Functional Oligosaccharides: Application and Manufacture

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Abstract

Oligosaccharides are attracting increasing interest as prebiotic functional food ingredients. They can be extracted or obtained by enzymatic hydrolysis from a variety of biomass sources or synthesized from simple oligosaccharides by enzymatic transfer reactions. The major prebiotic oligosaccharides on the market are inulin, fructo-oligosaccharides, and galacto-oligosaccharides. They have been evaluated using a range of in vitro and in vivo methods, although there is a need for more large-scale human trials using modern microbiological methods. Prebiotics are being studied for their effects on gut health and well being and specific clinical conditions, including colon cancer, inflammatory bowel disease (IBD), acute infections, and mineral absorption. Developing understanding of the functional ecology of the human gut is influencing current thinking on what a prebiotic might achieve and is providing new targets for prebiotic intervention.

INTRODUCTION

The Gut Microbiota in Health and Disease

The human gut plays host to probably the most complex microbial ecosystem yet studied. The microbial population and diversity increase from relatively low levels in the stomach (10^3 per ml) to approximately 10^4 to 10^7 per ml in the small intestine (Dethlefsen et al. 2006, Flint et al. 2007). The colon, however, is the site of heaviest microbial colonization with a microbial load of approximately 10^{12} bacteria per gram from more than 500–1000 species (Ley et al. 2006). This complex colonic ecosystem makes the colon possibly the most metabolically active organ in the body, and it is increasingly being realized that it has a significant impact on host health and well-being (Flint et al. 2007). The colonic microbiota, however, is still imperfectly understood, as more than 50% is not cultivable using current approaches (Xu et al. 2007). Using molecular sequence analysis, it has been estimated that there could be more than 7000 strains present (Backhed et al. 2005).

Most of our understanding of the colonic microbiota is based on the luminal microbiology, mainly from analyses performed on fecal samples. This clearly accounts for the majority of the bacteria present, but it does not give information on the mucosa-associated flora. Although this is a minority component, its association with the mucosa is likely to render it biologically significant. The information we do have comes from studies on biopsy samples (Zoetendal et al. 2002). The picture emerging is of a host-specific mucosal microbiota.

Of more significance is that we have an even poorer understanding of the functional roles that members of this ecosystem play. Although it may seem to be a simplistic approach to class species within the colonic microbiota as health positive or health negative, it is possible to identify certain individuals, notably the bifidobacteria and lactobacilli, that are exclusively benign and have identifiable health positive attributes. These organisms are saccharolytic in their metabolism and produce short-chain fatty acids (SCFA), which reduce luminal colonic pH and provide an inhibitory environment for exogenous pathogens. They can also produce a range of specific antimicrobial compounds to inhibit invading pathogens.

However, other members of the colonic microbiota, for example, many members of the clostridia, can produce very potent toxins under certain circumstances. Proteolytic bacteria in the colon produce a range of toxic compounds from protein catabolism. Our current understanding of the human colonic ecosystem is that these organisms live in a delicate and dynamic balance.

The gut microbiota is known to influence the development and activity of the immune system (Corthesy et al. 2007). The gut has the largest concentration of immune cells and is one of the primary interfaces with extraneous antigens.

Prebiotics and Synbiotics

Given that the colonic microbiota has an impact on host health, it is not surprising that there has been much interest over the years in identifying ways of modulating the activities of colonic bacteria and manipulating the composition in favor of health (Louis et al. 2007).

Historically, this has been attempted by consumption of live microbial supplements, usually bifidobacteria or lactobacilli, in an attempt to fortify the gut. This is the probiotic approach, and it has a long history (Rastall et al. 2005) with many successful trials. Probiotics, however, are live bacterial supplements that need to be kept viable in order to be active.

An alternative is the use of prebiotics. A prebiotic is defined as "a nondigestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a

limited number of bacteria in the colon, and thus improves host health" (Gibson & Roberfroid 1995). All known prebiotics are carbohydrates. The original definition calls for them to be nondigestible, although this is not strictly necessary—all that is needed is for substantial quantities to reach the colon intact. The key attribute of prebiotics, and the one that separates them from other dietary fibers, is that they are selectively metabolized by the more health positive members of the colonic microbiota, thus influencing the ecological balance.

Methods of Evaluation of Prebiotic Activity

Many different techniques have been used to evaluate the prebiotic potential of carbohydrates. Historically, researchers have made extensive use of in vitro techniques such as pure cultures and fermentation systems designed to model the gut to some degree. Ultimately, however, any claim for prebiotic activity must be supported by more than one human study in relevant population groups.

In vitro methods. Ultimately, any claim to prebiotic status for a food ingredient must be supported by more than one good-quality, well-designed human trial. These are, however, expensive, and it is ethically questionable to feed volunteers a test ingredient without some good reason to believe it will have activity. For this reason, it is common to evaluate candidate prebiotics initially using in vitro methods.

Historically, reliance has been placed on the use of pure culture testing of intestinal bacterial isolates. This approach does not, however, tell us anything at all about how a candidate prebiotic will behave in mixed culture systems; indeed, misleading conclusions would be drawn based on pure cultures alone.

A more useful initial test for prebiotic potential is the use of mixed fecal inocula in batch cultures. Given the huge variation seen between the gut microbiota of different individuals, these must be carried out with sufficient replication. The simplest forms of such cultures are non-pH controlled (Rycroft et al. 2001), and these can give a very preliminary view of the selectivity of fermentation.

A much more robust culture method is to use pH-controlled batch cultures. These can be set at varying pH values to model different regions of the colon.

Non-pH-controlled batch cultures have been scaled down to microcentrifuge tube size, and this allows an evaluation on 7 mg of test material per culture (Sanz et al. 2005). Although this is a very compromised model of the human colonic microbiota, it does allow preliminary testing of scarce carbohydrate candidates and has been used in a structure-function study to obtain information on the influence of linkage type, anomeric configuration, and glycosyl residue type on fermentation selectivity (Sanz et al. 2005). These authors validated the microscale method against pH-controlled batch cultures. Recently a pH-controlled microscale system has been developed (S. Kolida, unpublished data) that can evaluate 50 mg of carbohydrate per culture.

Batch culture testing should be followed by evaluation in a more sophisticated model of the human gut. There are several such systems in use around the world (Rumney & Rowland 1992). They vary in terms of their complexity and consequently in the type of information that they yield.

One complex intestinal model system is the SHIME (simulation of the human intestinal microbial ecosystem) reactor (Molly et al. 1993). This model simulates most of the attributes of the human intestinal tract, with the exception of the selective absorption of nutrients. The system consists of a series of five temperature (37°C)- and pH-controlled vessels that simulate the stomach; small intestine; and ascending (pH 5.6–5.9), transverse (pH 6.2–6.5), and descending (ph 6.6–6.9) colon. The total retention time is 76 hours, and the model is fed with a carbohydrate-rich medium that includes arabinogalactan, pectin, xylan, starch, and mucin.

A simpler system focusing on the luminal microbiology of the colon is the three-stage continuous culture model (Macfarlane et al. 1998). This model is the only one to have been validated against the colonic contents of sudden death victims. This is a cascade system of three culture vessels (37°C) to model the ascending, transverse, and descending colon, with varying dilution rate and pH (5.6, 6.2, and 7.0, respectively). It is fed with a medium designed to model the effluent of the small intestine and is rich in carbohydrates such as arabinogalactan, xylan, pectin, starch, inulin, and guar gum.

These complex models of the human gut are very important research tools as they allow experiments that are difficult, if not impossible, in human volunteers. For instance, they are very useful for assessing factors such as site of and persistence of fermentation through the gut. They are also useful for carrying out challenge experiments with pathogens.

One important consideration when studying the effects of prebiotics is the microbiology methods used. Traditional selective culture methods have been shown to be inadequate for the enumeration of the microbial groups in feces (Greetham et al. 2002). Such a complex ecosystem requires much more sophisticated methods, and a range of DNA-based methods have been introduced for this purpose (Blaut et al. 2002). One of the most widely used is fluorescent in situ hybridization (FISH). This approach utilizes fluorescently labeled gene probes targeted at the 16S rRNA sequences to give group-specific identifications (Blaut et al. 2002). Fluorescently labeled bacteria can then be counted microscopically, or by automated techniques such as flow cytometry or image analysis.

A complementary technique to give an overview of the diversity of the microbial ecosystem is denaturing gradient gel electrophoresis (DGGE). This technique involves a PCR amplification of diagnostic DNA sequences, generally the 16S rRNA sequences (McCartney 2002). Amplicons are separated on a polyacrylamide gel containing a gradient of urea or a temperature gradient. This results in sequence-specific separation of DNA bands that can be excised for subsequent DNA sequencing.

DGGE and sequencing can be usefully coupled with quantitative PCR (qPCR) (Matsuki et al. 2004) with a range of species-specific primers to obtain quantitative data at a species level based on the diversity revealed by the DGGE analysis.

Human studies. Ultimately, any claim to prebiotic properties must be established in human trials. Unfortunately, many of the early human data were acquired from small, uncontrolled studies using culture-based microbiology methods. It is important for the future development of the field that trials are carried out using robust study designs, preferably double-blind and placebo-controlled, with sufficient statistical power and clearly identified end points.

Comparative Studies of Prebiotics

Rycroft and colleagues (Rycroft et al. 2001) performed fermentation studies (non-pH-controlled fecal batch culture) on a range of oligosaccharide prebiotics and candidate prebiotics, including fructo-oligosaccharides (FOSs) and inulin. Microflora changes were determined and the evolution of gas and short-chain fatty acids measured. Distinct differences were seen between the oligosaccharides tested, although it must be borne in mind that they were commercial preparations of variable purity. Some of the prebiotics tested, notably the galacto-oligosaccharides, contained large quantities of carbohydrate that would, when ingested, be metabolized by the small intestine. These represent confounding factors in the batch cultures. This study found that the xylo-oligosaccharides were the most selective for bifidobacteria, followed by the fructans. These did, however, result in the highest gas evolution. The galacto-oligosaccharides represented the best

compromise between selectivity and gas production. Isomalto-oligosaccharides (IMOs) were also very selective toward bifidobacteria.

A subsequent comparative study (Palframan et al. 2002) investigated the fermentation selectivity of inulin, FOSs, galacto-oligosaccharides (GOSs), IMOs, and lactulose at pH 6.0 and 6.8 and at either 1% (w/v) or 2% (w/v) substrate. The fructans were most selective at pH 6.8 and 1% (equivalent to 4 g/day in human adults), whereas the others had highest selectivity at pH 6.0 and 2% (equivalent to 8 g/day).

PROPERTIES OF PREBIOTICS

Established Prebiotics

Although there are several carbohydrates marketed as prebiotics around the world, there are only four that are well supported by good quality data from human trials. These are the fructans inulin and FOS, GOS, and the synthetic disaccharide, lactulose.

Fructans. Inulin is a polysaccharide of the form Glu α1–2[β Fru 1–2]n, in which n > 10 (Crittenden & Playne 1996). The structural relatives of inulin, fructo-oligosaccharides (a lower molecular weight version) have been the best-documented oligosaccharides for their effect on intestinal bifidobacteria and are considered important prebiotic substrates. They are produced in large quantities in several countries and are added to various products such as biscuits, drinks, yogurts, breakfast cereals, and sweeteners. The term fructo-oligosaccharides may be used to represent two different preparations, either derived from inulin by hydrolysis or from sucrose by synthesis (see below). Inulin occurs naturally in Western foods such as onion, asparagus, leek, garlic, wheat, and artichoke, although to a lesser extent than in the commercial source chicory. Inulin-derived FOSs are Glu α1–2[β Fru 1–2]n, in which n = 2–9. Sucrose-derived FOSs are largely composed of a mixture of three oligosaccharides, i.e., 1-kestose (Glu-Fru_2), 1-nystose (Glu-Fru_3), and 1F-β-fructofuranosylnystose (Glu-Fru_4). Because of the low molecular weight of the sucrose-derived product, they are frequently referred to as short-chain FOSs or sc-FOSs.

Batch culture studies in which fecal slurries were incubated with inulin, FOS, starch, polydextrose, fructose, and pectin for 12 h (Wang & Gibson 1993) showed the greatest increase in bifidobacteria with FOSs and inulin, indicating the prebiotic nature of these substrates. Continuous culture systems inoculated with fecal slurries were later used to investigate FOSs fermentation (Gibson & Wang 1994a,b). In accordance with earlier studies, bifidobacteria, and to a lesser extent, lactobacilli, preferred FOS and inulin to glucose, whereas bacteroides could not grow on FOS. By varying parameters in the chemostat, the optimum conditions for growth of bifidobacteria, but inhibition of bacteroides, clostridia, and coliforms, were concluded to be low pH (pH 5.5), high culture dilution rate (0.3 h–1), and 1% (w/v) concentration of carbohydrate, i.e., similar to the physicochemical environment of the proximal colon. Three-stage gut models confirmed the enhanced proliferation of bifidobacteria by FOS in conditions resembling the proximal colon (Gibson & Wang 1994b, McBain & Macfarlane 1997).

A later single-stage chemostat study with FOSs (Sghir et al. 1998) demonstrated discrepancies between classical microbiological techniques and molecular approaches. Agar plate counts showed an increase in the combined populations of bifidobacteria and lactobacilli to reach 98.7% of the total bacterial flora by steady state. However, 16S rRNA genus-specific probes indicated an initial increase in the bifidobacterial population, which decreased after six days, whereas lactobacilli thrived in the low pH fermenter (pH 5.2–5.4) and maintained a high population at steady state.

Changes observed in the short-chain fatty acid profile corresponded well with the population data obtained through probe methods.

van de Wiele et al. (2004) studied the fermentation of inulin in a complex model of the human gut. They fed the system with a complex growth medium containing inulin at 2.5 g/day (equivalent to 5 g/day in adult humans) and monitored the microbiota using a multiphase approach consisting of plate counting, quantitative PCR, and DGGE. They also monitored metabolites and enzyme activities. The results showed a significant increase in lactobacilli in the transverse and descending colon vessels. Low levels of bifidobacteria were recorded in the colon vessels. DGGE analysis revealed that bacteria in the ascending colon vessel grouped together, as did bacteria in the other colon vessels. Bifidobacteria clustered according to time point rather than vessel. Quantitative PCR, however, revealed a significant increase in bifidobacterial populations in all three colon vessels. Inulin feeding also resulted in an increase in short-chain fatty acids, particularly propionate and butyrate, indicating a shift toward a more saccharolytic metabolism.

These authors have more recently used the same model system and metabolic analysis to investigate the effect of molecular weight of inulin on fermentation properties (van de Wiele et al. 2007). In this study, two fructan fractions, DP 2–20 (FOSs) and DP 3–60 (inulin), were fed to the SHIME system. qPCR revealed that both fructans were bifidogenic, with inulin resulting in a more pronounced and persistent bifidogenic effect. Addition of the fructans resulted in increased production of short-chain fatty acids, with inulin resulting in more propionate and butyrate than FOSs. Further, the inulin resulted in lower branched-chain fatty acid concentrations.

There are numerous human studies confirming the prebiotic status of inulin and FOSs based on assessments of the fecal microbiota. They have been evaluated in infants and adults and have been used in numerous studies on specific health attributes (see below). The studies have used different protocols and differing methods of microflora analysis (**Table 1**). They do, however, show a consistent bifidogenic effect with inconsistent effects on other groups of bacteria. Inulin and FOSs have been investigated in several disease states or with specific health outcomes in mind, and these will be discussed in more detail below.

The majority of studies on prebiotics have involved the examination of the microbiota of fecal samples. Owing to the practical difficulties involved, the mucosally associated flora has not been well studied. One study fed 15 g/day of a mixture of FOSs and inulin to 14 individuals awaiting colonoscopy for two weeks (Langlands et al. 2004). Fifteen control volunteers were not fed the fructan supplement. Biopsy samples from the cecum and descending colon were then analyzed for the associated microbiota and for cell proliferation markers. Mucosal associated bacteria were cultured anaerobically and characterized to species level using fatty-acid profiling. Significant increases were seen in populations of bifidobacteria, lactobacilli, and eubacteria. No effects on cell proliferation indices were seen.

Galacto-oligosaccharides. Galacto-oligosaccharides are galactose-containing oligosaccharides of the form Glu α1–4[β Gal 1–6]n, in which n = 2–5, and are produced from lactose syrup using the transgalactosylase activity of the enzyme β-galactosidase (Crittenden & Playne 1996).

The term galacto-oligosaccharides tends to be used generically for any oligosaccharide mixture resulting from the transgalactosylation activity of β-galactosidases. Early reports of the manufacture and evaluation of GOSs used the term transgalactosylated oligosaccharides (TOS) (Tanaka et al. 1983). These were produced using β-galactosidase from *Aspergillus oryzae*.

The commercial product Oligomate 55 is prepared using β-galactosidases from *A. oryzae* and *Streptococcus thermophilus* (Crittenden & Playne 1996) and contains 36% tri-, tetra-, penta-, and hexa-galacto-oligosaccharides, 16% disaccharides galactosyl glucose and galactosyl galactose, 38% monosaccharides, and 10% lactose.

Table 1 Studies in healthy human volunteers on prebiotic properties of inulin and FOSs[1]

Prebiotic	Study design	Microbiology	Dose	Outcome	Reference
FOSs and Inulin	Eight subjects on controlled diets fed FOSs, sucrose, or inulin per day for 15 days	Culture—principal groups	15 g/day	Significant increase in bifidobacteria; decreases in clostridia, bacteroides, fusobacteria	(Gibson et al. 1995)
sc-FOSs	Twelve healthy adults fed sc-FOSs in a controlled diet for 25 days	Culture—bifidobacteria, enterobacteria, total anerobes, and aerobes	4 g/day	Significant increase in bifidobacteria; decreases in β-glucuronidase and glycolic acid hydroxylase activities	(Buddington et al. 1996)
Inulin	Thirty-five elderly constipated individuals fed inulin or lactose for 19 days in a double-blind trial	Culture—principal groups	20 & 40 g/day	Significant increase in bifdobacteria; decrease in enterococci and enterobacteria. No change in fecal β-glucosidase or β-glucuronidase	(Kleessen et al. 1997)
Inulin	Eight subjects on low-fat diets fed inulin as a fat replacer for 64 days. Dose chosen to be isoenergetic	FISH—total bacteria and bifidobacteria only	Up to 34 g/day	Significant increase in bifidobacteria as a function of the total	(Kruse et al. 1999)
FOSs	Eight healthy volunteers fed FOSs for five weeks, comparison with baseline	Culture—principal groups	8 g/day	Significant increase in bifidobacteria	(Menne & Guggenbuhl 2000)
sc-FOSs	Nineteen elderly individuals fed FOSs for three weeks, comparison with baseline	Culture—principal groups	8 g/day	Increase in bifidobacteria and bacteroides, decreased phagocytic activity, reduced expression of IL-6	(Guigoz et al. 2002)
Inulin	Ten healthy adults fed for nine days, self-controlled, inulin compared to baseline	FISH & DGGE—principal groups	9 g/day	Significant increase in bifidobacteria and decrease in *Eubacterium rectale* probe group	(Harmsen 2002)
sc-FOSs	Forty healthy volunteers fed sc-FOSs or placebo for seven days	Culture—principal groups	2.5–10 g/day	Significant increase in bifidobacteria, dose-response relationship seen over all doses, no significant differences seen for other groups	(Bouhnik et al. 2006)
Inulin	Thirty-nine healthy volunteers fed inulin or placebo for four weeks on a double blind trial	Culture—principal groups	2.5 g/day	Significant increase in bifidobacteria, no change in other groups. Decreased β-glucuronidase	(Bouhnik et al. 2007b)
sc-FOSs	Twelve healthy elderly volunteers fed sc-FOSs and compared to baseline	Culture—principal groups	8 g/day	Significant increase in bifidobacteria, increased excretion of cholesterol in feces	(Bouhnik et al. 2007a)
Inulin	Thirty healthy volunteers fed inulin or maltodextrin for two weeks in a double-blind trial	FISH—principal groups	5 and 8 g/day	Significant increase in bifidobacteria and clostridia at both doses, no dose-response, response related to initial numbers	(Kolida et al. 2007)

[1]FOSs: fructo-oligosaccharides derived from inulin; sc-FOSs: short-chain fructo-oligosaccharides derived from sucrose.

The Dutch dairy company FrieslandCampina produce a GOS known as VivinalGOS, which is made using a *Bacillus circulans* enzyme. VivinalGOS is 59% GOS w/w and 41% glucose, galactose, and lactose. The oligosaccharides present are predominantly disaccharides (19%) and trisaccharides (23%). Nissin Sugar in Japan produce a product using *Cryptococcus laurentii* enzymes known as Cup-Oligo which is a β1–4 galactosyl lactose.

More recently, Clasado Ltd has begun to produce a GOS, BiMuno. This is a powdered product containing 52% GOS and a syrup. The product is produced with β-galactosidases from *Bifidobacterium bifidum*.

The choice of enzyme used in the manufacture of the GOS has a profound influence on the structure of the products (Rabiu et al. 2001). Oligomate is composed largely of β 1–6 linkages, whereas Vivinal and Cup-Oligo are largely β 1–4 linked, and BiMuno is mainly β 1–3 linkages.

GOS products have good selectivity using in vitro model systems using molecular microbiological methods of microbiota characterization. In a comparative in vitro study (Rycroft et al. 2001), GOSs gave the best compromise between selectivity and gas production. GOSs also performed well in the three-stage gut model (McBain & MacFarlane 2001). GOSs were only weakly bifidogenic in the first vessel (modeling the ascending colon) but were very stimulatory to lactobacilli. Vessels two and three (modeling transverse and descending colon, respectively) displayed very little change in microbiota, but there was a suppression of activities of β-glucosidase, β-glucuronidase, and arylsulphatase.

The properties of the BiMuno product have been investigated in a three-stage gut model (Tzortzis et al. 2005). BiMuno at 1% (w/v) had a strong bifidogenic effect that showed good persistence through the first two vessels, with a weaker response in the third. The BiMuno GOS is relatively low molecular weight, however, and was probably metabolized in the first two vessels.

GOSs have been evaluated in several animal studies, mainly in rats (Rowland & Tanaka 1993, Djouzi & Andrieux 1997, Morishita et al. 2002). Generally, these studies have shown a consistent bifidogenic effect, frequently at the expense of other microbial groups.

There have been several human trials of GOSs (**Table 2**) using a variety of methods and study designs. Early feeding studies showed prebiotic potential of GOSs but in small, uncontrolled studies. More recent studies, with larger groups of volunteers, have given rather mixed results. Several studies have failed to show any significant changes in fecal microbiota of volunteers (Alles et al. 1999, Alander et al. 2001, Satokari et al. 2001), whereas others have shown a response (Gopal et al. 2003, Bouhnik et al. 2004, Depeint et al. 2008).

Lactulose. Lactulose is a synthetic disaccharide in the form Gal β1–4 Fru. Lactulose was used originally as a laxative, as it is not hydrolyzed or absorbed in the small intestine (Saunders & Wiggins 1981). Lactulose has also received attention as a potential prebiotic. Lactulose increased lactobacilli and bifidobacteria and significantly decreased bacteroides in mixed continuous fecal culture (Fadden & Owen 1992), although total bacterial numbers decreased. Lactulose has been consistently found to have prebiotic potential in human trials (**Table 3**). Although lactulose looks to be a very promising prebiotic, it is not yet widely distributed as such. It has an established market as a medical product and would seem to have much value in the food sector.

Emerging Prebiotics

There are several other putative prebiotic oligosaccharides on the world market, mainly in Japan, that do not have robust data from human studies to support their status as prebiotics. Frequently,

the studies have been carried out with small cohort sizes, and the microbiology is often dependent on culture-based techniques. It will be interesting to see how these products develop in the future.

Xylo-oligosaccharides. Xylo-oligosaccharides (XOSs) are chains of xylose molecules linked by β1–4 bonds (Vázquez et al. 2000, Moure et al. 2006), although they can be substituted with arabinosyl, acetyl, or glucuronyl moieties. They are produced enzymatically by hydrolysis of xylan from birch wood, oats, or corn cobs, (Moure et al. 2006) and commercial products generally consist of xylobiose, xylotriose, and xylotetraose (Crittenden & Playne 1996). XOSs are predominantly used in Asia, particularly in Japan, where they enjoy FOSHU (Foods for Specified Health Use) status. The principal manufacturers are Suntory in Japan and Shandong Longlive Biotechnology in China. They are available in high purity forms containing up to 95% XOSs. They are increasingly widely used in the Asian market, and they are utilized in a wide range of food products, where their stability in heat (up to 100°C) and acid (pH 2.5–8.0) gives an advantage over other prebiotics (Vázquez et al. 2000).

XOSs have not been particularly well studied in terms of their prebiotic activity. They have been studied using in vitro batch culture systems (Rycroft et al. 2001, Zampa et al. 2004), where they have proven to be very selective for bifidobacteria at the expense of other groups. They have modest gas production compared with other prebiotics (Rycroft et al. 2001). The prebiotic potential has also been confirmed in animal studies (Campbell et al. 1997, Hsu et al. 2004, Santos et al. 2006).

Recently, interest has focused on wheat arabinoxylans as sources of prebiotic oligosaccharides (Hughes et al. 2007). There seems to be a clear influence of molecular weight, with low molecular weight materials showing more pronounced effects. Fractions with molecular weights of 354, 278, and 66 KDa have been evaluated in pH-controlled fecal batch culture systems, and they were selectively fermented by bifidobacteria. Fractions of a wheat pentosan concentrate with degrees of polymerization from 3 to 61 and arabinose:xylose ratios from 0.26 to 0.58 have been tested in a rat model (Van Craeyveld et al. 2008). The fractions, particularly the fractions with lower arabinose substitution, were bifidogenic. The higher degree of polymerization (DP) fractions resulted in lower levels of undesirable branched-chain fatty acids and elevated levels of acetate, propionate, and butyrate.

There are no well-designed and robust human intervention studies published to date for XOSs. Early human intervention studies carried out in Japan tended to be uncontrolled and involved small numbers of subjects (Barger-Lux et al. 1989, Ballongue et al. 1997, Boon et al. 2000, Blaut et al. 2002, Backhed et al. 2005, Bang et al. 2007) and the microbial analysis was performed using culture-based techniques (Vázquez et al. 2000). These studies did, however show selective increases in bifidobacteria and decreases in putrefactive products such as *p*-cresol, skatol, and indole. A more recent study fed 4 g XOSs per day for three weeks to 22 elderly (over 65) volunteers in a parallel placebo-controlled design (Chung et al. 2007). Bifidobacteria and *Clostridium perfringens* were enumerated by purportedly selective media. A significant increase in bifidobacteria relative to the sucrose placebo was seen after three weeks.

Resistant starch. Resistant starch is the term used for the fraction of starch that escapes digestion in the upper gastrointestinal tract and that reaches the colon to be fermented by the colonic microbiota (Topping et al. 2003). There are four classes of resistant starch. RS1 is starch in a physically inaccessible form such as whole grains and seeds; RS2 is granular starch such as that in green banana, potato, and legumes; RS3 is retrograded starch as found in cooked and cooled potatoes, bread, etc.; RS4 is chemically modified starch such as starch esters. In addition to resistant starch that falls into these categories, one must consider oligosaccharide breakdown products of digestion in the upper GIT that may act as prebiotics in the colon (Topping et al. 2003).

Table 2 Studies in healthy human volunteers on prebiotic properties of GOSs

GOS product[1]	Study design	Microbiology	Dose	Outcome	Reference
Oligomate[2]	Five healthy adults fed the lower dose for seven days, then the higher dose for 7 days	Culture—principal groups	43–143 mg/kg/day	Increase in bifidobacteria, decrease in bacteroides	(Tanaka et al. 1983)
Oligomate	Twelve healthy adults fed for seven days in a crossover study	Culture—principal groups	36, 71, or 143 mg/kg/day	Dose-dependant increase in bifidobacteria as a proportion of the total	(Ito et al. 1990)
Oligomate	Healthy individuals with low bifidobacterial numbers	Culture—principal groups	36 mg/kg/day	Significant increase in bifidobacteria and lactobacilli and decreases in bacteroides and candida. Fecal ammonia, indole, and cresol significantly decreased	(Ito et al. 1993)
Oligomate	Eight healthy adults fed for 21 days in an uncontrolled study. Counts compared to baseline	Culture—bifidobacteria and enterobacteria only	10 g/day	Significant increase in bifidobacteria, no change in enterobacteria	(Bouhnik et al. 1997)
Vivinal[3]	Forty healthy adults fed controlled diets supplemented with two GOS doses for three weeks in a placebo-controlled parallel design	Culture—principal groups except bacteroides	7.5 and 15 g/day	No significant differences in any group	(Alles et al. 1999)
Vivinal	Thirty healthy volunteers fed for two weeks in a parallel design to compare prebiotic and synbiotic effects, comparison with baseline	Culture—principal groups except bacteroides	8.1 g/day	No significant differences	(Alander et al. 2001)
Vivinal	The same study group as above	DGGE	8.1 g/day	No changes in bifidobacterial population profile	(Satokari et al. 2001)

BP*Oligo*[4]	Thirty healthy volunteers fed for four weeks in a parallel design to compare prebiotic and synbiotic effects, comparison with baseline	Culture—principal groups	2.4 g/day	Significant increase in bifidobacteria and lactobacilli	(Gopal et al. 2003)
Cup-Oligo[5]	Eight healthy volunteers fed one of the doses for one week in a placebo-controlled parallel study	Culture—principal groups	2.5, 5.0, 7.0 or 10 g/day	Significant increase in bifidobacteria, no dose-response effect	(Bouhnik et al. 2004)
Vivinal and Bimuno[6]	Thirty healthy volunteers fed for one week in a double-blind crossover study comparing Vivinal to the milk matrix; then 30 healthy volunteers in a double-blind crossover study comparing doses of Bimuno with sucrose	FISH—principal groups	4.9 g/day (Vivinal) 1.9 and 3.6 g/day (Bimuno)	Significant increase in bifidobacteria with both prebiotics. Dose response seen with Bimuno, Bimuno more bifidogenic than Vivinal	(De Preter et al. 2006)

DGGE: denaturing gradient gel electrophoresis; FISH: fluorescent in situ hybridization.

[1]Various commercial products have been used. These have different structural profiles and different content of effective prebiotics.

[2]Yakult Honsha, Japan.

[3]FrieslandCampina, The Netherlands.

[4]A milk product treated with β-galactosidase to generate GOSs from lactose in situ.

[5]Nissin Sugar, Japan.

[6]Clasado, United Kingdom.

Table 3 Studies in healthy human volunteers on prebiotic properties of lactulose

Study design	Microbiology	Dose	Outcome	Reference
Eight healthy volunteers fed for two weeks on a nonplacebo controlled study with reference to baseline	Culture—principal groups	3 g/day	Significant increase in bifidobacteria, significant decrease in clostridia and bacteroides. Decrease in fecal indole, skatole, phenols, β-glucuronidase, nitroreductase, and azoreductase	(Terada et al. 1992)
Thirty-six healthy volunteers fed for four weeks on a placebo controlled trial	Culture—principal groups	20 g/day	Significant increase in bifidobacteria and lactobacilli. Significant decrease in clostridia, coliforms, eubacteria, streptococci, and bacteroides. Reduced activities of azoreductase, 7α-dehydroxylase, β-glucuronidase, urease, and nitroreductase	(Ballongue et al. 1997)
Ten healthy adults fed for one week on a double-blind, placebo-controlled trial	Culture and FISH—principal groups	10 g/day	Significant increase in bifidobacteria and decrease in clostridia. No effect on fecal water genotoxicity	(Tuohy et al. 2002)
Eight healthy volunteers fed for one week in a placebo-controlled parallel study	Culture—principal groups	10 g/day	No significant changes	(Bouhnik et al. 2004)
Forty-three healthy adults fed for four weeks on a double blind placebo-controlled crossover study	rtPCR for bifidobacteria	20 or 30 g/day	Significant increase in bifidobacteria	(De Preter et al. 2006)

Resistant starch has been extensively investigated for its various effects on the gut (Lehmann & Robin 2007). Relatively few studies, however, have investigated its prebiotic potential. Most studies on prebiotic properties of resistant starch have been in animals. Studies in rats (Le Blay et al. 2003, Dongowski et al. 2005, Jacobasch et al. 2006), human-flora-associated rats (Silvi et al. 1999), and pigs (Brown et al. 1997) have generally shown an increase in bifidobacteria and short-chain fatty acids.

A human study designed to examine the effective dose of a type-3 resistant starch gave ambiguous results (Bouhnik et al. 2004). When eight subjects were fed 10 grams/day for seven days, a significant increase in bifidobacteria was seen relative to the placebo. Doses of 2.5, 5.0, 7.5, or 10 grams/day were subsequently fed to groups of 32 subjects for seven days, and no significant increases were recorded. This study, however, used culture-based microbiological counting with no subsequent speciation.

Soy oligosaccharides. The main oligosaccharides contained in soybeans are raffinose and stachyose, which are resistant to digestion in the human gastrointestinal tract. Pure culture studies showed them to be good growth promoters of *B. infantis* but not *E. coli*, *S. faecalis*, or *L. acidophilus*. Raffinose is metabolized effectively by *B. infantis*, *B. bifidum*, *B. longum*, *Bact. thetaiotaomicron*, and *Bact. fragilis* but not by *E. coli* or *C. difficile* in pure culture (Jaskari et al. 1998).

The addition of a low concentration (0.1%, w/v) of SOR to a two-stage continuous culture of fecal bacteria (Saito et al. 1992) resulted in a threefold increase in the proportion of bifidobacteria in the total bacterial count. As only these bacterial groups were enumerated, any other changes that occurred in the microflora were overlooked. A significant decrease in azoreductase activity was recorded as well as decreases in β-glucosidase and β-glucuronidase. These results do not

correspond fully with those from in vivo trials, however. There are few strong data in the literature from human studies on soy oligosaccharides. In uncontrolled feeding studies with small groups of volunteers, soy oligosaccharides have been shown to elicit a bifidogenic effect with doses varying from 0.6–15 g/day (Hayakawa et al. 1990, Bang et al. 2007). There are also few data on the selectivity of the population changes, and the studies have been performed using culture-based methods. Elevated levels of propionate and butyrate have been seen along with reduced levels of genotoxic enzymes (Bang et al. 2007). Soy oligosaccharides have been found to be bifidogenic in a comparative study including IMOs, GOSs, resistant starch, lactulose, and sc-FOSs (Bouhnik et al. 2004). Feeding doses from 2.5 to 10 g/day to groups of eight volunteers for one week in a placebo-controlled parallel study showed that soy oligosaccharides were bifidogenic at 10 g/day but did not result in a dose response. Soy oligosaccharides cannot be considered as prebiotics on the current evidence, and there is a need to evaluate these materials in well-designed human studies.

Gluco-oligosaccharides. A new oligosaccharide preparation has been enzymatically synthesized, using a glucosyl-transferase from *Leuconostoc mesenteroides*, to transfer glucose molecules from the sucrose donor to an acceptor, namely maltose (Valette et al. 1993). The fructose from the sucrose molecule was released, leaving a mixture of gluco-oligosaccharides of various DP. The mixture composed of 18% mono-, di-, and trisaccharides, 18% tetrasaccharides, 33% pentasaccharides, and 31% hexa- and heptasaccharides comprising glucose units linked by α1–6 and α1–2 glycosidic bonds. GOSs were poorly hydrolyzed and digested in the intestinal tract of gnotobiotic rats (Valette et al. 1993).

A defined mixed culture of *Bact. thetaiotamicron*, *B. breve*, and *Cl. butyricum* incubated with 0.5% (w/v) GOS (Djouzi et al. 1995) resulted in no change in the *Bact. thetaiotamicron* population but increased the *B. breve* count and reduced *Cl. butyricum* numbers. However, such data should be interpreted with caution, as the use of defined mixed culture does not allow the full diversity and complexity of the intestinal microflora to be determined.

Recently, gluco-oligosaccharides synthesized with dextransucrase and alternansucrase and based on maltose and gentiobiose as acceptors have been evaluated in small-scale batch cultures after fractionation (Sanz et al. 2006a,b). The synthetic products displayed a highly selective fermentation in non-pH-controlled batch cultures.

Isomalto-oligosaccharides. 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 (Crittenden & Playne 1996). 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).

In vitro pH-controlled fecal batch culture studies with molecular microbiology have shown encouraging results with IMOs selectively supporting growth of bifidobacteria (Rycroft et al. 2001, Palframan et al. 2002). Studies with the three-stage gut model have shown that longer-chain IMOs derived by dextran hydrolysis resulted in a lactic acid flora while also allowing the generation of butyrate (Olano-Martin et al. 2000). As this is thought to be a desirable metabolite of colonic function, it may be that IMOs could be considered effective prebiotics. There is, however, a question over the digestibility of these materials. They are substrates for the human intestinal brush border sucrase-isomaltase system. However, estimates of the degree of digestibility vary (Oku & Nakamura 2003). IMOs display a size-dependent slow digestibility in the jejunum of the rat (Kaneko et al. 1995). As it is likely that longer chain IMOs will be more resistant to digestion in

humans, alternative manufacturing routes might be desirable. Dextran can be used as a substrate to generate higher molecular weight IMOs (Mountzouris et al. 1999b, 2002).

There are few human data on IMOs in the literature, and the studies are uncontrolled, using small numbers of volunteers and culture-based microbiology. The minimum effective dose of IMOs is 8–10 g (Kohmoto et al. 1991). A subsequent study evaluated fractionated IMOs (Kaneko et al. 1995) and found that the DP had an effect on the prebiotic properties. Increasing DP from two to three and higher reduced the effective dose, with the trisaccharide and higher fraction having an effective dose of 5 g/day. A comparative study evaluating IMOs against GOSs, resistant starch, lactulose, and sc-FOSs fed 10 g/day for one week in a placebo-controlled parallel study (Bouhnik et al. 2004). No significant changes were seen in any of the microbial groups enumerated by culture-based methods.

As far as health benefits are concerned, IMOs supplementation (10 g) into low-fiber diets improved bowel movement and stool output, relieving constipation in seven elderly men (Chen et al. 2001). Finally, 20 hemodialysis patients, fed with 30 g IMOs for four weeks, experienced relief from constipation, and their total cholesterol and triglycerides were significantly lowered (Wang et al. 2001). Given the potential shown by IMOs in vitro, more human trials on these materials would seem to be justified.

Lactosucrose. Lactosucrose (LS) is a non-reducing trisaccharide (Gal β1–4 Glc α1–2β Fru) that is synthesized by β-fructosidase-catalyzed transfer reaction using sucrose as both donor and acceptor. A variety of forms are manufactured by Ensuiko Sugar Refining Company and Hayashibara Shoji, Inc.

There are very few published data on the prebiotic potential of LS, and there are no large-scale trials using modern microbiological techniques. LS has been shown to be selective for bifidobacteria in healthy adults fed LS at 8 g/day (Ohkusa et al. 1995). However, one study in inflammatory bowel disease (IBD) patients at 8.5 g/day did not show any bifidogenic effect (Teramoto et al. 1996).

Novel Candidate Prebiotics

A further set of carbohydrates can be identified that are currently under investigation for their prebiotic potential. These have data predominantly from in vitro approaches or animal studies and do not have good human data to support them.

Polydextrose. Polydextrose is a macromolecule of randomly polymerized glucose. It is a polydisperse molecule and has an average degree of polymerization (DP) of approximately 12 with DP values exceeding 30 and is only partly digested in the human gut (Makivuokko et al. 2005, Fava et al. 2007). Polydextrose is marketed as a dietary fiber, but there has been much interest in its putative prebiotic properties. Although there have been many studies showing desirable effects of polydextrose on various aspects of gut physiology and pathology, there have been few actually addressing the selectivity of its fementation effects in the colon. Polydextrose has been evaluated using in vitro systems (Probert et al. 2004), animals (Fava et al. 2007), and humans (Jie et al. 2000, Tiihonen et al. 2008). Polydextrose displayed a moderate bifidogenic effect in vitro using a three-stage model of the colon (Probert et al. 2004) with DNA-based microbiota analysis. No effect on the microbiota was seen, however, in a study in pigs (Fava et al. 2007). The human data published to date on polydextrose are inconclusive. A study in 120 human volunteers, who were fed various doses of polydextrose (0, 4, 8, and 12 g/day) for 28 days, found an increase in fecal bifidobacteria and lactobacilli and a decrease in bacteroides. Unfortunately, the authors did not

analyze any other major components of the gut microbiota or carry out a total count. Further, this study used counting on selective media, apparently with no subsequent speciation. A more recent study (Tiihonen et al. 2008) fed 5 g polydextrose per day accompanied by a cocktail of probiotic strains to 20 volunteers for 14 days. Significant increases in bifidobacteria and lactobacilli were seen. Unfortunately, these authors froze the fecal samples, a procedure known to result in differential effects on fecal bacteria. Further, the bacterial enumeration was performed by culture techniques with no subsequent speciation. No total bacterial count was performed. The totality of evidence published to date does not support prebiotic status for polydextrose.

Pectic oligosaccharides. There has been recent interest in the potential of generating prebiotic oligosaccharides from pectic materials. Pectins are heterogeneous polysaccharides with a diversity of structural elements (Mohnen 2008). Generically, they are built upon a partially methyl esterified homogalacturonan backbone with periodic interruptions by regions of alternating galacturonic acid and rhamnose residues. The rhamnose residues carry arabino-oligosaccharides, galacto-oligosaccharides, or arabino-galactan-oligosaccharides to form the rhamnogalacturonan I structure. The rhamnogalacturonan II structure consists of side chains attached to the homogalacturonan backbone. These side chains frequently contain uncommon sugars, including apiose and aceric acid, together with rhamnose.

Pectic oligosaccharides have been obtained by either enzymatic or physical methods. Enzymatic hydrolysis of citrus and apple pectins in membrane reactors has been used to manufacture oligosaccharides of 3–4 kDa molecular weight (Olano-Martin et al. 2001). The fermentation properties of these oligosaccharide preparations have been investigated using pH-controlled fecal batch cultures (Olano-Martin et al. 2002). The oligosaccharides were more selective for bifidobacteria and lactobacilli than were the parent high molecular weight molecules. The structures of the oligosaccharides present are not known with any certainty.

Pectic oligosaccharides can also be obtained by nonenzymatic processes. A nitric acid hydrolysis process to manufacture pectins from citrus peel gives rise to a low molecular weight coproduct stream (Fishman et al. 2000). This coproduct stream consists of arabinose-based oligosaccharides. The fermentation properties of these preparations have also been evaluated in the pH-controlled fecal batch fermentation system, and these materials proved to have higher selectivity for bifidobacteria than did the enzymatically derived materials when compared with FOSs (Manderson et al. 2005). To date, these materials have not been tested in humans, so they cannot, at the present time, be accorded prebiotic status.

Mechanisms of Action of Prebiotics

Despite the intense interest in recent years in prebiotics and the rapidly increasing number of publications documenting their manufacture and application, we still have a very poor understanding of the mechanisms by which they selectively stimulate populations and activities of only some members of the colonic microbiota. One paradigm of prebiotic action is that certain members of the colonic microbiota possessed cell-associated glycosidases, which allowed the hydrolysis of prebiotics and the subsequent uptake of the liberated monosaccharides. Cell-associated β-fructofuranosidases have indeed been isolated from *Bifidobacterium infantis* strains (Imamura et al. 1994, Perrin et al. 2001). In *B. infantis* ATCC 15697, the β-fructofuranosidase was found to be induced by fructose (Perrin et al. 2001) and to preferentially hydrolyze shorter chains, as would be expected with an exo-acting glycosidase. Although glucose was the preferred substrate for growth, fructose resulted in the highest levels of acetate and lactate.

An alternative paradigm, however, is that certain members of the gut microbiota possess oligosaccharide transport systems that can scavenge oligosaccharides from the environment for subsequent intracellular hydrolysis. Such a mechanism would provide a clear selective advantage as the monosaccharide products of hydrolysis would not be available in the environment for other, competing microorganisms. Transport systems of this kind have not been described in detail for bifidobacteria or lactobacilli, but their existence has been implied in several studies. In a comparative study of the carbohydrate preferences of nine *Bifidobacterium* species, *B. bifidum* displayed a significantly higher growth rate on xylo-oligosaccharides than on xylose, implying a transport system for the oligomers (Palframan et al. 2003). *Lactobacillus plantarum* NRRL 1195 (subsequently reclassified as *L. paracasei*) has been found to display a preference for tri- and tetrasaccharides when grown on fractionated FOSs (Kaplan & Hutkins 2000). *Lactobacillus rhamnosus* strain GG did not ferment these oligosaccharides, and neither strain metabolized the pentasaccharide. Further work on the *L. paracasei* NRRL 1195 using radiolabeled FOS fractions revealed that transport of the tri- and tetrasaccharide fraction was rapid, and the pentasaccharide was barely transported (Kaplan & Hutkins 2003).

Lactobacillus plantarum WCFS1 displayed a preference for the trisaccharide fraction of sc-FOSs (Saulnier et al. 2007). DNA microarrays revealed that β-fructofuranosidase, sucrose phosphoenolpyruvate transport system, fructokinase, α-glucosidase, and a sucrose operon repressor were up-regulated on sc-FOSs. A mannose phosphoenolpyruvate transport system likely to transport glucose was down-regulated.

Substrate preferences have also been seen with fractionated galacto-oligosaccharides (Gopal et al. 2001). *Lactobacillus rhamnosus* DR20 displayed a preference for monosaccharides and disaccharides, whereas *Bifidobacterium lactis* DR10 preferred tri- and tetrasaccharides.

APPLICATIONS OF PREBIOTICS

There is a steadily increasing body of published information on the health attributes of prebiotics. It must, however, be remembered that the concept was only defined in 1995 (Gibson & Roberfroid 1995), and we do not currently have anywhere near the level of experimental evidence that supports the health attributes of probiotics. One problem with many studies involving prebiotics and health is that researchers have often used them in conjunction with a probiotic, i.e., as a synbiotic, without comparing the synbiotic to the prebiotic and probiotic alone. For this reason, there is often ambiguity over where the health benefits actually reside.

Colon Cancer

Colon cancer is the second most common cancer in Western societies and the fourth most common worldwide (Boyle & Langman 2000). Although there is a known genetic component of approximately 15%, the major factors influencing colon cancer incidence are lifestyle-related. Of these, diet plays a large role, and one rich in processed meat and alcohol is believed to increase risk, whereas one rich in fiber and milk is believed to reduce risk. The sequence of molecular events involved in the so-called adenoma-carcinoma sequence leading to tumors is complex (Fearon & Vogelstein 1990). The gut flora, however, can interact with this sequence in several ways. There is a solid body of evidence showing that intestinal bacteria and their metabolic activities can participate in the formation of tumors (Heavey & Rowland 2004). The gut microbiota produces genotoxic and tumor-promoting compounds and enzymes capable of liberating such compounds from precursors. The enzyme β-glucuronidase, for instance, releases several known carcinogens from their noncarcinogenic glycosylated form. Similarly, β-glucosidase hydrolyzes plant

glycosides in the gut. These include cycasin, which is hydrolyzed to a carcinogen. There is, however, potential for hydrolysis products to be protective; quercitin is a flavonoid that is hydrolyzed to an anticarcinogenic form (Gorbach & Goldin 1990). The rationale for the use of prebiotics as preventative agents is based on the observation that health-positive bacteria such as bifidobacteria and lactobacilli do not produce these compounds or enzymes in any significant quantity (Nakamura et al. 2002). Increasing the proportion of these bacteria in the colon, therefore, might reduce the levels of tumor promoters and genotoxins. The gut microbiota is also responsible for producing tumor promoting deoxycholic and lithocholic acids from primary bile acids.

Several studies have looked for effects of prebiotics on these bacterial enzyme activities. In rats, FOSs and GOSs have consistently been shown to reduce the activities of these bacterial enzyme activities and of toxic phenolic compounds such as cresol (Rowland & Tanaka 1993, Rowland et al. 1998). Data from human studies, however, are inconsistent. Studies on GOSs (Tanaka et al. 1983), soy oligosaccharides (Hayakawa et al. 1990), and isomalt (Gostner et al. 2006) have failed to show major changes in bacterial enzyme activities, with the exception of β-glucuronidase, which was reduced by isomalt.

DNA damage is an early event in colon carcinogenesis, and this can be measured using cell-culture assays such as the Comet assay (Pool-Zobel et al. 1996). Lactulose has been shown to reduce DNA damage in rat colonic mucosa (Rowland et al. 1996). Rats were fed 3% lactulose in the diet and given 1,2-dimethylhydrazine (DMH) to induce DNA damage. Lactulose-fed animals displayed 33% fewer cells with severe damage than the control group.

A more direct anticancer assay involves inducing aberrant crypt foci (ACF) in rats by feeding carcinogens such as azoxymethane (AOM) or DMH. In this assay, prebiotics have given mixed results. Studies on inulin (Rao et al. 1998) and FOSs (Gallaher et al. 1996) failed to show any impact on ACF. Other studies on inulin (Reddy et al. 1997; Verghese et al. 2002a,b; Buddington et al. 2002), FOSs (Reddy et al. 1997, Buddington et al. 2002), lactulose (Challa et al. 1997), and XOSs (Hsu et al. 2004) have shown significant reductions in ACF on prebiotic feeding.

Data from human studies on anticancer activities of prebiotics are scarce. This is not surprising given the nature of the clinical outcome, but some studies have looked at the effect of prebiotics on biomarkers of colon carcinogenesis. sc-FOSs have been investigated in a study on adenoma-affected and adenoma-free patients (Boutron-Ruault et al. 2005). Feeding 10 g/day sc-FOSs resulted in positive effects in biomarkers in the adenoma-free patients. A recent human study investigated the effect of a FOS 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 cancer patients and polypectomized individuals. Colorectal cell proliferation and genotoxicity were significantly reduced, and the intestinal barrier function increased. The data on colon cancer, taken as a whole, would seem to justify further research on the potential of prebiotics.

Inflammatory Bowel Diseases (IBD)

IBD are chronic, recurring conditions characterized by uncontrolled inflammation in the gastrointestinal tract (Hoentjen et al. 2005, Geier et al. 2007). Two specific conditions are of most concern: Crohn's disease (CD) and ulcerative colitis (UC). Crohn's disease results in a range of symptoms including nonbloody diarrhea, cramping, and fever (Hoentjen et al. 2005). Individuals frequently experience weight loss and can develop fistulae to the skin and to other internal organs. UC produces diarrhea, abdominal pain, and rectal bleeding. Patients can also experience weight loss and fever (Hoentjen et al. 2005). Further, UC can predispose toward colorectal cancer. These are distinct in their pathologies and sites of onset. CD can occur anywhere in the GI tract, whereas

UC originates in the rectum and progresses proximally through the colon. The precise pathological mechanisms of each of these are still being elucidated. They both involve a dysregulation of the immune system in genetically susceptible individuals that are exposed to specific environmental triggers. They are characterized by an inappropriate immune response to the host microbiota (Hoentjen et al. 2005, Geier et al. 2007).

Although there is clearly both a genetic component and an environmental component to IBD, it is also clear that there is a key role played by the gut microbiota. Diversion of the fecal stream in CD reduces inflammation (Geier et al. 2007), which is reverted by infusion of intestinal contents. In animal models of UC, rearing of the animals in germ-free conditions prevents development of disease (Geier et al. 2007). Further, in both cases treatment with broad-spectrum antibiotics is a viable treatment option, particularly in CD. Human studies have frequently shown differences in the microbiota of IBD patients compared with healthy individuals (Sokol et al. 2006). IBD patients often display significant components of the colonic microbiota that would be considered unusual in healthy individuals (Sokol et al. 2006).

As a consequence of the role of the gut microbiota in IBD, there has been interest in the use of prebiotics to ameliorate the conditions (Guarner 2007). Several studies have been carried out to date (**Table 4**). Most of the current studies have been in experimental animals. A commonly used model is the rat fed with dextran sulfate sodium (DSS). Feeding with DSS for 2–3 days causes mucosal barrier damage and symptoms similar to the human disease (Videla et al. 2001). An alternative means of inducing disease symptoms in rats is trinitrobenzene sulfonic acid (TNBS) (Cherbut et al. 2003). Inulin (Videla et al. 2001, Osman et al. 2006), FOSs (Cherbut et al. 2003, Moreau et al. 2003, Osman et al. 2006), and lactulose (Rumi et al. 2004, Camuesco et al. 2005) have all been evaluated in these disease models (**Table 4**) and have generally shown beneficial effects with reductions in disease scores and frequently shown reductions in proinflammatory immune regulators. Another animal model that has been used is the HLA-B27 rat. These animals are transgenic for the human HLA-B27 β2-microglobulin gene, and they spontaneously develop colitis at 2–4 months (Hoentjen et al. 2005). Inulin and FOSs (Schultz et al. 2004, Hoentjen et al. 2005) have shown significant beneficial effects in these models too.

There have been few human studies on IBD with prebiotics (**Table 4**). Lactulose has shown beneficial effects in a study of CD and UC patients (Szilagyi et al. 2002), with a reduction in disease symptoms relative to controls on consumption of 20 g lactulose per day. FOSs and inulin have given mixed results in UC patients, with a significant reduction in disease severity in one small open-label study (Lindsay et al. 2006) and no significant effect in a much larger study (Chermesh et al. 2007). This latter study, however, only fed 2.5 grams of inulin per day, less than the 5–10 g/day generally recognized as necessary for a prebiotic effect. More cohesive data have been obtained in two studies on UC patients. A mixture of FOSs and inulin has shown significant reductions in disease severity indices, reduction in proinflammatory immune markers, and a reduction in calprotectin, a validated marker of intestinal inflammation (Konikoff & Denson 2006). Given the potential shown in animal studies and the few human studies carried out to date, more studies on prebiotics in IBD would seem to be warranted.

Acute Infections

Bifidobacteria and lactobacilli are known to produce a range of antimicrobial agents from short-chain fatty acids to peptides (Collado et al. 2005a,b; Trejo et al. 2006). For this reason, there has been much interest in the potential of using prebiotics to reduce risk of acute infections. FOSs, inulin, and GOSs have been found to be effective in in vitro fecal cultures at inhibiting colonization by *Clostridium difficile* (Hopkins & Macfarlane 2003). Prebiotics have also been evaluated for their

Table 4 Studies on prebiotics in IBD

Prebiotic	System	Design	Outcome	Reference
Inulin	Rats + DSS	Matched groups of rats fed inulin orally (400 mg/day) or by enemas	Reduced inflammatory lesions, lower release of inflammatory mediators	(Videla et al. 2001)
Lactulose	CD and UC patients	Prospective cohort study (20 g/day for three weeks)	Reduced symptoms in patients relative to controls, lower colonic adaptation than controls	(Szilagyi et al. 2002)
Inulin	Pouchitis patients	Randomized double-blind, placebo-controlled crossover. 20 subjects, 24 g/day for 3 weeks	Reduced inflammation by endoscopy and histology, significantly reduced Pouchitis Disease Activity Index	(Welters et al. 2002)
FOSs	Rats + TNBS	Intragastric infusion (1 g/day) for 14 days	Significant reduction in inflammation, myeloperoxidase activity, and pH	(Cherbut et al. 2003)
FOSs RS	Rats + DSS	FOSs or RS-containing diet compared with basal diet	RS but not FOSs significantly reduced macroscopic and histolytical scores of inflammation	(Moreau et al. 2003)
Lactulose	Rats + DSS	Lactulose (0.3–1 g/kg) fed orally twice daily for 6 days	Dose-dependent reduction in colonic ulceration, diarrhea, and myeloperoxidase activity	(Rumi et al. 2004)
Inulin[1]	HLA-B27 rats	Rats fed synbiotic or control in drinking water for 2 months	Histological scores of inflammation significantly reduced	(Schultz et al. 2004)
Lactulose	Rats + TNBS	Rats fed lactulose solution (2..5%, w/w) or water for three weeks over initiation of colonic damage	Reduction in myeloperoxidase activity, colonic TNF-α, and leucotriene B4	(Camuesco et al. 2005)
FOSs + inulin[2]	UC patients	Randomized, placebo-controlled, double-blind study. 18 subjects in parallel groups fed 12 g/day for 1 month	Significant reduction in proinflammatory cytokines (TNF-α, IL-1β) and defensins. Histology of biopsies showed reduction in inflammation and crypt abscesses	(Furrie et al. 2005)
FOSs + inulin	HLA-B27 rats	Rats fed 5 g/kg body weight in drinking water against water control	Significant decrease in inflammatory scores in cecum and colon. Significant decrease in proinflammatory IL-1β and increase in regulatory TGF-β	(Hoentjen et al. 2005)
FOSs + inulin	CD patients	10 patients, open label trial, 15 g/day	Significant reduction in disease severity indices	(Lindsay et al. 2006)
FOSs + inulin	Rats + DSS	Oral administration of 0.5 g/animal twice daily for seven days	Significant reduction in disease activity index and myeloperoxidase activity. Significant decrease in proinflammatory IL-1β. Significant reduction in bacterial translocation	(Osman et al. 2006)
FOSs + inulin	UC patients	Prospective, randomized, placebo-controlled trial. Ten patients fed 12 g/day for 2 weeks	Significant reduction in calprotectin by day 7. Significant reduction in dyseptic symptom scores	(Casellas et al. 2007)
Inulin[3]	CD patients	Prospective, randomized, placebo-controlled, double-blind trial. 2.5 g/day for 24 months	No significant difference in endoscopic or clinical relapse rate	(Chermesh et al. 2007)

DSS: dextran sulfate sodium, TNBS: trinitrobenzene sulfonic acid, FOSs: fructooligosaccharides, RS: resistant starch.

[1]Administered in the form of a synbiotic with a mixture of lactobacilli and bifidobacteria.

[2]Administered in the form of a synbiotic with *Bifidobacterium longum*.

[3]Administered together with several nonprebiotic dietary fibers and a cocktail of probiotics.

ability to act synergistically with probiotics to inhibit pathogens (Fooks & Gibson 2002, Tzortzis et al. 2004) with good effect in vitro.

The data from human studies on acute infection are, however, very variable. Prebiotics have been investigated for their potential benefits in traveler's diarrhea. A study of 244 volunteers traveling to destinations classed as medium to high risk of infection fed 10 g/day of FOSs or placebo for two weeks prior to traveling and for two weeks while on holiday (Cummings et al. 2001). FOS consumption increased perceived well-being but had no significant impact on the incidence of diarrhea. There may be several reasons for the failure of prebiotics to provide protection. In studies on traveler's diarrhea, the causative agent is rarely identified, and there are many potential causes, both bacterial and viral. Given that prebiotics can only be expected to have a significant effect in the colon, any pathogen targeting the small intestine is not likely to be inhibited.

Prebiotics have also been evaluated as preventative agents in antibiotic-associated diarrhea (Lewis et al. 2005). Increasingly, this is caused by *Clostridium difficile*, and the hypothesis is that the normal gut microbiota prevents *C. difficile* populations from becoming problematic. Broad-spectrum antibiotics disrupt the normal barrier function and allow proliferation of *C. difficile* with concomitant toxin generation. 435 elderly patients (over 65) on broad-spectrum antibiotics were fed 12 g/day of FOSs in a double-blind, placebo-controlled study design. Volunteers received treatments while undergoing antibiotic therapy and for a week post cessation of the antibiotics. The FOS group displayed significantly increased levels of bifidobacteria but there were no differences in incidence of diarrhea or *C. difficile* infection between the two groups.

FOSs did, however, result in a significant decrease in diarrhea, vomiting, and fever in a study of young children in a day-care center (Waligora-Dupriet et al. 2007). Infants were fed 2 g/day of FOSs or placebo for 21 days in a double-blind trial. Using culture-based methods, a nonsignificant increase in bifidobacteria was seen with a significant decrease in clostridia.

Mineral Absorption

There has been a lot of interest in the potential of prebiotics to increase mineral absorption from the gut. This has generally focused on calcium with a view to potentially increasing bone mineral density (Coxam 2007, Scholz-Ahrens & Schrezenmeir 2007). Most of the absorption of essential minerals from the diet takes place in the small intestine; in the case of calcium, approximately 95% is absorbed in the small intestine (Barger-Lux et al. 1989). Prebiotics may, however, have a benefit in maximizing the scavenging of the calcium that does enter the colon.

Much of our knowledge of the effects of prebiotics, mainly inulin and FOSs, on calcium absorption comes from animal studies, generally rats. These studies show that fructan consumption can increase calcium absorption (Ohta et al. 1998a,b; Scholz-Ahrens et al. 2001; Coudray et al. 2005, 2006).

There have been relatively few studies in humans (**Table 5**). The overall conclusion from these studies is that prebiotics do indeed increase calcium absorption, albeit by a small percentage. Further, it is apparent that some individuals do not respond to prebiotic consumption with an increased calcium absorption (Abrams et al. 2007, Holloway et al. 2007).

Although the mechanism for the effect of prebiotics on calcium absorption is not known with any certainty, there have been several hypotheses put forward (Coxam 2007). One such hypothesis is that fermentation of prebiotics increases passive absorption through the colonocytes. Fermentation of prebiotics to short-chain fatty acids reduces luminal pH, and this increases bioavailability of calcium. It is also possible that prebiotics may increase paracellular calcium transport via tight junctions; the nondigestible oligosaccharides melibiose and difructose anhydride have been found to open up tight junctions in rat intestinal epithelia (Mineo et al. 2004).

Table 5 Human studies on the effect of prebiotics on calcium absorption

Study population	Prebiotic[1]	Design	Outcome	Reference
Nine healthy young men	Inulin	Control diet compared to inulin or sugar beet fiber at 40 g/day	Significant increase in absorption and balance	(Coudray et al. 1997)
Twelve healthy young men	Inulin, FOSs, and GOSs	Treatments (15 g/day) compared to basal control diet for 21 days in a randomized crossover trial. Stable isotope method	No significant effect on calcium or iron absorption	(van den Heuvel et al. 1998)
Twelve healthy male adolescents	FOSs	Inulin (15 g/day) compared to sucrose for nine days in a randomized double-blind crossover study	Significant increase in calcium absorption	(van den Heuvel et al. 1999)
Twelve postmenopausal women	GOSs	GOSs (20 g/day) compared to sucrose for nine days in a double-blind randomized crossover study. Stable isotope method	Significant increase in calcium absorption	(van den Heuvel et al. 2000)
Fifty-nine healthy adolescent girls	FOSs and inulin + FOSs	Prebiotics or sucrose placebo (8 g/day) fed for three weeks in a randomized double-blind crossover study. Stable isotope method	Significant increase in calcium absorption with FOSs + inulin but not with FOSs	(Griffin et al. 2002)
Fifty-five healthy adolescent girls	Inulin + FOSs	Inulin + FOSs or sucrose placebo (8 g/day) fed for three weeks in a randomized double-blind crossover study. Stable isotope method	Significant increase in calcium absorption	(Griffin et al. 2003)
Twelve healthy post-menopausal women	sc-FOSs	FOSs (10 g/day) fed for five weeks in a randomized double-blind crossover study. Stable isotope method	No significant effect on calcium absorption seen	(Tahiri et al. 2003)
One hundred young adolesents	Inulin + FOSs	Inulin + FOSs or maltodextrin placebo (8 g/day) fed for twelve months in a placebo-controlled study. Stable isotope method	Significant increase in calcium absorption; significant increase in bone mineral content and bone mineral density after one year	(Abrams et al. 2005)
Thirteen young adults	Inulin + FOSs	Inulin + FOSs (8 g/day) eight weeks. Stable isotope kinetic study	Significant increase in calcium absorption in individuals who responded	(Abrams et al. 2007)
Fifteen post-menopausal women	Inulin + FOSs	Inulin + FOSs or maltodextrin (10 g/day) fed for six weeks in a double-blind placebo-controlled crossover study	Increased calcium absorption on prebiotic consumption; increased bone turnover markers in individuals who responded to prebiotic consumption	(Holloway et al. 2007)

[1]FOSs: fructo-oligosaccharides derived from inulin; sc-FOSs: short-chain fructo-oligosaccharides derived from sucrose; GOSs: galacto-oligosaccharides.

MANUFACTURE OF PREBIOTICS

Extraction from Biological Materials

Some prebiotics and candidate prebiotics are naturally present in plant materials. Fructans such as inulin can be readily extracted from sources such as chicory, the main industrial source, and agave. Soy oligosaccharides are extracted from soybeans. Extraction from an easily grown crop such as chicory provides an economic advantage for inulins as prebiotic products.

Inulin. Inulins are present in a variety of plant species, mainly Liliales such as garlic, asparagus, leek, and onion, and Compositae, primarily dahlia, Jerusalem artichoke, and chicory. Chicory (*Cichorium intybus*) is the principal commercial source of food grade inulin. The tubers contain approximately 15–20% (w/w) inulin, and this is extracted in a process similar to the extraction of sucrose from sugar beet (Smits 2003). Chicory roots are sliced into chips, then inulin is extracted with hot water. Carbonatation then removes proteinaceous and other contaminants. Raw inulin is then chromatographically demineralized and decolored prior to spray drying. In the Beneo-Orafti process, this produces inulin ST. This can then be fractionated to remove the fraction with a DP less than 10 to produce inulin HP, rich in high molecular weight chains.

Soy oligosaccharides. Soybean oligosaccharides (SOSs) are α-galactosyl sucrose derivatives (Crittenden & Playne 1996). SOSs are extracted directly from soybean whey. The main oligosaccharides contained are raffinose (Gal α1–6 Glc α1–2β Fru) and stachyose (Gal α1–6 Gal α1–6 Glc α1–2β Fru). The commercial product from Calpis Food Industry Company is a concentrated syrup, containing 75% (w/v) solids, 35% of which are SOSs (Crittenden & Playne 1996).

Manufacturing Routes by Polysaccharide Hydrolysis

It often seems to be the case that although polysaccharides are fermented by the colonic microbiota, the selectivity for health-promoting bacterial groups is increased by partial hydrolysis. For this reason, plant polysaccharides such as inulin, starch, and xylan can be considered as sources of prebiotics.

Fructans. Fructo-oligosaccharides can be manufactured by the hydrolysis of inulin, and this is the manufacturing route for the Beneo-Orafti range of FOS products. Chicory inulin is partially hydrolyzed by endo-inulinase (EC3.2.1.7) to produce a mixture of fructo-oligosaccharides with a DP of 2 to 7 with an average of 4. The oligosaccharides produced are largely nonglucose terminated and are reducing. The oligosaccharide product can also be spray dried with inulin HP to form a product known as Synergy 1. Synergy 1 has a wide distribution of short and long chains.

Xylo-oligosaccharides. The major commercial XOS products are manufactured from corn cobs by enzymatic hydrolysis using endo-xylanases (Vázquez et al. 2000). Xylan and arabinoxylan polysaccharides are, however, widely distributed in plants, and there are many more potential sources of XOSs that have not yet been developed commercially (Moure et al. 2006). One attractive source is wheat bran. Oligosaccharides derived from wheat arabinoxylans have been investigated for their prebiotic potential in vitro (Hughes et al. 2007). These enzymatically derived materials displayed a marked influence of molecular weight on fermentation selectivity with lower DP resulting in increased selectivity. Recently, wheat bran-derived XOSs with varying structures have been evaluated in rats for prebiotic potential (van Craeyveld et al. 2008). Low DP

oligosaccharides had a more pronounced selectivity for bifidobacteria, but higher DP fractions resulted in lower branched-chain fatty acids, suggestive of a shift away from protein metabolism. Optimum prebiotic properties were found with a fraction of DP of 5 and a degree of arabinose substitution of 0.27. Given the structure-function relationships found so far, it would seem fruitful to investigate the rational enzymatic tailoring of such molecules for optimum prebiotic activity. There is potential in developing economical autohydrolysis approaches for the manufacture of XOSs from brewery spent grains (Carvalheiro et al. 2004, 2005), but the prebiotic potential of such materials is not yet characterized.

Isomalto-oligosaccharides. The commercial IMOs are low molecular weight and are partially digested by the human small intestine (see above). Consequently, there is interest in generating higher molecular weight IMOs. One way of achieving this is by using industrial dextran as a starting material. Controlled hydrolysis of dextrans has been achieved through the use of membrane reactors. Batch membrane reactors have been used (Mountzouris et al. 1999a) to produce high molecular weight IMOs, or oligodextrans, using an endodextranase (Dextranase 50 liters from *Penicillium lilacinum*). Product molecular weight was controlled by the 10,000 nominal molecular weight cut-off (NMWCO) membrane and by varying the enzyme:substrate ratio. The products formed contain an oligosaccharide fraction (DP2–4) and a higher molecular weight fraction with average molecular weights in the range of 2 to 74 kDa. Similar products can also be manufactured on a continuous basis in enzyme membrane reactors (Mountzouris et al. 2002) with recycle of the high molecular weight substrate.

Manufacturing Routes by Enzymatic Glycosyl Transfer

The third general approach to manufacture of prebiotics involves enzyme-catalyzed transfer reactions. Typically, a readily available substrate such as sucrose or lactose is used, and a suitable glycosyltransferase or glycosidase enzyme is used to produce novel oligosaccharides.

Fructo-oligosaccharides. In addition to the inulin-derived manufacturing route described above, fructo-oligosaccharides can also be manufactured from sucrose by glycosyl transfer reactions (Yun 1996). In this case, sucrose acts as the glycosyl donor and as the glycosyl acceptor in competition with water (hydrolysis). The FOSs produced from sucrose have a much lower DP range (2–4) than inulin-derived FOSs and are frequently described as short-chain FOSs. The enzymatic synthesis route to FOSs was first developed in Japan using fructosyltransferase (EC 2.4.1.9) from *Aspergillus niger* ATCC 20611. The enzyme produced FOSs in a yield of 55–60% (w/w) and was used by Meiji Seika Co. to develop the commercial product, Neosugar. Industrial processes for the production of sc-FOSs are generally based on batch reactors with soluble or immobilized enzymes (Yun 1996). After the reaction is complete, sc-FOSs can be purified using chromatographic or membrane processes such as nanofiltration. There have been many reports of fructosyltransferases with the potential to manufacture prebiotics and to offer some process enhancements including thermophilic enzymes (Katapodis et al. 2003) and recombinant enzymes with exciting biocatalytic properties (Zuccaro et al. 2008).

Galacto-oligosaccharides. GOSs are also produced using glycosyl transfer reactions (Rustom et al. 1998). The biocatalyst is β-galactosidase (EC 3.2.1.23) and suitable industrial catalysts can be obtained from bacteria and fungi. In high concentrations of lactose, β-galactosidase catalyzes the transfer of the galactosyl moiety from lactose onto another lactose molecule as an acceptor. This reaction takes place in competition with the hydrolysis reaction. The reaction is kinetically

controlled, and so the optimum yield of products is dependent on lactose concentration and reaction time. Commercial GOS products are usually approximately 55% oligosaccharides with the balance being made up of lactose, glucose, and galactose (Crittenden & Playne 1996). The enzyme will transfer the galactosyl moiety onto any of the free hydroxyl groups on the lactose acceptor, depending on the specificity of the enzyme active site. Further, the products of the reaction then act as acceptors, potentially leading to very complex mixtures of linear and branched oligosaccharides in the DP range of 2–6. In reality, enzyme specificity and thermodynamic considerations act to restrict product spectrum.

There has been much activity in developing enzyme reactors for GOS synthesis (Boon et al. 2000), and a variety of batch and continuous systems using free and immobilized enzymes have been described in the literature. Although continuous systems have clear advantages in terms of efficiency, batch systems are more widely used as they are simpler, cheaper, and tend to be more robust in operation.

As discussed, GOS products can be very complex, and one of the major controlling factors of this complexity is the nature of the enzyme used in the synthesis (Rabiu et al. 2001). This has led to the intriguing possibility of generating GOS mixtures tailored toward particular probiotic bacteria. The hypothesis is that, if β-galactosidases from probiotic bacteria are used as the synthetic biocatalyst, the resultant GOS mixture will contain a mixture of linkages that will be efficiently hydrolyzed by the producing organism. This idea has been evaluated to some degree (Rabiu et al. 2001) using a selection of bifidobacteria. The bifidobacterial strains were used as whole cell catalysts to make GOS mixtures with varying linkage profiles. The growth rates of the same strains were then determined in pure culture. In most cases, the bifidobacteria had the highest growth rate on their own GOS product. The commercial product BiMuno, which is manufactured by Clasado, is synthesized by *Bifidobacterium bifidum* NCIMB 41171. Interestingly, most of the bifidobacterial isolates obtained from fecal samples obtained during a human volunteer trial on BiMuno were *B. bifidum* strains (Depeint et al. 2008). Further, the bifidobacterial isolates all had broadly similar growth rates on Vivinal GOS and BiMuno, with the exception of the *B. bifidum*, which had significantly higher growth rates on BiMuno.

Gluco-oligosaccharides. Sucrose can also be used as a substrate for the manufacture of α-linked gluco-oligosaccharides with prebiotic potential. Glucosyl transferases such as dextransucrase and alternansucrase can be used to synthesize high molecular weight polymers such as dextran and alternan (Koepsell et al. 1953, Monchois et al. 1999). In aqueous solution, these enzymes will build high molecular weight polymers from sucrose. In the presence of oligosaccharides as alternative glycosyl acceptors to water, however, they catalyze the synthesis of oligosaccharides (Côté & Fobyt 1982). Dextransucrase from *Leuconostoc mesenteroides* NRRL B-1299 has been used to synthesize oligosaccharides using maltose as an acceptor (Sanz et al. 2006a). The oligosaccharides have maltose as the reducing sequence and a combination of α-1,6 and α-1,2-linked glucosyl sequences at the nonreducing end. These structures are exciting candidate prebiotics (see above) and are currently under evaluation.

Isomalto-oligosaccharides. IMOs are manufactured commercially from starch by enzymatic transfer reactions (Crittenden & Playne 1996). Starch is first hydrolyzed to a mixture of malto-oligosaccharides using α-amylase (EC 3.2.1.1) together with pullulanase (EC 3.2.1.41) to remove branching residues and produce linear maltodextrins. The maltodextrins are then used as substrates in a glycosyl transfer reaction catalyzed by α-glucosidase (EC 3.2.1.20), which converts the α-1,4 linked maltodextrins into α-1,6 linked isomalto-oligosaccharides. Glucose is then removed from the product.

Sucrose has the potential to be an alternative substrate to starch for the manufacture of IMOs. Dextransucrase together with dextranase (EC 2.4.1.5) have been used in a recycle membrane reactor fed with sucrose to produce IMOs of varying molecular weight (Goulas et al. 2004). The dextransucrase builds α-1,6-linked dextran polymers from the sucrose that are then partially hydrolyzed by the dextranase. Products from the reactor contain a mixture of branched and linear IMOs with DP values from 2 to more than 27. By controlling the ratio of the two enzymes and the enzyme:substrate ratio, some degree of control over the DP distribution is possible.

Chemical Synthesis

Lactulose is unusual among prebiotics as it is the only one manufactured by chemical synthesis (Timmermans 2007). It is manufactured by a Lobry de Bruyn-Alberda van Ekenstein isomerization of lactose catalyzed by sodium hydroxide or borate, converting the glucosyl moiety into fructose. Solvay is the largest manufacturer of lactulose in Europe.

FUTURE PERSPECTIVES

The Evolving Definition of Prebiotics

Since the first definition of prebiotics in 1995 as "a nondigestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health," (Gibson & Roberfroid 1995) there have been several evolutionary changes in our view of the concept. Early work in this field tended to rely on culture-based microbiological methods involving selective media and subsequent speciation. The focus was firmly on bifidobacteria and lactobacilli as the most health-significant members of the gut microbiota. The rapid deployment of molecular microbiology methods in the late 1990s led to a significant increase in our appreciation of the complexity of the gut microbiota as well as an improvement in our ability to experimentally characterize the microbiological consequences of prebiotic fermentation (Blaut et al. 2002, McCartney 2002, Flint et al. 2007). It became apparent that an undue focus on bifidobacteria and lactobacilli was no longer appropriate, and it seems certain that other health-positive bacteria will be identified in the future. More recently, researchers have begun to unravel at least some of the functional and nutritional interactions between members of the colonic ecosystem (Dethlefsen et al. 2006; Flint et al. 2007; Falony et al. 2009a,b), and this is providing us with new outcomes to target in prebiotic interventions. This coincides with a new focus on the products of bacterial metabolism as important indicators of prebiotic action (Flint et al. 2007). Our understanding of the biological properties of metabolites is increasing, as is our ability to determine the systemic metabolic consequences of prebiotic action through metabonomic approaches (Waldram et al. 2009).

Consequently, there have recently been attempts to redefine prebiotics. In 2004, the definition was modified slightly to remove the criterion of nondigestibility and to encompass the gastrointestinal tract rather than specifically the colon. Well-being was also included as an outcome (Gibson et al. 2004), and a prebiotic was defined as "a selectively fermented ingredient that allows specific changes both in the composition and/or activity in the gastrointestinal microflora that confers benefits upon host well being and health."

In 2007, an FAO technical meeting sought to redefine the term prebiotic as "a nonviable food component that confers a health benefit on the host associated with modulation of the microbiota." This is a considerably broader definition than the earlier ones and is significant in that it does not specify that a prebiotic should be selectively metabolized by members of the gut microbiota. This leads to the rather unhelpful situation in which any dietary fiber could be classed as a prebiotic.

The most recent attempt to define prebiotics took place at the November 2008 Meeting of the International Scientific Association of Probiotics and Prebiotics (ISAPP) in London, Ontario. A working group of 22 international experts define a prebiotic as "a dietary prebiotic is 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." This new definition reaffirms that prebiotics act by inducing specific changes but allows changes in wording such that the term could be used for extraintestinal applications.

SUMMARY POINTS

1. Prebiotic oligosaccharides are gaining increasing recognition as agents to modulate the colonic microbiota in humans and animals.
2. The current range of prebiotics have varying degrees of robust data to support their prebiotic status, and there is a need for more large scale, well designed human trials with clearly defined outcomes using modern molecular microbiological methods.
3. Accumulating data are supporting the role of prebiotics in dietary intervention in several disease conditions, including colon cancer, acute infections, and inflammatory bowel disease. They also have potential in promoting mineral scavenging from the colon with potential long-term health benefits.
4. There is great potential for the generation of new prebiotics from plant biomass sources.

FUTURE ISSUES

1. What are the long-term consequences of prebiotic consumption? Most feeding trials are short term, and there are very few data on long-term intake.
2. Can we rationally design oligosaccharides for specific health benefits? We do not currently have a sound understanding of the structure-function effects in prebiotic oligosaccharides or the mechanisms behind their specific metabolism. With this knowledge and developments in enzyme engineering, it may be possible to produce designer prebiotics in the future.
3. Which microbiological outcomes should prebiotics target? We currently have a relatively poor understanding of the functional ecology of the human colon. It seems certain that as our knowledge improves interventions with prebiotics will become more sophisticated.

DISCLOSURE STATEMENT

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Errata

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