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Dietary modulation of the human colonic microbiota: updating the concept of prebiotics

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Prebiotics are non-digestible (by the host) food ingredients that have a beneficial effect through their selective metabolism in the intestinal tract. Key to this is the specificity of microbial changes. The present paper reviews the concept in terms of three criteria: (a) resistance to gastric acidity, hydrolysis by mammalian enzymes and gastrointestinal absorption; (b) fermentation by intestinal microflora; (c) selective stimulation of the growth and/or activity of intestinal bacteria associated with health and wellbeing. The conclusion is that prebiotics that currently fulfil these three criteria are fructo-oligosaccharides, galacto-oligosaccharides and lactulose, although promise does exist with several other dietary carbohydrates. Given the range of food vehicles that may be fortified by prebiotics, their ability to confer positive microflora changes and the health aspects that may accrue, it is important that robust technologies to assay functionality are used. This would include a molecular-based approach to determine flora changes. The future use of prebiotics may allow species-level changes in the microbiota, an extrapolation into genera other than the bifidobacteria and lactobacilli, and allow preferential use in disease-prone areas of the body.

Prebiotics: Criteria for prebiotic classification: Gut flora: Oligosaccharides

Introduction

A prebiotic was first defined as a 'non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health' (Gibson & Roberfroid, 1995). However, a prebiotic effect has been attributed to many food components, sometimes without due consideration to the criteria required. In particular, many food oligosaccharides and polysaccharides (including dietary fibre) have been claimed to have prebiotic activity, but not all dietary carbohydrates are prebiotics. There is, therefore, a need to establish clear criteria for classifying a food ingredient as a prebiotic. Such classification requires a scientific demonstration that the ingredient:

(1) resists gastric acidity, hydrolysis by mammalian enzymes and gastrointestinal absorption;
(2) is fermented by the intestinal microflora;
(3) stimulates selectively the growth and/or activity of intestinal bacteria associated with health and wellbeing.

As with functional foods or ingredients (Diplock *et al*. 1999), the final demonstration should be carried out *in vivo*, through appropriate nutritional feeding trials in the targeted species (i.e. man, livestock or companion animals). The methodologies used must be validated and supported by sound science.

Although each of these criteria is important, the third, concerning the selective stimulation of growth and/or activity of bacteria, is the most contentious and difficult to fulfil. Indeed, it requires anaerobic sampling followed by reliable and quantitative microbiological analysis of a wide variety of bacterial genera, for example, total aerobes and anaerobes, *Bacteroides*, *Bifidobacterium*, *Clostridium*, enterobacteria, *Eubacterium*, and *Lactobacillus*. Simply reporting fermentation in pure cultures of single microbial strains or an increase in a limited number of bacterial genera in complex mixtures of bacteria (for example, faecal slurries) either *in vitro* or *in vivo* cannot confirm a prebiotic effect. This is because it does not take bacterial interactions into account. Molecular-based microbiological methodologies have been

Abbreviations: DP, degree of polymerisation; Dp_{av}, average degree of polymerisation; FISH, fluorescence *in situ* hybridisation; $G_{py}F_n$, α-D-glucopyranosyl-[-β-D-fructofuranosyl]$_{n-1}$-β-D-fructofuranoside; IMO, isomalto-oligosaccharides; TOS, transgalacto-oligosaccharides; XOS, xylo-oligosaccharides.

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developed and should be applied to prebiotic demonstrations (as discussed later; p. 261).

Regarding the stimulation of bacterial activity, the patterns of production of organic acids, gases and enzymes have been used. However, these have not been validated as biomarkers of specific bacterial genera.

In light of these criteria and the aforementioned considerations, the aim of the present paper is to review and discuss methodologies to demonstrate scientifically a prebiotic effect as well as to evaluate evidence available for proving the prebiotic nature of candidate ingredients (hitherto these are all carbohydrates). The present paper also updates the initial definition of a prebiotic and reviews the status 8 years from its first introduction (Gibson & Roberfroid, 1995). The information in the present review is based upon literature searches last updated in July 2003.

Testing methodologies

It is apparent that if good-quality and biologically meaningful data are to be collected on different prebiotics, standardised testing methodologies are needed. We have suggested a scheme for the evaluation of a candidate prebiotic (Gibson *et al*. 1999). Such rigorous testing of candidate molecules is essential if we are to have confidence in any health claims made by manufacturers of functional foods. It is important also that the rationale behind the prebiotic effect is elucidated through mechanistic explanations of the effect. In this context, several genes specific for oligosaccharide metabolism have been identified in bifidobacteria to help explain the selective action of prebiotics that is clearly an integral part of the overall prebiotic effect (Schell *et al*. 2002).

Non-digestibility: testing prebiotic resistance to gastric acidity, hydrolysis by mammalian enzymes and gastrointestinal absorption

This first criterion must be fulfilled and can be demonstrated both *in vitro* and *in vivo*.

In vitro *methods*. *In vitro* demonstration includes determining resistance to acidic conditions (for example, those that occur in the stomach) and enzymic (saliva, pancreatic and small intestinal) hydrolysis (Oku *et al*. 1984; Ziesenitz & Siebert, 1987; Nilsson & Bjorck, 1988; Molis *et al*. 1996). After an appropriate incubation, hydrolysis products are assayed chemically or enzymically using standard methods (Dahlqvist & Nilsson, 1984).

In vivo *models*. Resistance to any endogenous digestive process can be shown by measuring the recovery in faeces of an oral dose given to germ-free rats or after antibiotic pre-treatment to suppress the intestinal flora (Nilsson *et al*. 1988). Other, more invasive methods involve intubation into the gastrointestinal system of living anaesthetised rats (Nilsson *et al*. 1988).

Models applicable to man involve either the direct recovery of non-digested molecules or an indirect assessment that neither glycaemia nor insulinaemia are increased significantly following oral administration. Direct approaches include either oral intubation to allow distal ileum fluid sampling (Molis *et al*. 1996) or the use of individuals who have been subjected to proctocolectomy, the so-called ileostomy patients (Bach Knudsen & Hessov, 1995; Ellegard *et al*. 1997). This model is widely accepted as a valuable alternative to study the small-intestinal excretion of nutrients (Langkilde *et al*. 1990; Cummings & Englyst, 1991). The intubation technique, with an unabsorbable marker, is used to quantitatively assess ileal flow (Phillips & Giller, 1973; Levitt & Bond, 1977).

Fermentation by intestinal microflora

In vitro *methods*. The most commonly used *in vitro* models to study anaerobic fermentation of carbohydrates by mixed bacterial populations, particularly faecal bacteria, are batch and continuous culture fermentation systems. Batch culture fermenters are inoculated with either pure culture(s) of selected species of bacteria or, preferably, with a faecal slurry and the carbohydrate to be studied.

Multi-chamber continuous culture systems have been developed to reproduce the physical, anatomical and nutritional characteristics of gastrointestinal regions (Macfarlane *et al*. 1998; Gmeiner *et al*. 2000). These models are useful for predicting both the extent and site of prebiotic fermentation.

In vivo *methods*. The *in vivo* fermentation of non-digestible carbohydrates can be studied in laboratory and companion animals, in livestock and in human subjects.

In laboratory animals, often rats, the prebiotic under investigation is added to food or drinking water but can be administered by gastric intubation. Animals are then anaesthetised and killed at pre-determined time points. Faecal samples, and the contents of the gastrointestinal segments, are collected for analysis. One interesting model by which to study carbohydrate fermentation in experimental animals is the heteroxenic rat harbouring a human faecal flora.

To study the fermentation of dietary carbohydrates in human subjects, two major approaches are used. The first is indirect, in which exhaled air is collected at regular time intervals to measure the concentration of gases, essentially H_2, in volunteers previously given a single oral dose of the carbohydrate (Christl *et al*. 1992). The other approach consists of collecting faeces after oral feeding and measuring the recovery of the test carbohydrate.

Selective stimulation of growth and/or activity of intestinal bacteria

As the field of prebiotics has developed, so has the methodology for investigating functionality; in particular, flora compositional changes as a response to the selective fermentation. Much of the early (and some of the current) literature describes studies performed on pure cultures. Typically, this involves the selection of a range of strains of *Bifidobacterium* spp., *Lactobacillus* spp. and other bacteria such as *Bacteroides* spp., *Clostridium* spp., *Eubacterium* spp. and *Escherichia coli*. The number of strains tested varies with different reports. The problem with this

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approach is, of course, that the strains selected cannot truly be considered as representative of the colonic microbiota. This is further compounded in some studies as authors have used a wide range of bifidobacteria and lactobacilli but only one or two strains of the 'undesirable' species. Such studies cannot establish that the test carbohydrate is metabolised selectively and should be used for initial screening purposes only.

A more meaningful *in vitro* method for studying prebiotic oligosaccharides is the use of faecal inocula, which ensures that a representative range of bacterial species is exposed to the test material. Study of the changes in populations of selected genera or species can then establish whether the fermentation is selective. The use of faeces probably gives an accurate representation of events in the distal colon. However, more proximal areas will have a more saccharolytic nature, and both the composition and activities of the microbiota indigenous to the colon are variable, dependent upon the region being sampled. This has been confirmed through studies on sudden-death victims, where the colon contents were sampled shortly following death (Macfarlane *et al*. 1992, 1998). The complex gut models, which replicate different anatomical areas, attempt to overcome this and should be used in concert with human trials.

Culture on selective media. One major problem with the use of faecal inocula is identification of the genera and species present. Traditionally, this has been accomplished by culturing on a range of purportedly selective agars followed by morphological and biochemical tests designed to confirm culture identities (van Houte & Gibbons, 1966; Finegold *et al*. 1974). This approach is adequate to establish that a prebiotic selectively enriches defined 'desirable' organisms and depletes 'undesirable' organisms but does not give a true picture of the population changes occurring. This is unavoidable when using a selective culture, as it is estimated that only about 50 % of the diversity present in the human colon has yet been characterised (Suau *et al*. 1999).

A much more reliable approach involves the use of molecular methods of bacteria identification. These have advantages over culture-based technologies in that they have improved reliability and can encompass the full flora diversity. Examples of the molecular procedures are given below.

Fluorescence in situ *hybridisation*. Modern techniques are now available whereby bacterial enumeration can be carried out in a quick, culture-independent and reliable manner such as fluorescence *in situ* hybridisation (FISH). This technique involves the use of group-specific (and in some cases species-specific) oligonucleotide probes that target discrete discriminatory regions of the rRNA molecule. By targeting highly conserved areas of the rRNA, specific groups of bacteria can be distinguished from others in a mixed culture.

A host of phylogenetic probes are currently available for the enumeration of faecal bacteria, whilst more are being designed and validated. Groups targeted include *Bacteroides* spp. (Manz *et al*. 1996), *Bifidobacterium* spp. (Langendijk *et al*. 1995) and *Lactobacillus* and *Enterococcus* spp. (Harmsen *et al*. 1999), *Eubacterium* (Franks *et al*. 1998), and *Clostridium* (Tuohy *et al*. 2001).

As well as being a relatively quick technique, this method removes the ambiguity that is a prominent feature of traditional selective agars. Additionally, FISH provides a means through which hitherto unculturable bacterial species of the gut may be investigated, since this is a culture-independent technique and therefore does not require prior, often anaerobic, growth of an organism upon laboratory media (Liesack & Stackebrandt, 1992).

Polymerase chain reaction. Due to the ambiguity inherent in using purportedly selective agars, only tenuous identifications of bacteria can be made using this methodology. Bacterial ribosomes offer the means by which identifications can be made at a molecular level. The genes that code for the 16S subunits of the bacterial ribosomes are comprised of both conserved and variable regions, and sequencing of the 16S rRNA gene enables bacterial identifications to be made. By using a process known as PCR, segments of this gene can be amplified to a level whereby their sequence can be determined (Steffan & Atlas, 1991).

Direct community analysis. Characterisation of both the culturable and non-culturable components of a microbial mixture may be achieved via direct community analysis. This process characterises the 16S rRNA diversity of the sample of interest. The total bacterial DNA is extracted from the sample and partial 16S rDNA genes are amplified (using universal primers) via PCR (Suau *et al*. 1999). The purified amplification products are subsequently cloned into *E. coli*, and clones containing the 16S rDNA inserts are sequenced and identified by comparison with database 16S rDNA sequences.

Denaturing and temperature-gradient gel electrophoresis. Another method to evaluate the genetic diversity of the intestinal microflora is denaturing gradient gel electrophoresis or temperature-gradient gel electrophoresis. These approaches separate amplified DNA fragments of the same size based on the extent of the sequence divergence between different PCR products (Muyzer & Smalla, 1998). A whole community PCR is carried out and partial 16S rDNA sequences are amplified from the different bacterial species present. Separation occurs due to the decreased electrophoretic mobility of the partially melted, double-stranded DNA molecule in polyacrylamide gels containing either a temperature or chemical denaturant gradient (Muyzer & Smalla, 1998). Identification can be carried out either by excising fragments from the gel and sequencing them, or by comparing their motility with that of known control sequences. As with FISH, both culturable and unculturable populations can be characterised and this relatively rapid technique also offers the potential of monitoring gut flora over time (Zoetendal *et al*. 1998). Table 1 summarises the principal techniques used for evaluating bacterial populations in faeces, along with some of their advantages and disadvantages.

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Table 1. Principal methodologies employed to enumerate colonic bacteria

Method	Advantages	Disadvantages
Selective culturing and biochemical characteristics	Straightforward, relatively inexpensive, a large number of replicates can be carried out	Operator subjectivity, applicable only to culturable bacteria, selectivity of media is ambiguous, metabolic plasticity of organisms may introduce error
Fluorescence *in situ* hybridisation	Can be used on unculturable as well as culturable bacteria, highly specific	Can probe only for known bacteria, more time-consuming than culture procedures
PCR	High fidelity, reliable, allows placement of previously unidentified bacteria, can be used for unculturable bacteria	Expensive, time-consuming. Some bias in the PCR process
Direct community analysis	Culture independent, the diversity of entire samples can be elucidated	Some loss of bacterial diversity due to the bias introduced by PCR
Denaturing and temperature-gradient gel electrophoresis	Rapid, can be used for both culturable and unculturable bacteria	Qualitative rather than quantitative, bias introduced by the PCR process may lead to a loss of diversity

Flow cytometry. Flow cytometry can be used to quantify bacteria using the FISH procedure. However, in the hands of the authors of the present review it has been problematic for assaying complex communities such as mixed faecal culture. This is because a great deal of background 'noise' exists. However, the throughput currently seen for pure or co-culture studies may eventually be realised for complex gut communities through further refinement of the flow cytometry techniques and improved discrimination as a result.

Review of candidate prebiotics

For each candidate, a brief introduction will give a description of the chemistry and manufacturing process followed by a review of the data available to fulfil the three criteria for prebiotic classification, which are:

(1) resistance to gastric acidity, hydrolysis by mammalian enzymes and gastrointestinal absorption;
(2) fermentation by intestinal microflora;
(3) selective stimulation of the growth and/or activity of intestinal bacteria associated with health and wellbeing.

Inulin

Chemistry, nomenclature and manufacture of inulin. From a chemical point of view, the linear chain of inulin is either an α-D-glucopyranosyl-[-β-D-fructofuranosyl]$_{n-1}$-β-D-fructofuranoside ($G_{py}F_n$) or a β-D-fructopyranosyl-[β-D-fructofuranosyl]$_{n-1}$-β-D-fructofuranoside. The fructosyl–glucose linkage is always β(2↔1) as in sucrose, but the fructosyl–fructose linkages are β(1←2).

Chicory inulin is composed of a mixture of oligomers and polymers in which the degree of polymerisation (DP) varies from two to approximately sixty units with an average DP (DP_{av}) of twelve. About 10 % of the fructan chains in native chicory inulin have a DP ranging between two (F_2) and five (GF_4). The partial enzymic hydrolysis of inulin using an endo-inulinase (*EC* 3.2.1.7) produces oligofructose, which is a mixture of both $G_{py}F_n$ and β-D-fructopyranosyl-[β-D-fructofuranosyl]$_{n-1}$-β-D-fructofuranoside molecules, in which the DP varies from two to seven with a DP_{av} of four. Oligofructose can be obtained by enzymic synthesis (transfructosylation) using the fungal enzyme β-fructosidase (*EC* 3.2.1.7) from *Aspergillus niger*. In such a synthetic compound, the DP varies from two to four with a DP_{av} of 3·6, and all oligomers are of the $G_{py}F_n$ type. By applying specific separation technologies, the food industry also produces a long-chain inulin known as high-polymer inulin (DP of ten to sixty) with a DP_{av} of twenty-five. Finally, by mixing oligofructose and long-chain inulin, specific products known as Synergy® (Orafti NV, Tienen, Belgium) have been developed. The different industrial products vary in DP_{av}, maximum DP, and DP distribution, and they have varying properties (Franck, 2002).

Inulin is a generic term that covers all β(1←2) linear molecules. In any circumstances that justify the identification of the oligomers *v.* polymers, the terms oligofructose and/or inulin can be used, respectively. Even though the inulin hydrolysate and the synthetic compound have a slightly different DP_{av} (four and 3·6, respectively), the term oligofructose can be used to identify both. Indeed, oligofructose and fructo-oligosaccharides are considered to be synonymous names for the mixture of small inulin oligomers with maximum DP of less than ten (Quemener, 1994; Roberfroid *et al*. 1998; Coussement, 1999; Roberfroid, 2002).

Criterion 1: resistance to gastric acidity, hydrolysis by mammalian enzymes and gastrointestinal absorption. The resistance of inulin to digestive processes has been studied extensively by applying all the methods (both *in vitro* and *in vivo*) described earlier (p. 260) in the section regarding testing methodologies. Inulin is a non-digestible oligosaccharide that, for nutritional labelling, is classified as dietary fibre.

Criteria 2 and 3: fermentation by intestinal microflora and selective stimulation of the growth and/or activity of intestinal bacteria associated with health and wellbeing. *In vitro* data supporting the selective stimulation of bacterial growth by inulin has been generated in numerous studies that are summarised in Table 2. This has been carried out in defined pure culture fermentation and by using a mixed faecal inocula in both batch and continuous culture (Wang & Gibson, 1993; Gibson & Wang, 1994*a*; Roberfroid *et al*. 1998).

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As well as *in vitro* work, *in vivo* studies have been carried out using animal models, for example with germ-free rats associated with a human faecal flora. A bifidogenic effect was observed in rats fed oligofructose, whilst lactobacilli were mostly increased in rats fed oligofructose alone or a mixture of oligofructose and inulin. This same mixture led to smaller numbers of clostridia, whilst short-chain fructo-oligosaccharides and/or inulin increased the relative proportion of butyrate (Levrat *et al*. 1991; Campbell *et al*. 1997; Kleessen *et al*. 2001; Poulsen *et al*. 2002).

Human trials with oligofructose and inulin include those with a controlled diet, and cross-over feeding trials, although the dose, substrate, duration and volunteers vary (Mitsuoka *et al*. 1987; Gibson *et al*. 1995; Buddington *et al*. 1996; Kleessen *et al*. 1997*b*; Bouhnik *et al*. 1999; Menne *et al*. 2000; Tuohy *et al*. 2001) (Table 3).

The efficacy of inulin has been evaluated with a view to its administration to formula-fed infants (Coppa *et al*. 2002). Moro *et al*. (2002) observed an increase in bifidobacteria and lactobacilli in infants who received formula milk supplemented with a mixture of inulin and galacto-oligosaccharides, indicating its prospects in infant nutrition.

In these *in vivo* trials, there were large variations between the subjects in their microflora compositions and response to the substrates (Hidaka, 1986; Williams *et al*. 1994), particularly between Western and Eastern subjects (Buddington *et al*. 1996). Another general observation was the decrease in bifidobacteria once administration of the oligofructose and inulin ceased (Bouhnik, 1994; Gibson *et al*. 1995; Buddington *et al*. 1996).

Conclusion. Together, the evidence available today both from *in vitro* and *in vivo* experiments supports the classification of inulin and oligofructose as prebiotic, since they fulfil all three criteria.

Transgalacto-oligosaccharides

Chemistry and manufacture of transgalacto-oligosaccharides. Transgalacto-oligosaccharides (TOS) are a mixture of oligosaccharides derived from lactose by enzymic transglycosylation (Crittenden, 1996). The product mixtures depend upon the enzymes used and the reaction conditions. They generally consist of oligosaccharides from tri- to pentasaccharide with β(1→6), β(1→3) and β(1→4) linkages (Matsumoto *et al*. 1993). This diversity must be borne in mind when considering some of the early studies on these materials; different studies have almost certainly used oligosaccharide mixtures with different compositions. It is thus essential that the exact composition of the mixture be given in reports of studies.

Criterion 1: resistance to gastric acidity, hydrolysis by mammalian enzymes and gastrointestinal absorption. The data on non-digestibility do not fully match the criteria. However, there are suggestions that TOS do reach the colon intact (Tomomatsu, 1994).

Criteria 2 and 3: fermentation by intestinal microflora and selective stimulation of the growth and/or activity of intestinal bacteria associated with health and wellbeing. An early study by Minami *et al*. (1983) examined the fermentation of 'isogalactobiose' of unknown linkage and found that one strain of each of *Bifidobacterium infantis*, *B. longum*, *B. adolescentis* and *Lactobacillus acidophilus* metabolised it, whilst one strain each of *Enterococcus fecalis* and *E. coli* could not. A more extensive study found that many strains of enteric bacteria could not metabolise the isogalactobiose. Tanaka *et al*. (1983) also studied enzymically synthesised TOS in a pure culture study. These authors found that all of the bifidobacteria tested, all of the bacteroides, most lactobacilli and enterobacteria and some streptococci

Table 2. Studies carried out demonstrating the *in vitro* selectivity of inulin in pure culture, mixed batch culture and mixed continuous culture fermentation

Study	Observations	Reference
Examining the growth of bifidobacteria on different types of oligofructose in pure culture. Eight species tested as well as species of *Clostridium*, *Bacteroides*, *Enterococci* and *Escherichia coli*	Linear oligofructose had more of a bifidogenic effect than greater molecular-mass molecules and branched-chain varieties. *Bifidobacterium* species showed a preference for fructans compared with glucose	Gibson & Wang (1994*b*)
Species of *Bifidobacterium* (*longum*, *breve*, *pseudocatenulatum*, *adolescentis*) were tested in pure culture for their ability to ferment oligofructose	*B. adolescentis* was seen to grow best and was able to metabolise both short- and long-chain oligofructose	Marx *et al.* (2000)
The ability of *Bifidobacterium* and *Lactobacillus* to grow on MRS agar containing oligofructose was investigated	Seven out of eight bifidobacteria and twelve out of sixteen lactobacilli were able to grow on agar containing oligofructose	Kaplan & Hutkins (2000)
Batch culture using faecal inocula to study fermentation of inulin, oligofructose, starch, polydextrose, fructose and pectin	*Bifidobacteria* most increased with oligofructose and inulin whilst populations of *E. coli* and *Clostridium* were maintained at relatively low levels	Wang & Gibson (1993)
Batch culture using faecal inocula to study fermentation of oligofructose, branched fructan, levan, maltodextrin	Fluorescence *in situ* hybridisation revealed that branched fructan had the best prebiotic effect, followed by oligofructose	Probert & Gibson (2002)
Continuous culture fermentation to study fermentation of oligofructose	Selective culturing showed *Bifidobacterium* and, to a lesser extent, *Lactobacillus*, preferred oligofructose to inulin and sucrose. *Bacteroides* could not grow on oligofructose	Gibson & Wang (1994*b*)

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Table 3. Studies carried out demonstrating the *in vivo* selective stimulation of bacterial growth by inulin and oligofructose in human feeding trials

Study	Observations	Reference
Twenty-three subjects fed 8 g oligofructose/d for 2 weeks	Increase in faecal bifidobacteria by about ten times and decrease in stool pH	Mitsuoka *et al*. (1987)
Eight subjects on a controlled diet were fed 15 g oligofructose/d for 15 d, then four of these subjects were fed 15 g inulin/d for 15 d	Selective agars showed that oligofructose increased faecal bifidobacteria and decreased *Bacteroides*, clostridia and fusobacteria. Inulin increased bifidobacteria and decreased Gram-positive cocci	Gibson *et al*. (1995)
Twenty subjects were fed 12·5 g oligofructose/d for 12 d	Significant increase in bifidobacteria by about ten times was demonstrated on selective agars	Bouhnik *et al*. (1996)
Twelve young subjects were administered 4 g oligofructose/d for a period of 2 weeks	The bifidobacteria increased by 0·8 log unit	Buddington *et al*. (1996)
Ten female elderly subjects were given 20 and 40 g inulin/d	On selective agars a tenfold increase in bifidobacteria and significant decreases in *Bacteroides* were observed	Kleessen *et al*. (1997*b*)
Oligofructose (2·5, 10, and 20 g/d) fed for 7 d in a trial involving forty subjects	Selective agars showed that bifidobacteria were most increased by 10 and 20 g doses of oligofructose compared with 2·5 g and that the optimum dose of oligofructose was found to be 10 g/d	Bouhnik *et al*. (1999)
Chicory inulin hydrosylate (8 g/d) fed to eight subjects in a controlled feeding study	Selective agars showed an increase in faecal bifidobacteria	Menne *et al*. (2000)
Controlled feeding study where up to 34 g inulin/d were given to eight subjects for a period of 2 months	FISH revealed an increase in bifidobacteria from 9·8 to 11·0 $\log_{10}$ cells/g dry faeces. The effect lasted for the whole 2 months that the volunteers received the prebiotic	Kruse *et al*. (1999)
Oligofructose (5 g/d) was given to eight young and healthy volunteers for a period of 3 weeks	By means of selective agars, an increase in faecal bifidobacteria was observed	Rao (2001)
Biscuits containing oligofructose and partially hydrolysed guar gum and placebo biscuits were fed to thirty-one subjects for two 21 d cross-over periods	FISH revealed an increase in faecal bifidobacteria	Tuohy *et al*. (2001)
Nineteen elderly patients fed 8 g oligofructose/d for 3 weeks	Increase in faecal bifidobacteria of approximately 2·8 log cfu/g of faeces	Guigoz *et al*. (2002)
Fourteen adult volunteers were given 9 g long-chain inulin/d for a period of 2 weeks	Quantification of all bacteria, bifidobacteria, the Erec group, *bacteroides*, and *Eubacterium* were counted with FISH probes. A significant increase in bifidobacteria and a significant decrease in the Erec group was observed	Harmsen *et al*. (2002)

FISH, fluorescence *in situ* hybridisation; Erec, *Eubacterium rectale – Clostridium coccoides*.

metabolised the TOS, with bifidobacteria displaying the most vigorous growth. However, the available *in vitro* data do not fully demonstrate a selective stimulation of bacterial growth.

In a study by Rowland & Tanaka (1993), gnotobiotic rats inoculated with human faecal flora were fed a TOS-containing diet before being killed. Caecal contents analysed on selective agars revealed significant increases in bifidobacteria and lactobacilli, and a significant decrease in enterobacteria. Bifidobacteria decreased as a percentage of total anaerobes, suggesting growth of other anaerobic bacteria not enumerated by the selective agars. These authors found significant decreases in nitrate reductase and β-glucuronidase.

This was followed by an *in vivo* volunteer feeding study that showed significant increases in bifidobacteria. This study, however, fed subjects for only 1 week per dose and there was no reported washout period between treatments.

More recently, Bouhnik *et al*. (1997) found a significant increase in faecal bifidobacteria whilst populations of enterobacteria did not change following TOS feeding. Ito *et al*. (1990) fed TOS to male volunteers and found significant increases in bifidobacteria and lactobacilli. Similarly, Ito *et al*. (1993) found a significant increase in bifidobacteria and lactobacilli, and significant decreases in *Bacteroides* and *Candida*. They found significant decreases in ammonium, cresol, indole, propionate, valerate, isobutyrate and isovalerate, but no change in acetate or butyrate.

Infant formula milk supplemented with a mixture of oligosaccharides (90 % galacto-oligosaccharide and 10 % inulin) has been shown to increase faecal bifidobacteria in both preterm and term infants (Dubey & Mistry, 1996; Knol, 2001; Rivero-Urgell & Santamaria-Orleans, 2001; Boehm *et al*. 2002; Moro *et al*. 2002; Vandenplas, 2002).

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

Lactulose

Chemistry and manufacture of lactulose. Lactulose is manufactured by the isomerisation of lactose to generate the disaccharide galactosyl β-(1→4)fructose. It has found widespread application in the medical world as a laxative (Tamura, 1983).

Criterion 1: resistance to gastric acidity, hydrolysis by mammalian enzymes and gastrointestinal absorption. Investigations of the enzymic degradation of lactulose have found that human and calf intestinal β-galactosidases did not degrade lactulose (Gibson & Angus, 2000).

Criteria 2 and 3: fermentation by intestinal microflora and selective stimulation of the growth and/or activity of intestinal bacteria associated with health and wellbeing. One of the earliest studies on lactulose fermentation was that conducted by Sahota *et al*. (1982), who used thirty-seven species of bacteria in pure culture. They found that *Bacteroides oralis*, *Bacteroides vulgatus*, *Bifidobacterium bifidum*, *Clostridium perfringens*, *L. casei* sub. *casei* and four other strains of *Lactobacillus* spp. fermented lactulose. The *in vitro* data presently available do not demonstrate a selective stimulation of bacterial growth.

Tomoda *et al*. (1991) fed yoghurt made with lactulose to healthy volunteers. Faecal samples were analysed on 'selective' agars. These authors found a significant increase in bifidobacteria but no total anaerobic count was performed and no other bacteria were enumerated, providing no evidence of selective stimulation of growth.

A more microbiologically rigorous study was subsequently performed by Terada *et al*. (1993). Faecal samples were again analysed on agars as well as for enzymes and putrefactive products. Selective and significant increases in bifidobacteria and decreases in *C. perfringens*, streptococci, bacteroides and lactobacilli were found.

A study by Ballongue *et al*. (1997) provided more evidence for a prebiotic effect for lactulose. In a parallel-group, randomised, double-blind, placebo-controlled trial, significant increases in *Bifidobacterium*, *Lactobacillus* and *Streptococcus* were found concomitant with significant decreases in *Bacteroides*, *Clostridium*, coliforms and *Eubacterium*. Concentrations of acetate and lactate were increased, whilst concentrations of butyrate, propionate and valerate decreased. All of the enzyme activities measured were lowered significantly (25–45 %). A recent study by Tuohy *et al*. (2002) has demonstrated, using FISH, that a statistically significant and selective increase in bifidobacteria occurred, following the feeding of lactulose.

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

Isomalto-oligosaccharides

Chemistry and manufacture of isomalto-oligosaccharides. Isomalto-oligosaccharides (IMO) are manufactured from starch, which is hydrolysed by the combined action of α-amylase and pullulanase, and the resultant malto-oligosaccharides are acted upon by α-glucosidase (Kohmoto *et al*. 1988, 1991). α-Glucosidase catalyses a transfer reaction converting the α(1→4)-linked malto-oligosaccharides into α(1→6)-linked IMO. Commercial IMO consist of a mixture of oligosaccharides of differing molecular mass.

Criterion 1: resistance to gastric acidity, hydrolysis by mammalian enzymes and gastrointestinal absorption. Studies carried out by Kaneko *et al*. (1995) using rats demonstrated that IMO was digested slowly in the jejunum and that components with a higher DP were less digestible and that the hydrogenated derivative of IMO was non-digestible. As such, it enters the colon in variable amounts. No human data are available and it cannot be concluded that IMO are non-digestible or only partly so.

Criteria 2 and 3: fermentation by intestinal microflora and selective stimulation of the growth and/or activity of intestinal bacteria associated with health and wellbeing. The fermentation properties of IMO have been tested by a combination of pure culture studies and human volunteer trials.

Kohmoto *et al*. (1988) conducted a pure culture study in which they tested isomaltose, isomaltotriose, panose, and the commercial product Isomalto-9000 (Hyashibara Co. Ltd, Okayama, Japan). They found that *B. adolescentis*, *B. longum*, *B. breve*, and *B. infantis* (not *B. bifidum*) metabolised the test sugars. IMO were also metabolised by *Bacteroides*, *Enterococcus faecalis* and *C. ramnosum* but not by a range of other enteric bacteria. At present, there appears to be no continuous culture fermentation work with IMO. The available *in vitro* data do not demonstrate a selective stimulation of bacterial growth. *In vivo*, the same authors carried out a volunteer trial that involved feeding IMO. Bacterial populations were determined by culture on selective agars. Significant increases in bifidobacteria were found.

The dose–response of IMO has been investigated by Kohmoto *et al*. (1991) in a volunteer trial involving feeding different doses. This study found a significant increase in bifidobacteria as determined by culture on agars that were only purportedly selective.

Because commercial IMO products contain a mixture of oligosaccharides, the influence of DP on fermentation, *in vivo*, has been studied by Kaneko *et al*. (1994). However, since these authors determined the counts of only bifidobacteria and the total microflora and no other bacterial group, the data do not fit the criteria for prebiotic effect.

Conclusion. Some of the evidence for prebiotic status for IMO appears to be promising but still not sufficient. In conclusion, IMO cannot, presently, be classified as prebiotics.

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Lactosucrose

Chemistry and manufacture of lactosucrose. Lactosucrose is produced from a mixture of lactose and sucrose using the enzyme β-fructofuranosidase (Playne & Crittenden, 1996). The fructosyl residue is transferred from sucrose to the C_1 position of the glucose moiety in the lactose, producing a non-reducing oligosaccharide (Hara *et al*. 1994).

Criterion 1: resistance to gastric acidity, hydrolysis by mammalian enzymes and gastrointestinal absorption. There appears to be no data on this.

Criteria 2 and 3: fermentation by intestinal microflora and selective stimulation of the growth and/or activity of intestinal bacteria associated with health and wellbeing. The properties of lactosucrose have been studied by Kumemura *et al*. (1992) in chronically constipated patients. Faecal bacteria were enumerated on agars, although the follow-up characterisation procedures are not clear. These authors found a significant increase in bifidobacteria and a significant decrease in clostridia.

Ohkusa *et al*. (1995) carried out a volunteer study involving feeding a normal diet supplemented with lactosucrose. Faecal samples were collected and plated onto agars. A significant increase in bifidobacteria compared with pre-trial values was seen, together with a significant decrease in bacteroides compared with samples 1 week after termination.

Conclusion. The evidence for the prebiotic status of lactosucrose is still not sufficient. In conclusion, lactosucrose cannot, presently, be classified as prebiotic.

Xylo-oligosaccharides

Chemistry and manufacture of xylo-oligosaccharides. Xylo-oligosaccharides (XOS) are manufactured by the enzymic hydrolysis of xylan from maize cobs (*Zea mays*). The commercial products are predominantly composed of the disaccharide xylobiose with small amounts of higher oligosaccharides (Yamada, 1993).

Criterion 1: resistance to gastric acidity, hydrolysis by mammalian enzymes and gastrointestinal absorption. The parent molecule, xylan, is recognised as a dietary fibre, indicating that oligosaccharide versions may reach the colon intact. No data were found to support this assumption, however.

Criteria 2 and 3: fermentation by intestinal microflora and selective stimulation of the growth and/or activity of intestinal bacteria associated with health and wellbeing. The most informative studies on XOS are those carried out by Okazaki *et al*. (1990). These authors carried out an initial pure culture study involving a wide range of bacteria. This indicated that XOS were metabolised by the majority of bifidobacteria and lactobacilli tested but by few other bacteria, notable exceptions being *Bacteroides* and *C. butyricum*. A recent pure culture study by Jaskari *et al*. (1998) has shown that XOS from oat-spelt xylan was metabolised by bifidobacteria but also by bacteroides, *C. difficile* and *E. coli*. Lactobacilli did not metabolise the XOS. Although this study appears to show a lack of selectivity in the fermentation of XOS in contradiction to the studies reported on earlier, studies relying on pure cultures do not represent the situation in the colon. Crittenden & Playne (2002) suggested that bifidobacteria were able to utilise XOS but not xylan.

The *in vitro* data presently available do not demonstrate a selective stimulation of bacterial growth.

A study in rats was carried out by Campbell *et al*. (1997). The authors examined faecal and caecal bacteria. Although only bifidobacteria, lactobacilli, total anaerobes and total aerobes were determined, significant increases in bifidobacteria occurred.

A volunteer trial involving feeding XOS to healthy men has been carried out (Okazaki *et al*. 1990). Bacteria were counted on agars and samples were analysed for SCFA. Significant increases were found in bifidobacteria and *Megasphaera*. There was also a significant increase in the concentration of organic acids in the faeces.

Conclusion. The evidence for the prebiotic status of XOS is still not sufficient.

In conclusion, therefore, XOS cannot, presently, be classified as prebiotic.

Soyabean oligosaccharides

Chemistry and manufacture of soyabean oligosaccharides. Soyabean oligosaccharides are α-galactosyl sucrose derivatives (raffinose, stachyose). They are isolated from soya beans and concentrated to form the commercial product (Crittenden, 1996).

Criterion 1: resistance to gastric acidity, hydrolysis by mammalian enzymes and gastrointestinal absorption. Raffinose and stachyose have been suggested to reach the colon after feeding to human subjects (Oku, 1994).

Criteria 2 and 3: fermentation by intestinal microflora and selective stimulation of the growth and/or activity of intestinal bacteria associated with health and wellbeing. The fermentation properties of these oligosaccharides have been studied either as mixtures of oligosaccharides or as individual components. In an early study, Minami *et al*. (1983) studied the fermentation of raffinose in pure cultures and found it to be metabolised by bifidobacteria and a range of enteric organisms whereas *L. acidophilus*, *Enterococcus faecalis* and *E. coli* could not. Hayakawa *et al*. (1990) compared pure raffinose and stachyose with refined soyabean oligosaccharides. In a pure culture study, bifidobacteria (with the exception of *B. bifidum*) and lactobacilli (with the exception of *L. casei*) metabolised the test sugars, whilst a range of other enteric bacteria did not metabolise them or did so poorly. A pure culture study by Jaskari *et al*. (1998) found that *L. acidophilus*, *B. infantis*, *B. bifidum*, *B. longum*, *Bacteroides thetaiotomicron*, and *Bacteroides fragilis* grew well on raffinose; *E. coli* grew poorly, whilst *C. difficile* did not grow.

The *in vitro* data presently available do not demonstrate a selective stimulation of bacterial growth.

A volunteer trial (Hayakawa *et al*. 1990) in healthy male adults found a significant increase in bifidobacteria with no change in putrefactive compounds.

Conclusion. The evidence for the prebiotic status of soyabean-oligosaccharides is still not sufficient.

In conclusion, and mostly because of the unreliable microbial methods, soyabean oligosaccharides cannot, presently, be classified as prebiotic.

Gluco-oligosaccharides

Chemistry and manufacture of gluco-oligosaccharides. Gluco-oligosaccharides are synthesised by the action of the enzyme dextran sucrase (*EC* 2.4.1.5) on sucrose in the presence of maltose. The resulting oligosaccharides contain α(1→2) linkages such as the following tetrasaccharide:

glucosylα(1→2)glucosylα(1→6)glucosylα(1→4)glucose.

Gluco-oligosaccharides can be produced via fermentation using *Leuconostoc mesenteroides*.

Criterion 1: resistance to gastric acidity, hydrolysis by mammalian enzymes and gastrointestinal absorption. These oligosaccharides were not digested in a germ-free rat model system (Valette *et al*. 1993).

Criteria 2 and 3: fermentation by intestinal microflora and selective stimulation of the growth and/or activity of intestinal bacteria associated with health and wellbeing. Branched-chain oligomers produced using *Leuconostoc mesenteroides* B-742 have been shown to be utilised readily by bifidobacteria and lactobacilli in a pure culture study by Chung & Day (2002) but not by *Salmonella* spp. or *E. coli*.

The fermentation properties of these oligosaccharides have been studied by Djouzi *et al*. (1995), who carried out a pure culture study and found that gluco-oligosaccharides were utilised by *B. breve*, *B. pseudocatenulatum* and *B. longum* but not by *B. bifidum*. They were utilised also by *Bacteroides* spp. and *Clostridium* spp. but not by lactobacilli. They then carried out artificial mixed culture studies in anaerobic culture vessels using *B. thetaiotomicron*, *B. breve* and *C. butyricum*. Gluco-oligosaccharides were then fed to germ-free rats inoculated with the three cultures used *in vitro* (Djouzi *et al*. 1995). In this model, the gluco-oligosaccharides had no effect on bacterial populations.

Conclusion. The evidence for the prebiotic status of gluco-oligosaccharides is still not sufficient. In conclusion, gluco-oligosaccharide cannot, presently, be classified as prebiotics.

Miscellaneous carbohydrates

The prebiotic potential of several other compounds has been investigated. However, evidence pointing towards any prebiotic effect is too sparse to justify a detailed review or a classification as prebiotic at the present time. These compounds include:

Germinated barley foodstuffs (Kanauchi *et al*. 1998*a*,*b*,*c*; Kanauchi, 2003);
Oligodextrans (Olano-Martin *et al*. 2000);
Gluconic acid (Tsukahara *et al*. 2002);
Gentio-oligosaccharides (Rycroft *et al*. 2001);
Pectic oligosaccharides (Olano-Martin *et al*. 2002);
Mannan oligosaccharides (White *et al*. 2002);
Lactose (Szilagyi, 2002);
Glutamine and hemicellulose-rich substrate (Bamba *et al*. 2002);
Resistant starch and its derivatives (Silvi *et al*. 1999; Lehmann *et al*. 2002; Wang *et al*. 2002);
Oligosaccharides from melibiose (van Laere *et al*. 1999);
Lactoferrin-derived peptide (Lipke *et al*. 2002);
N-Acetylchito-oligosaccharides (Chen *et al*. 2002);
Polydextrose (Murphy, 2001);
Sugar alcohols (Piva *et al*. 1996).

Prebiotic responses

With regards to the selective stimulation of specific bacteria, the questions of the dose–effect relationship and of the comparison of prebiotic effects of different compounds have caused some discussion. Regarding a dose–effect relationship, initial numbers of the bacteria that will be selectively stimulated to grow (the number before prebiotic administration) strongly determines the extent of stimulation (i.e. low if the initial number is high, but high if the initial number is low) (Roberfroid *et al*. 1998). A dose–effect relationship can thus be demonstrated only if the same group of volunteers having similar initial numbers of the different bacteria are used to test the different doses. Comparing the effect of prebiotics, especially with the aim to compare potency in terms of active dose, in different groups of volunteers having different initial numbers of bacteria cannot be made. In addition, the biological significance of changes in numbers of bacteria is limited if these numbers are expressed in logarithmic values. Indeed, in absolute numbers (decimal values), it is the case that even a small logarithmic increase (for example, +0·1 $\log_{10}$) can still represent a large increase in bacterial cell population (if the initial $\log_{10}$ number is 7 or 9, such an increase corresponds to $+10^6$ and $+10^8$ respectively or 100 times greater in the latter than in the former). This can have important consequences in terms of biological activity of the microflora.

Use of prebiotics for domestic animals

Companion animals are an extremely fruitful area for prebiotic use. Indeed, recent trials have emphasised the use of prebiotics in both dried and wet foods, whereby they seek to increase indigenous levels of lactobacilli and bifidobacteria (Swanson, 2002). For example, the use of oligofructose and mannose-oligosaccharides increased faecal bifidobacteria and ileal lactobacilli in dogs (Hussein *et al*. 1998; Hussein, 1999). Generally, for companion animal nutritional research, prebiotic use may reduce small-intestinal bacterial overgrowth, improve colonic bacterial profile, decrease faecal putrefactive compounds, and affect faecal characteristics and nutrient digestibility. Glucose-based oligosaccharides increased bifidobacteria in canine faeces (Flickinger *et al*. 2000). In contrast, oligofructose as a

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canine prebiotic generated only the sporadic isolation of bifidobacteria (Willard *et al*. 2000). The feeding of oligofructose to cats elevated lactobacilli by 164-fold, but no information was provided on the bifidogenic effect (Sparkes *et al*. 1998). The use of inulin and oligofructose in livestock and companion animals was reviewed recently by Flickinger *et al*. (2003).

Prebiotics, like probiotics, have been used in livestock applications (Morisse, 1993; Waldroup *et al*. 1993; Oyarzabal & Conner, 1996; Hu & Wang, 2001; Xu *et al*. 2002*a*,*b*; Yusrizal & Chen 2003*a*,*b*). Here, the main intentions are to reduce gastrointestinal infections, improve yield, carcass quality, reduce odour, etc.

The dietary supplementation of livestock feeds with inulin and oligofructose is a promising field of research. Increased numbers of bifidobacteria by oligofructose have been observed in pigs and quails, and it has been shown that oligofructose and, to a lesser extent, inulin reduced pathogen colonisation and contamination in poultry. In young pigs, oligofructose increased caecal and colonic epithelial cell proliferation. In pigs and rabbits, both inulin and oligofructose were shown to reduce intestinal concentrations of NH_3 (Flickinger *et al*. 2000). Inulin and oligofructose shift the excretion of N compounds from urine to faeces and reduce the production of putrefactive fermentation endproducts. This approach may be valid for growing–finishing pigs, being largely responsible for manure production and the environmental pollution of mainly N and minerals. The observed beneficial effects of inulin and oligofructose on pig-meat quality deserve further research attention, as meat safety and eating quality are major consumer concerns (Janssens *et al*. 2003). Acceptable (from both an economic and nutritional point of view) inclusion levels for inulin and/or oligofructose in piglet diets vary from 0·1 to 1 %, depending on the weaning age, diet composition and infection pressure.

Supplementing broiler diets with oligofructose improved ($P<0·05$) body-weight gain, feed conversion, carcass weight, carcass percentage and increased the small intestine length for female birds. Both inulin and oligofructose reduced ($P<0·05$) serum cholesterol and abdominal fat in broilers. Oligofructose-treated females had a denser distribution of ileal villi in the small intestine. Lactobacilli counts in the female birds were increased when the diets were supplemented with either inulin or oligofructose. Among the microflora tested, the *Campylobacter* count of the male birds and the *Salmonella* counts of the female birds were lower in the caecal contents for the prebiotic-supplemented birds (Yusrizal & Chen, 2003*b*).

Future perspectives and conclusions

Prebiotics have great potential as agents to improve or maintain a balanced intestinal microflora to enhance health and wellbeing. They can be incorporated into many foodstuffs (Table 4). There are, however, several questions that still need to be answered. For example, the present review has based its conclusions on prebiotic classification from current evidence. As this continues to accumulate, the picture will become clearer, for example in classifying certain carbohydrates where evidence is currently sparse or absent. Moreover, as better information on structure–function relationships accrues, as well as individual metabolic profiles of target bacteria, then it may be easier to tailor prebiotics into specific health attributes. Much more information is needed on the fine structure of the changes brought about by the regular intake of prebiotics. With the new generation of molecular microbiological techniques now becoming available, it will be possible to gain definitive information on the species rather than genera that are influenced by the test carbohydrate. If comparative information is to be gathered on structure–function relationships in prebiotic oligosaccharides, a rigorous approach to the evaluation of these molecules will be required. Such thorough comparative studies will allow intelligent choices when incorporating prebiotics into functional foods and should increase confidence amongst consumers and regulatory authorities. Similarly, it may be possible to incorporate further biological functionality into the concept; for example, an increase in beneficial bacteria while suppressing pathogens at the same time, perhaps through anti-adhesive approaches (Gibson, 2000).

The current most popular targets for prebiotic use are lactobacilli and bifidobacteria. This is based largely upon their success in the probiotic area (Fuller, 1997; Hamburger, 1997; Majamaa, 1997; Roberfroid, 1998; Gibson, 2000; Kazuhiro Hirayama, 2000; Capurso, 2001; Fooks & Gibson, 2002; Tannock, 2002). However, as our knowledge of the gut flora diversity improves (through using the molecular procedures described earlier; p. 261), then it may

Table 4. Possible foodstuffs that can be fortified with prebiotics

Dairy products
Beverages and health drinks
Spreads
Infant formulae and weaning foods
Cereals
Bakery products
Confectionery, chocolates, chewing gum
Savoury products, soups
Sauces and dressings
Meat products
Dried instant foods
Canned foods
Food supplements
Animal feeds
Petfoods

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become apparent that other micro-organisms should be fortified through their use. One example may be the eubacteria that produce butyric acid, a metabolite seen as beneficial for gut functionality and potentially protective against bowel cancer (Antalis, 1995; D'Argenio, 1996).

The concept currently targets microbial changes at the genus level. Future developments may elucidate molecules that induce species-level effects. This is because certain species of bifidobacteria and lactobacilli may be more desirable than others. It is important for colonic function to identify molecules that can be fermented distally, the principal site of chronic gut disorders such as bowel cancer and ulcerative colitis.

At the end of the present review aimed at updating the prebiotic definition, it must be underlined that only three carbohydrates, essentially non-digestible oligosaccharides, today fulfil the criteria for prebiotic classification (Table 5). For the other candidates, data are promising but more studies are still required. In particular, it must be stressed that data to fulfil criterion 1, i.e. 'resistance to gastric acidity, hydrolysis by mammalian enzymes and gastrointestinal absorption', are lacking. Similarly (more) *in vitro* data in mixed culture systems and (more) *in vivo* data, especially, in reliable human nutrition intervention studies, are required.

The original definition of a prebiotic considers only microbial changes in the colonic ecosystem of man. However, it may be timely to extrapolate this into other areas that may benefit from a selective targeting of particular micro-organisms. As such, we propose a refining of the original definition to:

'A prebiotic is a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microflora that confers benefits upon host wellbeing and health.'

The real drive is the nutritional, physiological and microbial benefits of prebiotics that have been published so far (Table 6) and their future exploitation in authentic health issues.

Table 5. Summary and conclusion on the prebiotic effect of various oligosaccharides

Carbohydrate	Non-digestibility	Fermentation	Selectivity	Prebiotic status
Inulin	Yes	Yes	Yes	Yes
Transgalacto-oligosaccharides	Probable	?	Yes	Yes
Lactulose	Probable	?	Yes	Yes
Isomalto-oligosaccharides	Partly	Yes	Promising	No
Lactosucrose	NA	NA	Promising	No
Xylo-oligosaccharides	NA	NA	Promising	No
Soyabean oligosaccharides	NA	NA	NA	No
Gluco-oligosaccharides	NA	NA	NA	No

?, Preliminary data, but further research still needed; NA, data not available.

Table 6. Reported nutritional and physiological effects and health outcomes associated with prebiotic intake both in experimental animals and in human subjects

Health aspect	Summary of the effect	Reference
Bowel cancer	Positive effects on biomarkers of colonic cancer through probiotic, prebiotic and synbiotic use	Rafter (2002)
	Reduction in aberrant crypt foci with inulin + *Lactobacillus acidophilus*	Bolognani *et al.* (2001)
	Both probiotics and prebiotics are protective	Wollowski *et al.* (2001)
	Positive effects with both inulin + *Lactobacillus acidophilus* in apoptosis stimulation, using the rat colon	Hughes & Rowland (2001)
	Synbiotics are effective at tumour suppression	Burns & Rowland (2000)
	Both probiotics and prebiotics exert inhibitory effects on aberrant crypt foci in animal models	Brady *et al.* (2000)
	Inulin as a prebiotic was effective	Gallaher & Khil (1999)
	Inulin can exert several positive effects	Pool-Zobel *et al.* (2002)
	Positive effects with synbiotic use (*Bifidobacterium longum*, inulin) on NH_3, colonic lesions, genotoxic enzymes	Rowland *et al.* (1998)
	In rats treated with carcinogens, inulin with bifidobacteria repressed effects but this did not occur with soya or wheat	Gallaher & Khil (1999)
	Dietary inulin reduced aberrant crypt foci incidence in the colon in rats, caecal weight was increased and caecal pH decreased	Verghese *et al.* (2002)
	Addition of oligofructose to a standard diet reduced the number of experimentally induced breast tumours in female rats	Taper & Roberfroid (1999)
	Oligofructose reduced the incidence of colon tumours and concomitantly developed gut-associated lymphoid tissue in Min mice	Pierre *et al.* (1997)
	Growth of mouse tumours was significantly inhibited by supplementation of the diet with either inulin or oligofructose	Taper *et al.* (1998)
	Aberrant crypt foci were significantly inhibited by inulin and oligofructose feeding	Reddy *et al.* (1997)
	Aberrant crypt multiplicity incidence in rats fed galacto-oligosaccharides was reduced	Wijnands *et al.* (2001)
	Prebiotic administration in the diet inhibited carcinogenesis in rats	Femia *et al.* (2002)
Inflammatory bowel disease	Lactulose reduced symptoms in patients with inflammatory bowel disease but the duration was too short (3 weeks) to generate significance	Szilagyi *et al.* (2002)
	Summarises promising trials with probiotics and prebiotics	Katz (2002)

Continued

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Table 6. *Continued*

Health aspect	Summary of the effect	Reference
Pathogenic agents	Oligofructose and inulin protected against *Listeria monocytogenes* and *Salmonella typhimurium* as well as chemically induced tumours	Buddington *et al.* (2002)
	Reduced incidence of travellers' diarrhoea with inulin	Cummings *et al.* 2001
	Inulin affects immunology through macrophage activation and through cell-wall fragments of bifidobacteria	Meyer *et al.* (2000)
	Inulin in an oral electrolyte solution accelerated beneficial bacteria and recovery from diarrhoea	Oli *et al.* (1998)
	Prebiotic fermentation increased organic acids which may be useful for suppressing pathogens	Kleesen *et al.* (1997*a*)
	Bifidobacterium breve plus transgalactosylated oligosaccharides inhibited *Salmonella enteritica*	Asahara *et al.* (2001)
CHD	Inulin decreased triacylglycerols, trend towards decreased cholesterol also	Brighenti *et al.* (1999)
	Inulin + lactobacilli decreased serum total cholesterol, and also LDL-cholesterol and the LDL:HDL ratio	Schaafsma *et al.* (1998)
	Oligofructose supplementation resulted in a decrease in postprandial triacylglycerolaemia and protected rats against an increase in non-esterified cholesterol serum level induced by a high-fat diet	Kok *et al.* (1998)
	Chronic feeding of rats with oligofructose significantly reduced the capacity of isolated hepatocytes to synthesise triacylglycerols	Fioraliso *et al.* (1995)
	Triacylglycerol and phospholipid concentrations in the liver and blood were decreased with oligofructose	Kok *et al.* (1996)
	Addition of oligofructose prevented some lipid disorders, lowered fatty acid synthase activity in the liver of rats	Agheli *et al.* (1998)
Necrotising enterocolitis	Oligofructose enhanced the bifidogenic effect, decreased severity and necrotising enterocolitis lesions in a quail model	Catala *et al.* (1999)
	Gnotobiotic quails as a model showed that oligofructose could act as an anti-infective agent and decrease the occurrence and severity of lesions in necrotising enterocolitis	Butel *et al.* (2002)
	Dietary inulin improves distal colitis induced by dextran sodium sulfate in the rat	Videla *et al.* (2001)
Mineral availability	Prebiotics have good possibilities for osteoporosis protection	Cashman (2002)
	An inulin–oligofructose mixture generated increased Ca absorption from orange juice	Griffin *et al.* (2002)
	Oligofructose improved Mg absorption in post-menopausal women	Tahiri *et al.* (2001)
	An increase in true fractional Ca absorption was seen after ingestion of oligofructose	van der Heuvel *et al.* (1999)
	Ovariectomy-induced loss of bone structure in the tibia of rats was prevented by oligofructose	Scholz-Ahrens *et al.* (2002)
	Mg and Ca absorption were raised significantly by oligofructose feeding	Beynen *et al.* (2002)
	Feeding inulin decreased faecal excretion of Ca, Mg, Fe, Zn and Cu	Delzenne *et al.* (1995)
	Beagle dogs fed inulin had a higher capacity for carrier-mediated glucose uptake than dogs fed cellulose	Buddington *et al.* (1999)
	Lactulose stimulated Ca absorption in rats	Brommage *et al.* (1993)
Obesity	Oligofructose could counteract both the fat mass development and the hepatic steatosis that occurs in obese Zucker rats	Daubioul *et al.* (2000)

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