli and bifidobacteria and significantly decreased bacteroides in a mixed continuous faecal culture (Fadden and Owen 1992). The feeding of lactulose to rats significantly increased bifidobacteria; however, only a limited number of bacterial groups were enumerated in this trial (Suzuki *et al*. 1985).

In a human trial, bifidobacteria significantly increased while clostridia, bacteroides, streptococci and Enterobacteriaceae decreased on the feeding of 3 g/day lactulose to eight volunteers (5 male, 3 female) for 14 days (Terada *et al*. 1992). Small decreases in bacteroides and lactobacilli during the test period were also determined. In addition, decrease in the detrimental metabolites (ammonia, indole, phenol, *p*-cresol and skatole) and enzymes (β-glucuronidase, nitroreductase and azoreductase) supported the beneficial claims of lactulose.

Lactulose has been used in pharmaceutical products for the control of constipation and it has been used, in early studies, as an additive in infant formula feed to stimulate lactobacilli (MacGillivray *et al*. 1959).

Ballongue *et al*. (1997) carried out a volunteer trial to confirm the prebiotic nature of lactulose. The feeding was a parallel group, randomised, double-blind, placebo-controlled study with 12 healthy volunteers per group. Two weeks baseline was followed by 4 weeks of treatment and a 3 week follow-up. Subjects were fed with 2 × 10 g/day of lactulose or placebo of 50:50 glucose and lactose. Faecal samples were taken throughout and bacteria were determined by selective agars.

A volunteer trial carried out at the University of Reading (UK) used a placebo-controlled parallel study volunteer trial to ascertain the prebiotic effects of 10 g/day lactulose. The bacterial changes were identified using both plate culture, with genotypic characterisation, as well as fluorescent *in situ* probing (Tuohy *et al*. 2002). This trial confirmed the prebiotic effects of dry lactulose powder. This was further shown by Bouhnik *et al*. (2004a, 2004b) who also looked at associated enzyme activities.

4.2 Inulin-type fructans

These fructans contain both GpyF*n* (α-D-glucopyranosyl-[β-D-fructofuranosyl]$_{n-1}$-D-fructofuranoside) and FpyF*n* (β-D-fructopyranosyl-[β-D-fructofuranosyl]$_{n-1}$-D-fructofuranoside) molecules, with the number of fructose units varying from 2 to more than 70 units of which FOS are a subcategory of up to nine units. Fructans of all chain lengths are well documented for their effect on intestinal bifidobacteria and are considered important prebiotic substrates. Inulin naturally occurs in hundreds of different plant foods (van Loo *et al*. 1995), with garlic, onions, asparagus, chicory, artichokes, bananas, wheat and leeks being especially rich (Gibson *et al*. 1994).

Two different types of FOS are common. First, inulin extracted from chicory roots can be hydrolysed under controlled conditions by the enzyme inulinase (Crittenden 1999) to produce short-chain inulin molecules known as OF. A new product called 'Synergy' combines short-chain OF and long-chain inulin. Another FOS product known as 'scFOS' is essentially a mixture of three oligosaccharides of DP3–DP5 (Hidaka *et al*. 1986). The mixture is enzymatically synthesised from sucrose by the transfructosylation action of β-fructofuranosidase from the fungus *Aspergillus niger* (Hidaka *et al*. 1986).

It is accepted that fructans are not degraded or absorbed in the upper human GI tract. As such, they enter the colon intact where they are susceptible to metabolism by the resident microbiota. The β-configuration of anomeric C_2 in fructose monomers is thought to make fructans resistant to hydrolysis by human digestive enzymes that are mostly specific for α-glycosidic linkages. The most conclusive evidence comes from the ileostomy model as used by Bach Knudsen and Hessov (1995) and Ellegärd *et al*. (1997). Both studies reported that the vast majority of ingested fructans could be recovered in ileostomy fluid.

In pure cultures, most species of bifidobacteria are adept at the use of inulin-type fructans (Gibson and Wang 1994a). Many other bacteria are also capable of metabolising these substrates, including *Klebsiella pneumoniae*, *Staphylococcus aureus*, *S. epidermidis*, *Enterococcus faecalis*, *E. faecium*, *Bacteroides vulgatus*, *B. thetaiotaomicron*, *B. ovatus*, *B. fragilis*, *Lactobacillus acidophilus* and *Clostridium* spp. (mainly *C. butyricum*; Roberfroid *et al*. 1998). However, in mixed batch and continuous culture studies, it has been demonstrated that both inulin and its hydrolysate selectively stimulated the growth of the bifidobacteria, at the expense of more pathogenic bacteria, which, at the end of the incubation period, became numerically predominant, indicating good prebiotic activity (Wang and Gibson 1993; Gibson and Wang 1994b).

Rats that were previously fed with tyrosine and tryptophan (capable of producing putrefactive products) were administered a 10% (w/v) fructan diet, and this resulted in increased SCFA, decreased faecal pH and significantly decreased concentrations of the tyrosine derivatives phenol and *p*-cresol (Hidaka *et al*. 1986). A study using human flora-associated rats has also indicated the prebiotic effect of OF (Djouzi and Andrieux 1997).

Several studies have been conducted using human subjects although the dose, substrate, duration and volunteers vary (Table 1). A general observation was the greater bifidogenic effect of substrates in subjects with a low initial

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bifidobacterial count (10^7 per gram of faeces) than in those with high initial numbers ($10^{9.5}$ per gram of faeces; Hidaka *et al*. 1986).

Gibson *et al*. (1995) carried out a crossover volunteer trial with adult subjects on strictly controlled diets supplemented with 15 g/day of FOS. Sucrose was used as the control and faecal samples were processed, in a blind manner, within 30 min of passage. This study showed that the intake of both OF and inulin stimulated the growth of bifidobacteria which, after 2 weeks of the feeding period, became by far the most numerically predominant bacterial group. In addition, the inulin and OF feeding significantly reduced counts of bacteroides, fusobacteria and clostridial populations. These effects lasted for as long as the prebiotic was consumed.

Similar human studies in adult European, Japanese and North American populations have also been reported for these fructans at various doses (from 4 to 40 g/day; Hidaka *et al*. 1986; Mitsuoka *et al*. 1987; Williams *et al*. 1994; Bouhnik *et al*. 1996, 1999; Buddington *et al*. 1996; Kleessen *et al*. 1997, 2007; Tuohy *et al*. 2001a, 2001b; Guigoz *et al*. 2002; Harmsen *et al*. 2002; Kolida *et al*. 2007; de Preter *et al*. 2008). The data are striking as large variations existed between the subjects in their microflora compositions, yet the fructans were always efficient prebiotics. As expected, the main genus of gut bacteria to respond was the bifidobacteria.

Bifidobacteria are able to breakdown and use fructans due to their possession of a competitive β-fructofuranosidase enzyme (Imamura *et al*. 1994). It would appear that this enzyme is elaborated at a high level by bifidobacteria in mixed cultures.

In pure culture experiments, other bacteria may also take advantage of fructans. This is also true for the human situation. In healthy volunteers, the daily intake of 10 g of inulin stimulated both bifidobacteria and *Faecalibacterium prausnitzii* (Ramirez-Farias *et al*. 2009). The latter species is depleted in patients suffering from inflammatory bowel diseases and exerts beneficial effects when applied to mice with experimental colitis (Sokol *et al*. 2009). The immunomodulating effects of *F. prausnitzii* are possibly mediated by a secreted metabolite blocking NFk-B activation and IL-8 production. These studies demonstrate that it is possible to identify non-lactic acid producers contributing to host health in a very specific manner. Such bacteria might be new targets for prebiotic research.

4.3 Trans-*galactooligosaccharides (TOS)*

TOS are galactose-containing oligosaccharides of the form Glu α1-4[β-Gal 1-6]$_n$, where n = 2–5, and are produced from lactose syrup using the transgalactosylase activity of the enzyme β-galactosidase (Crittenden 1999).

Tanaka *et al*. (1983) tested eight *Bifidobacterium*, five *Bacteroides*, three *Fusobacterium*, six *Eubacterium*, eight *Clostridium*, one *Propionibacterium acnes*, eight *Lactobacillus*, eight *Streptococcus*, four Enterobacteriaceae and one *Staphylococcus aureus* for their ability to use TOS. These studies showed good growth of all the bifidobacteria strains tested, as well as two *Bacteroides fragilis* strains, four lactobacilli strains and four enterobacteria. Although this study set out to compare monocultures of gut bacteria, TOS was concluded to be a suitable bifidobacterial-promoting substrate.

TOS have bifidogenic properties after feeding to rats associated with a human microflora (Rowland and Tanaka 1993). The model used was germ-free animals that are inoculated with human faeces. In this study, six rats were used as controls, and six were fed with a TOS-containing diet at 5% (w/w). After 4 weeks, the rats were sacrificed, and the bacteriology of caecal contents was analysed using selective agars. The data showed a significant increase in bifidobacteria and lactobacilli.

Ito *et al*. (1990) recruited 12 male volunteers divided into four groups of three to receive 0, 2.5, 5 and 10 g/day of TOS for 8 weeks. These data showed a significant increase in bifidobacteria as well as lactobacilli at all the test doses. Ito *et al*. (1993) followed up this study by feeding volunteers 15 g/day TOS for 6 days and again found a selective effect on bifidobacteria and lactobacilli. Moreover, certain putrefactive enzymes were reduced during the test period.

In humans, 10 g/day of TOS significantly reduced breath hydrogen (Bouhnik *et al*. 1997), whereas this increased in human flora-associated rats fed with 5 or 10% (w/v) TOS (Andrieux and Szylit 1992). Bouhnik *et al*. (1997) also reported an increased number of bifidobacteria in faeces.

A new type of TOS has been synthesised using enzymes from *Bifidobacterium bifidum* 41171. This is known as Bimuno and has been tested for its prebiotic effect *in vitro*, in pigs and in humans (Tzortzis *et al*. 2005a, 2005b, 2009; Depeint *et al*. 2008).

4.4 Dietary ingredients that are candidate prebiotics

The following materials are being explored for prebiotic activity. However, at present and using the three criteria set out earlier (Gibson *et al*. 2004), the evidence is not as convincing as for the fructans or galactans.

4.4.1 Polydextrose

Polydextrose is a glucose polymer. The bonds are random, with 1–6 being predominant. Because of the random assortment, its molecular weight varies (Figdor and Rennhart 1981).

In vitro, the use of a multiple-stage continuous culture system showed that polydextrose had a consistent stimula-

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tory effect upon bifidobacteria (Probert *et al*. 2004). As this model has been validated against colonic contents of sudden death victims, the prebiotic capacity of polydextrose is promising. However, there are two human trials with 8 and 15 g of polydextrose per day in which no change in bifidobacteria could be found, although one study targeted specific species only and the other did show decreased clostridia (Endo *et al*. 1991; Hengst *et al*. 2008). One human study by Jie *et al*. (2000) reported increased bifidobacteria and lactobacilli after feeding of polydextrose. However, it is difficult to be convinced on the basis of the microbial techniques used and conditions of faecal storage. Human trials using up-to-date molecular-based methodologies for microflora identification are needed.

4.4.2 Soybean oligosaccharides

The predominant oligosaccharides in soybeans are the trisaccharide raffinose and the tetrasaccharide stachyose which are thought to stimulate bifidobacteria (Oku 1994).

In pure culture studies, soybean oligosaccharides were fermented to a far greater degree by bifidobacteria than other organisms tested (Hayakawa *et al*. 1990). Pure raffinose and stachyose and refined soybean oligosaccharides were the test materials. *Bifidobacterium longum*, *B. breve*, *B. infantis* and *B. adolescentis* (but not *B. bifidum*) metabolised the test substrates, as did *Lactobacillus acidophilus*, *L. gasseri* and *L. salivarius* (but not *L. casei*). The same authors then carried out a feeding study with six healthy male volunteers, in which the oligosaccharides were fed in conjunction with a pure culture of *B. longum* for 3 weeks. This resulted in enhanced recovery of bifidobacteria from stools. Similar data were obtained from the volunteer trials carried out by Wada *et al*. (1992).

The addition of a low concentration of soybean oligosaccharides to a two-stage continuous culture of faecal bacteria (Saito *et al*. 1992) resulted in a three-fold increase in the proportion of bifidobacteria in the total bacterial count.

4.4.3 Lactosucrose

Lactosucrose is produced from a mixture of lactose and sucrose using the enzyme β-fructofuranosidase (Playne and Crittenden 1996) and has been found to be bifidogenic in pure culture studies (Tamura 1983; Fujita *et al*. 1995).

A later pure culture study compared lactosucrose with lactulose, fructans, soybean oligosaccharides, raffinose and glucose for its utilisation by various intestinal bacteria (Hara *et al*. 1994). Six bifidobacteria and three lactobacilli strains grew to the same extent (comparable end pH) on lactosucrose and glucose, whereas all the other organisms tested preferred glucose.

4.4.4 Isomaltooligosaccharides

Isomaltooligosaccharides (IMOs) are composed of glucose monomers linked by α1-6 glucosidic linkages. A commercial mixture known as Isomalto-900 has been produced by incubating α-amylase, pullulanase and α-glucosidase with cornstarch (Kohmoto *et al*. 1988). The major oligosaccharides in this mixture are isomaltose (Glu α1-6 Glu), isomaltotriose (Glu α1-6 Glu α1-6 Glu) and panose (Glu α1-6 Glu α1-4 Glu).

Human studies have also been conducted to determine the effect of IMOs on the colonic microflora. Kohmoto *et al*. (1988) fed 20 g/day IMO to six healthy adult males for 10 days and to 18 elderly men and women for 14 days. All groups demonstrated a significant increase in bifidobacteria. Similar data were obtained by Kohmoto *et al*. (1991) with 12 healthy volunteers fed with different IMO doses for 10 days and with IMO of varying chain length.

4.4.5 Glucans

To produce glucans consisting of oligosaccharides and polysaccharides, but generally referred to as glucooligosaccharides (GlOS), the glucosyltransferase from *Leuconostoc mesenteroides*, transfers glucose molecules from a sucrose donor to a maltose acceptor (Valette *et al*. 1993). The fructose from the sucrose molecule is then released, leaving a mixture of different sized GlOS.

In pure cultures, GlOS were used by *Bifidobacterium breve*, *B. pseudocatenulatum* and *B. longum*, and not by *B. bifidum*. They were also used by bacteroides and clostridia but not by lactobacilli (Djouzi *et al*. 1995). In the same study, a defined culture containing *Bacteroides thetaiotamicron*, *Bifidobacterium breve* and *Clostridium butyricum* repressed growth of the latter with GlOS fermentation. After 8 h, approximately equal numbers of the bacteroides and bifidobacteria were found. After 24 h, *B. thetaiotamicron* dominated the culture.

GlOS has been shown to be poorly hydrolysed and digested in the intestinal tract of gnotobiotic rats (Valette *et al*. 1993). In trixenic rats, decreased butyrate and hydrogen production suggested that clostridia did not degrade the GlOS to a great extent, as observed in a co-culture study (Djouzi *et al*. 1995).

Data on human studies with GlOS are needed for further assessment of its prebiotic potential.

4.4.6 Xylooligosaccharides

Xylooligosaccharides (XOS) are chains of xylose molecules linked by β1-4 bonds and mainly consist of xylobiose, xylotriose and xylotetraose. They can be enzymatically produced by hydrolysis of xylan from birch wood (Campbell *et al*. 1997), oats (Jaskari *et al*. 1998) or corncobs (Playne and Crittenden 1996).

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Okazaki *et al*. (1990) carried out pure culture work with a wide variety of bacteria and found that XOS were metabolised by the majority of bifidobacteria and lactobacilli. In a follow-up human trial, the volunteers showed some variation in their response to the XOS, but overall, the effect was to significantly increase faecal bifidobacteria and decrease bacteroides.

Campbell *et al*. (1997) carried out a feeding trial with rats in which the animals were given free access to feed containing XOS at 6% (w/w). The effect was a stimulation of caecal and faecal bifidobacteria.

4.5 Miscellaneous

A number of materials have the potential to act as prebiotics, but current confirmatory evidence in humans is scant or even absent.

Using *in vitro* static batch culture, gentiooligosaccharides were shown to possess bifidogenic activity when incubated with human faecal bacteria (Rycroft *et al*. 2001).

Pectic oligosaccharides were shown to have selective fermentation by bifidobacteria in pH-controlled batch culture fermentations (Olano-Martin *et al*. 2002). These oligosaccharides were extracted from orange peel (Manderson *et al*. 2005) and the active structures were rich in arabinooligosaccharides (Hotchkiss *et al*. 2009). Butyrate was produced and selective fermentation by eubacteria was also observed in mixed batch faecal cultures that included orange peel pectic oligosaccharides (Manderson *et al*. 2005). Selective fermentation by both bifidobacteria and lactobacilli was reported for pectic oligosaccharides extracted from bergamot peel (Mandalari *et al*. 2007).

Mannanoligosaccharides from yeasts allowed small changes in flora composition after feeding to pigs but did affect certain immune traits (White *et al*. 2002).

Several studies have examined the potential of resistant starch and its derivatives to act as prebiotics. Wang *et al*. (2002) fed Balb/c mice with diets containing various amounts of amylomaize starch and modified amylomaize starches. An increase in bifidobacteria was seen for all starches fed. Silvi *et al*. (1999) investigated the effect of sucrose and resistant starch on human flora-associated rats. Bifidobacteria and lactobacilli were increased, and enterobacteria were decreased with starch when compared with rats fed with sucrose. Evidence from human studies is lacking, however.

A multiple-stage fermenter system (SHIME) was used to monitor the effects of oat bran fermented with *Lactobacillus rhamnosus* on human microflora. The reactor was colonised with the probiotic bacteria; oat bran feeding favoured the growth of bifidobacteria (Knotula *et al*. 1998).

Bifidogenic factors exist in human milk, probably explaining the predominance of bifidobacteria in breast-fed infants over infants receiving formula feeds (Kanyshkova *et al*. 2002).

Detailed information on microflora changes with β-glucan fermentation are lacking, although end products are affected (Wood *et al*. 2002), i.e. no evidence for prebiotic properties yet.

In non-competitive pure culture studies, *N*-acetylchitooligosaccharides stimulated both bifidobacteria and eubacteria (Chen *et al*. 2002); again, this suggests that there is, as yet, no evidence for prebiotic properties.

Sugar alcohols such as lactitol, isomalt, sorbitol and maltitol may also be of use as prebiotics. In particular, lactitol (4-*O*-β-D-galactopyranosyl-D-glucitol) and isomalt (mixture of 1-*O*-α-D-glucopyranosyl-D-glucitol and 1-*O*-α-D-glucopyranosyl-D-mannitol) have been tested in humans (Ballongue *et al*. 1997; Gostner *et al*. 2005).

5. Determination of prebiotic effects

For prebiotics, many human volunteer trials have already been carried out in order to elucidate their effects. There are several approaches that can be used to support such trials, which are summarised below.

5.1 Pure cultures

This involves challenging the test material with pure cultures of selected microorganisms. The substrate would be added to a basal growth medium and bacterial growth determined during a time course incubation (Gibson and Wang 1994a). This approach gives a reasonable comparative assessment of metabolism in monocultures, but does not induce any element of competition. Thus, the approach cannot identify true selectivity and therefore the prebiotic activity of a particular substrate. A more complex approach is to use mixed culture experiments with selected gut microbial species. This introduces some competition between the microorganisms, but again does not adequately resemble complex interactions that occur in the human gut microflora.

5.2 Mixed culture bacterial fermenters

A common approach towards the determination of gut microbial activities is to use batch culture fermenters inoculated with faecal bacteria (Wang and Gibson 1993). These would be kept anaerobic by the infusion of an oxygen-free gas such as nitrogen, pH controlled and mixed. However, these are closed systems where the substrate is limited, and therefore they are only appropriate for short-term experiments.

A further approach is continuous culture, whereby a constant input of nutrients may be supplied and other physiological parameters such as varying dilution rate are imposed (Gibson and Wang 1994b). Semi-continuous culture is one variable in which the medium is added and spent culture removed at specific intervals. The drawback

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is that the one-stage continuous culture chemostat is a homogeneous system and varying physicochemical determinants cannot be imposed.

5.3 In vitro *gut models*

The large gut is composed of various anatomically distinct regions such as the caecum, ascending colon, transverse colon, descending colon and sigmoid rectum. In the proximal region, there is a ready supply of substrate. Hence, bacteria grow quickly and the pH is acidic (due to the formation of acidic end products). In the left side, bacteria grow more slowly, the nutrient supply is diminished and the pH is more neutral.

One model to mimic these different physicochemical parameters is the three-phase continuous culture. This system has been validated against samples taken at autopsy and gives a very close approximation to fermentative bacterial events that occur *in situ* (Macfarlane *et al*. 1998). Studies on the development of the microbial flora in the three vessels can be performed such that fermentation characteristics in the varying areas may be predicted. The system consists of three vessels, of increasing size, aligned in series such that a sequential feeding of growth medium occurs. The vessels are pH regulated to reflect *in vivo* differences. One such model has been validated against gut contents from sudden death victims. Five-stage continuous fermenters have also been used to simulate the intestinal tract from the jejunum to the descending colon (Molly *et al*. 1993).

5.4 Animal methods

Animals, often rats or mice, have been used to determine the prebiotic nature of a substrate (Rowland and Tanaka 1993). Conventional or germ-free rats or those inoculated with one or a limited number of microorganisms may be used to investigate prebiotic interactions, although this does not resemble the usual situation in the gut. Rats may also be associated with a human faecal flora and give a further representation of the situation in the human intestinal tract, although the intestinal physiology is not the same.

5.5 Human trials

The ultimate assessment of prebiotic effect is to feed candidate substrates or foods to human volunteers and assess microbiological changes in stools (Table 1). As faeces are the only readily accessible area of gut contents, it is difficult to predict fermentation reactions in more proximal gut contents. Human trials may be carried out on volunteers who are on controlled diets or are free living. To ensure consistency and exclude incidental findings, usually more than one human trial is needed, and the totality of several human studies for a candidate prebiotic should be considered.

Because of limitations in culture-based approaches for microbiota characterisation, there has been a move towards more reliable molecular-based methodologies such as quantitative real-time PCR or fluorescent *in situ* hybridisation (Langendijk *et al*. 1995; Kullen and Klaenhammer 1999; O'Sullivan 2000; McCartney 2002).

A prebiotic dose of 5 g/day of FOS and TOS should be sufficient to elicit a positive effect upon the gut microbiota (in some exceptional cases, this may be nearer to 8 g/day). A possible side effect of prebiotic intake is intestinal discomfort from gas production. However, bifidobacteria and lactobacilli cannot produce gas as part of their metabolic process. Therefore, at a rational dose of up to 20 g/day, gas distension should not occur. If gas is being generated, then the carbohydrate is not acting as an authentic prebiotic. This is perhaps because dosage is too high and the prebiotic effect is being compromised, i.e. bacteria other than the target organisms are becoming involved in the fermentation (Gibson and Roberfroid 1995; Probert and Gibson 2002).

6. Prebiotic use in infants

At birth, the GI tract is essentially germ-free, with initial microbial colonisation occurring during birth or shortly afterwards. The GI tract of newborns is primarily inoculated by organisms originating from the maternal microbial flora of the genital tract and colon, and from the environment (e.g. through direct human contact and hospital surroundings; Holzapfel *et al*. 1998; Mountzouris *et al*. 2002). Bacterial populations in infants develop during the first few days of life (Collins and Gibson 1999), and the intestinal flora develops as a result of the influence of intestinal physiology and diet upon acquired bacteria (Drasar and Barrow 1985). Significant differences in the composition of the gut flora have also been recognised in response to infant feeding regimes. The microflora of breast-fed infants is dominated by populations of bifidobacteria and this may explain the purported healthier outlook of breast-fed infants when compared with their formula-fed counterparts (Harmsen *et al*. 2000). Formula-fed infants have a more complex microbiota, with bifidobacteria, bacteroides, lactobacilli, clostridia and streptococci all being prevalent (Stark and Lee 1982; Benno *et al*. 1984; Harmsen *et al*. 2000). It is thought that the presence of certain glycoproteins and soluble oligosaccharides in human breast milk is selectively stimulatory for bifidobacteria (Petschow and Talbott 1991).

Therefore, the classical prebiotic is human breast milk. One approach to fortify the microbiological role of formula feeds has been to use prebiotics as stimulants for bifidobacteria and thereby aim to improve the gut microbiota composition (to better resemble that seen with breast feeding). Table 2 summarises studies on this specific aspect of prebiotic use.

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Table 2. Studies with prebiotics in infants

Test oligosaccharide	Study design	Dose	Evidence of prebiotic efficacy	Reference
TOS and polydextrose, lactulose	226 healthy formula-fed term infants, assigned to treatment groups of 76 parallel design followed up to 120 days old.	4 or 8 g/L prebiotic/ formula	Normal growth and stool characteristics similar to breast-fed infants.	Ziegler *et al.* 2007
TOS and long-chain FOS (inulin)	Healthy bottle-fed infants, randomised, double-blind, parallel design followed up to 6 weeks of age.	4 g/L prebiotic/ formula or standard formula (no prebiotic)	Significant decrease in clostridia (FISH), trend of increased bifidobacteria and *Escherichia coli*, higher stool frequency softer stools with respect to control group.	Costalos *et al.* 2008
TOS and FOS (inulin)	20 pre-term infants on enteral nutrition, assigned into 2 groups, placebo-controlled, double-blind, 14 day supplementation.	10 g/L prebiotic/ formula or standard formula	Significant reduction in gastrointestinal transit time and stool frequency, well tolerated.	Mihatsch *et al.* 2006
TOS and FOS (inulin)	199 formula-fed infants with colic, 96 prebiotic, aged >4 months, 103 standard formula parallel randomised, 2 weeks.	8 g/L prebiotic/ formula (90% TOS), formula and simethicone (6 mg/kg)	Significant reduction in crying episodes after 7 and 14 days when compared with standard formula.	Savino *et al.* 2006
TOS and FOS (inulin)	35 formula-fed infants in weaning, aged 4-6 months, double-blind, randomised, 6 week supplementation	4.5 g/day prebiotic in weaning food or weaning food (no prebiotic)	Significant increase in bifidobacteria % (FISH) with prebiotic significantly different to control.	Scholtens *et al.* 2006
TOS and FOS (inulin)	Two groups of 10 healthy, formula-fed infants, 28–90 days old, parallel study.	8 g/L prebiotic/ formula (90% TOS); breast-fed control group	Real-time PCR analysis, similar flora composition between formula- and breast-fed infants.	Haarman and Knol 2005; Haarman and Knol 2006
TOS and FOS; *Bifidobacterium animalis*	Three groups of 19 healthy, formula-fed infants, 63 breast-fed (reference group) randomised, double-blind parallel, from birth to 16 weeks.	6 g/L prebiotic/ formula; 6×10^{10} viable *B. animalis*/L formula; standard formula	Similar metabolic activity of the flora in TOS/FOS group as breast-fed, *B. animalis* group similar to standard formula.	Bakker-Zierikzee *et al.* 2005
TOS and FOS	Healthy, formula-fed infants, 28 days of feeding period.	8 g/L prebiotic/ formula; maltodextrin control	Significantly higher bifidobacteria with prebiotic when compared with control.	Moro *et al.* 2005
TOS	69 healthy term infants fed TOS, parallel study, 59 fed formula, 124 mixed; 6 month intervention.	2.4 g/L prebiotic/ formula; formula; mixed (breast-fed and prebiotic formula)	Significant increases in bifidobacteria, lactobacilli and stool frequency in prebiotic and mixed groups but not the standard formula group.	Ben *et al.* 2004
TOS and FOS (inulin)	19 pre-term infants on prebiotic, 19 maltodextrin placebo, 12 fortified breast milk parallel study, 28 day intervention.	10 g/L prebiotic/ formula (90% TOS)	Significantly higher bifidobacteria when compared with placebo group, similar to breast-fed group; significantly higher stool frequency when compared with placebo and breast-fed.	Boehm *et al.* 2002
Native inulin	14, 12.6 week formula-fed healthy infants, 6 week intervention (3 week inulin, 3 weeks without).	0.25 g/kg/day native inulin	Inulin significantly increased lactobacilli and bifidobacteria, stool frequency was not affected.	Kim *et al.* 2007

FOS, fructooligosaccharides; FISH, fluorescent *in situ* hybridisation; TOS, *trans*-galactooligosaccharides.

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7. Beneficial effects of prebiotics

Prebiotics have been used as food ingredients to maintain or restore a 'healthy' gut microflora. The majority of successful human trials on prebiotics show significantly increased intestinal levels of bifidobacteria (and sometimes lactobacilli). Often, these are associated with well-characterised and accepted markers of health.

7.1 Inflammatory bowel disease

Data from animal studies suggest that prebiotic administration can prove effective in ulcerative colitis (UC) management (Videla 1999; Videla *et al*. 2001; Cherbut *et al*. 2003). One successful randomised, double-blind, crossover placebo-controlled study with 24 patients with pouchitis has shown that the intake of 24 g/day of inulin for 3 weeks reduced the endoscopic and histological pouchitis disease index score, lowered gut pH, and reduced secondary bile acid and *Bacteroides fragilis* in faecal samples (Welters *et al*. 2002). A later study by Furrie *et al*. (2005) showed improved UC symptomology with a prebiotic (inulin) approach that increased gut bifidobacteria.

A prebiotic (lactulose) has shown beneficial effects in a study of patients with Crohn's disease (CD) and UC (Szilagyi *et al*. 2002), with a reduction in disease symptoms relative to controls on consumption of 20 g lactulose per day. OF and inulin have given positive results in patients with CD, with a significant reduction in disease severity in one small open label study (Lindsay *et al*. 2006). A mixture of OF and inulin has shown significant reductions in disease severity indices, reduction in pro-inflammatory immune markers and a reduction in calprotectin, a validated marker of intestinal inflammation (Konikoff and Denson 2006).

7.2 Antibiotic-associated diarrhoea

Daily ingestion of 12 g of OF reduced episodes of diarrhoea in 142 patients with *Clostridium difficile*-induced diarrhoea (Lewis *et al*. 2005).

7.3 Traveller's diarrhoea

One study with a prebiotic has been published for the treatment and prevention of diarrhoea (Cummings *et al*. 2001). Here, 244 healthy subjects travelling to high- or medium-risk destinations for traveller's diarrhoea received either 10 g of inulin or placebo for 2 weeks before travelling and then for the 2 weeks they were away. The prevalence of diarrhoea was lower in the prebiotic group, and less severe attacks of diarrhoea were also recorded.

7.4 Colon cancer

Effects have been reported to be associated with gut flora-mediated fermentation and production of protective metabolites such as butyrate (Reddy 1998, 1999; Rowland *et al*. 1998; Pool-Zobel 2005). The rationale for the use of prebiotics as preventative agents is based on the observation that health-positive bacteria such as bifidobacteria do not produce carcinogenic or genotoxic compounds. Increasing the proportion of these bacteria in the colon, therefore, might reduce the levels of tumour promoters and genotoxins, as shown in rat studies (Rowland and Tanaka 1993; Rowland *et al*. 1998). In humans, scFOS has been investigated in a study on adenoma and adenoma-free patients (Boutron-Ruault *et al*. 2005). Feeding 10 g/day of scFOS resulted in positive effects in biomarkers in the adenoma-free patients. A recent human study has investigated the effect of an OF and inulin mixture (Synergy 1) together with *Lactobacillus rhamnosus* GG and *Bifidobacterium lactis* Bb-12 on biomarkers of cancer (Rafter *et al*. 2007). The study was a 12 week double-blind placebo-controlled trial in patients with cancer and polypectomised individuals. Colorectal cell proliferation and genotoxicity were significantly reduced, and the intestinal barrier function increased.

7.5 Calcium absorption and bone health

Animal studies with rats have shown enhancement of calcium absorption with prebiotics, mainly fructans (Delzenne *et al*. 1995; Roberfroid *et al*. 2002). Similar trials have been reported in humans (Coudray and Fairweather-Tait 1998; van den Heuvel *et al*. 2000; Tahiri *et al*. 2003). Recent advances in this field show that inulin-type fructans enhanced calcium absorption (Abrams *et al*. 2007). Crude fractions of chicory (a source of inulin) have shown improved bone parameters relative to native or reformulated inulin in rats, suggesting possible synergies between inulin-type fructans and other nutrients (Demigne *et al*. 2008). A study in which 100 young adolescents received 8 g/day of short- and long-chain inulin fructans for a year showed a significant increase in calcium absorption and led to greater bone mineral density (Abrams *et al*. 2005). This is thought to be attributed to a lowering of gut pH as a result of organic acid production (from bifidobacteria) following prebiotic fermentation.

7.6 Other beneficial effects

The use of a prebiotic resulted in a significant decrease in diarrhoea, vomiting and fever in a study of young children in a day care centre (Waligora-Dupriet *et al*. 2007). Infants were fed with 2 g/day scFOS or placebo for 21 days in a double-blind trial. Using culture-based methods, an increase in bifidobacteria was seen, with a significant decrease in clostridia. A study by Silk *et al*. (2009) showed improvements in IBS following prebiotic (TOS)-based modulation of gut bifidobacteria. This included sta-

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tistically significant effects on gut function and typical IBS symptoms.

There is also interest in effects of prebiotics on lipid metabolism, immunomodulation of the gut immune system, glycaemic control, gut hormones, weight loss and satiety (Parnell and Reimer 2009), as well as behavioural effects and obesity. The works by Tannock (2002), Gibson and Rastall (2006), Gibson and Roberfroid (2008), Versalovic and Wilson (2008), Charalampopoulos and Rastall (2009) all contain reviews that provide information on the health attributes of prebiotic intake.

8. Conclusions

The development of prebiotics was an extension of the probiotic concept for the management of gut microbiota. It has similarities with dietary fibre functionality in that microbial fermentation of carbohydrate occurs. In contrast to fibre, selectivity of fermentation is the key to success. In future, the prebiotic concept may be expanded in terms of its functionality. Although many dietary prebiotic candidates exist, the strongest data to date are summarised in Table 1. As our knowledge of gut flora diversity improves and the outcomes of prebiotic metabolism expanded, it is probable that the list of accepted forms will increase. New research on metabolic interactions with the host and gut bacteria will also propel dietary prebiotic use.

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