Prebiotics: Concept, Definition, Criteria, Methodologies, and Products

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Concept, Definition, and Criteria

In 1995, Gibson and Roberfroid defined a prebiotic 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.” This definition only considers microbial changes in the human colonic ecosystem. Later, it was considered timely to extrapolate this into other areas that may benefit from a selective targeting of particular microorganisms and to propose a refined definition of a prebiotic as (Gibson et al. 2004):

a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microflora that confers benefits.

These definitions have attracted, and still continue to attract, a great deal of interest in the field of nutrition both in scientific research and in food applications. Consequently and over the years, prebiotic activity has been attributed to many food components, particularly oligosaccharides and polysaccharides (including some dietary fibers), but sometimes without due consideration to the criteria required. In particular it must be stressed that not all dietary nondigestible carbohydrates and certainly not all dietary fibers are prebiotics.

In a handbook of prebiotics, there is, therefore and more than anywhere else, a need to establish clear criteria for classifying a food ingredient as a prebiotic. Indeed, such classification requires a scientific demonstration that the ingredient:

- Resists gastric acidity
- Is not hydrolyzed by mammalian enzymes
- Is not absorbed in the upper gastrointestinal tract
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TABLE 3.1
Criteria for Classification of a Food Ingredient as Prebiotic

- Resistance to digestive processes in the upper part of the GI tract
- Fermentation by intestinal microbiota
- Selective stimulation of growth and/or activity of a limited number of the health-promoting bacteria in that microbiota

- Is fermented by the intestinal microflora
- Selectively stimulates the growth and/or activity of intestinal bacteria potentially associated with health and well-being

These requirements have been classified as the three prebiotic criteria (Table 3.1) (Gibson and Roberfroid 1995).

As with any functional food or ingredient and according to the European Consensus on Scientific Concepts of Functional Foods in Europe (Diplock et al. 1999), the final demonstration of these prebiotic attributes should include in vivo nutritional feeding trials in the targeted species (i.e., humans, livestock, or companion animals) using validated methodologies that are supported by sound science.

Although each of these criteria is important, demonstrating selectivity in the stimulation of growth and/or activity of bacteria remains the most contentious and difficult to fulfil. Indeed, it requires reliable and quantitative microbiological analysis of a wide variety of bacterial genera, for example, total aerobes/anaerobes, bacteroides, bifidobacteria, clostridia, enterobacteria, eubacteria, lactobacilli after anaerobic sampling of suitable biological materials, most usually feces, but sometimes also biopsies of colonic materials. As it does not take bacterial interactions into account, simply reporting in vitro fermentation in cultures of single microbial strains or even an increase in vitro in a limited number of bacterial genera in complex mixtures of bacteria (e.g., fecal slurries) is not proof of a prebiotic effect.

Regarding the stimulation of bacterial activity, patterns of production of organic acids, gases, and enzymes have been used as biomarkers of specific bacterial genera. However, these have not yet been validated and changes should be interpreted with caution.

Moreover, it is also important that the rationale behind a claimed prebiotic effect is elucidated through mechanistic explanations of effect. In this context, several bacterial genes specific for the metabolism of oligosaccharides have recently been identified. In particular, this is the case for a gene, in bifidobacteria, that codes for an enzyme that specifically hydrolyzes inulin-type fructans, thus explaining the selectivity in the action of these prebiotics (Schell et al. 2002). In light of the three criteria and the above considerations, the present chapter aims to review and discuss methodologies to scientifically demonstrate a prebiotic effect as well as evaluate evidence available for proving the prebiotic nature of candidate food ingredients (hitherto these are all carbohydrates).
Testing Methodologies

By referring to the criteria just described, a scheme has been proposed for the evaluation of a candidate prebiotic (Gibson et al. 1999). However, if good quality and biologically meaningful data are to be collected on different prebiotics, such an evaluation requires standardized testing methodologies that remain essential if we are to have confidence in any health claims on prebiotic functional foods.

Resistance to Digestive Processes in the Upper Part of the GI Tract

Resistance to digestive processes includes prebiotic resistance to gastric acidity, hydrolysis by mammalian enzymes, and gastrointestinal absorption. Both in vitro and in vivo methods are available to demonstrate this resistance in the candidate prebiotic.

In Vitro Methods

In vitro methods are applied to demonstrate resistance to acidic (i.e., those conditions which occur in the stomach) and enzymatic hydrolysis (i.e., saliva, pancreatic, and small intestinal enzymes; Oku et al. 1984; Ziesenitz and Siebert 1987; Nilsson and Bjorck 1988; Molis et al. 1996). With such methods and after an appropriate incubation, products of hydrolysis are quantified using standard chemical, physicochemical, or enzymatic methods (Dahlqvist and Nilsson 1984).

In Vivo Models

Resistance to any endogenous digestive process can be shown in experimental animals by measuring the fecal recovery of an oral dose given in germ-free conditions or after suppression of the intestinal flora by antibiotic pretreatment (Nilsson et al. 1988). Other, more invasive methods involve intubation into the gastrointestinal system of living anaesthetized rats (Nilsson et al. 1988).

In human volunteers, direct or indirect approaches are applicable following oral administration of the candidate prebiotic. Models that involve the direct recovery of nondigested molecules include oral intubation and distal ileum fluid sampling (Molis et al. 1996) or use proctocolectomized individuals, the so-called ileostomy patients (Bach Knudsen and Hessov 1995; Ellegard et al. 1997), a widely accepted alternative to study the small intestinal excretion of nutrients (Langkilde et al. 1990; Cummings and Englyst 1991). The intubation technique, with a nonabsorbable marker is also used to quantitatively assess ileal flow (Phillips and Giller 1973; Levitt and Bond 1977).

For indirect assessment of resistance to any endogenous digestive process, measurement of changes, as a function of time, in blood/serum concentration of either products of hydrolysis (e.g., glucose or fructose) or insulin as a
marker of glucose absorption can be used. However, if the candidate prebiotic is not composed of glucose or eventually fructose, such tests are not always applicable.

**Fermentation: Testing for Prebiotic Fermentation by Intestinal Microbiota**

**In Vitro Methods**

Batch and continuous culture fermentation systems are the most commonly used *in vitro* models to study anaerobic fermentation of carbohydrates both by pure selected species of bacteria or by mixed bacterial populations such as fecal microbiota. In such methodologies, disappearance of the candidate prebiotic is quantified as a function of time using standard chemical, physicochemical, or enzymatic methods. Batch culture fermenters are inoculated with either pure culture(s) of selected species of bacteria or with a fecal slurry and the candidate prebiotic to be studied. Multichamber continuous culture systems have been developed to reproduce physical, anatomical, and nutritional characteristics of gastrointestinal regions (Macfarlane et al. 1998; Gmeiner et al. 2000). These models, that are most exclusively used to study fermentation by mixed bacterial populations as in fecal slurry, are useful for predicting both the extent and site of prebiotic fermentation.

**In Vivo Methods**

*In vivo* fermentation of nondigestible carbohydrates can be studied in laboratory and companion animals, livestock, and humans. The heteroxenic animal harboring a human fecal flora is a particularly interesting model by which to study carbohydrate fermentation in experimental animals. In these animals, often rats, the candidate prebiotic is added to food or drinking water but can also be administered by gastric intubation. Animals are then anaesthetized and sacrificed at predetermined time intervals to collect contents of the gastrointestinal segments and/or fecal samples for analysis of fermentation products like gases and short chain fatty acids such as acetate, propionate, butyrate, lactate.

To study the fermentation in humans, previously given a single oral dose of the candidate prebiotics, two major approaches are used: an indirect approach that collects breath gas, at regular time intervals, to measure the concentration of gases, essentially hydrogen, a common end product of anaerobic fermentation (Christl et al. 1992), and a direct approach that consists of collecting feces and measuring recovery of the tested food ingredient.

**Selective Stimulation of Growth of Intestinal Bacteria**

Much of the early (and still some of the current) literature describes studies performed on pure cultures with the aim to show that selected bacterial
species or strain(s) ferment the candidate prebiotic with the tentative conclusion that such fermentation is “selective.” Typically this involves the selection of a range of strains of *Bifidobacterium* spp., *Lactobacillus* spp., and other gut bacteria such as *Bacteroides* spp., *Clostridium* spp., *Eubacterium* spp., and *Escherichia coli* and incubating them in the presence of the food ingredient under investigation. The number of strains tested varies with different reports. The problem with this approach is, of course, that the species/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 strains of bifidobacteria and lactobacilli but only one or two species and/or strains of the “undesirable” species. Such studies cannot establish that the test carbohydrate is selectively fermented and should be used for initial screening purposes only.

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. In this context, a more meaningful *in vitro* method for studying potential prebiotic oligosaccharides is the use of fecal inocula that ensures that a representative range of bacterial species is exposed to the test material. Study of the changes in populations of selected genera or species can then establish whether or not the fermentation is selective. The use of feces probably gives an accurate representation of events in the distal colon. However, both the composition and activities of the microbiota indigenous to the colon is variable, dependent upon the region being sampled. In particular, bacterial populations in more proximal areas will have a more saccharolytic nature compared to those in median or distal areas. This has been confirmed through studies on sudden death victims, where the colon contents of the different segments were sampled shortly following death (Macfarlane et al. 1992, 1998). The complex *in vitro* gut models, which replicate different anatomical areas, attempt to overcome this and should be used in concert with human trials.

**Identification of Changes in Composition of the Microbiota**

Major problems with the use of fecal inocula or any kind of mixed population of microorganisms include identification of the groups/genera and species present as well as quantitative assessment of changes in microflora composition. Traditionally, this has been accomplished by culturing on a range of purportedly selective agars followed by morphological and biochemical tests designed to confirm culture identity and finally counting of the colonies (Van Houte and 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 as it is estimated that, using selective culture, only about 50–60% of the diversity present in
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the human colon have yet been characterized (Suau et al. 1999; Marteau et al. 2001).

A much more reliable approach involves the use of molecular based methods of bacterial identification. These have advantages over culture-based technologies in that they have improved reliability and can encompass the full flora diversity including phyla, groups/genera or species that, up to now, have not been cultured. Using such methods, bacterial enumeration can be carried out in a rapid, culture-independent and reliable manner. The most frequently used molecular procedures are based on the observation that bacterial ribosomes offer a unique tool to identify and quantify bacteria at a molecular level. Indeed, the genes that code for the 16S subunits of the bacterial ribosomes (16S rRNA) are comprised of both conserved and variable regions, and sequencing of that particular gene enables bacterial identifications to be made. These methods remove the ambiguity that is a prominent feature of traditional selective agars. Additionally, they provide means by which hitherto bacterial species of the gut that cannot be cultured in vitro may be investigated. Indeed these are culture-independent techniques that do not require prior, often anaerobic, growth of a microorganism with laboratory media (Liesack and Stackebrandt 1992). The most frequently used methodologies for evaluating bacterial populations in feces are given below and Table 3.2 summarizes them, along with some of their advantages and disadvantages.

Fluorescence In Situ Hybridization

Fluorescence in situ hybridization (FISH) involves the use of group (and in some cases species) specific oligonucleotide probes that target discrete discriminatory, highly conserved regions of the rRNA molecule allowing specific groups of bacteria to be distinguished from others in a mixed culture. A variety of phylogenetic probes are currently available for the enumeration of fecal bacteria, while more are being designed and validated (Wang et al. 2002a,b). Groups targeted include Bacteroides spp. (Manz et al. 1996), Bifidobacterium spp. (Langendijk et al. 1995), Lactobacillus/Enterococcus spp. (Harmsen et al. 1999), Eubacterium (Franks et al. 1998), Clostridium (Tuohy et al. 2001), and Ruminococcus (Zoetendal et al. 2002).

Polymerase Chain Reaction

By using a process known as polymerase chain reaction (PCR) segments of rRNA genes can be amplified to a level whereby their sequence can be subsequently determined (Steffan and Atlas 1991). Community profiling techniques based on PCR, such as denaturing gradient gel electrophoresis (DGGE), may be applied to fecal samples to examine the predominant components (see below). In addition to PCR-cloning and PCR-DGGE community profiling assays, standard PCR techniques have been used to determine the presence or absence of and/or activity of particular bacterial groups (Sharkey et al. 2004).
### TABLE 3.2
Summary Presentation of Current Methodologies Applicable to Enumerate Bacteria in Fecal Microbiota

<table>
<thead>
<tr>
<th>Method</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Selective culturing and biochemical</td>
<td>• Straight forward</td>
<td>• Operator subjectivity</td>
</tr>
<tr>
<td>characteristics</td>
<td>• Relatively inexpensive</td>
<td>• Applicable only to culturable bacteria</td>
</tr>
<tr>
<td></td>
<td>• Possibility to carry out a large number of replicates</td>
<td>• Ambiguity of selectivity of media</td>
</tr>
<tr>
<td></td>
<td>• Possibility of error due to metabolic plasticity of organisms</td>
<td></td>
</tr>
<tr>
<td>FISH Fluorescence in situ hybridization</td>
<td>• Applicable on unculturable as well as culturable bacteria</td>
<td>• Probe available for known bacteria only</td>
</tr>
<tr>
<td></td>
<td>• Highly specific</td>
<td>• More time consuming than culture procedures</td>
</tr>
<tr>
<td>PCR Polymerase chain reaction</td>
<td>• High fidelity</td>
<td>• Expensive</td>
</tr>
<tr>
<td></td>
<td>• Reliability</td>
<td>• Time consuming</td>
</tr>
<tr>
<td></td>
<td>• Allows placement of previously unidentified bacteria</td>
<td>• Possibility of bias</td>
</tr>
<tr>
<td></td>
<td>• Applicable to unculturable bacteria</td>
<td></td>
</tr>
<tr>
<td>Direct community analysis</td>
<td>• Culture-independent</td>
<td>• Some loss of bacterial diversity due to the bias introduced by PCR</td>
</tr>
<tr>
<td></td>
<td>• Possibility to elucidate diversity of entire samples</td>
<td>• Qualitative rather than quantitative</td>
</tr>
<tr>
<td>D/TGGE Denaturing/temperature gradient</td>
<td>• Rapidity</td>
<td>• Some loss of bacterial diversity due to the bias introduced by PCR</td>
</tr>
<tr>
<td>gel electrophoresis</td>
<td>• Applicable to both culturable and unculturable bacteria</td>
<td></td>
</tr>
</tbody>
</table>

**Direct Community Analysis**

This process characterizes 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 via PCR (using universal primers) (Suau et al. 1999). The purified amplification products are subsequently cloned into *Escherichia coli*, and clones containing the 16S rDNA inserts are sequenced and identified by comparison to database 16S rDNA sequences.

**Denaturing/Temperature Gradient Gel Electrophoresis (DGGE or TGGE)**

These approaches separate amplified DNA fragments of the same size based on the extent of the sequence divergence between different PCR products (Muyzer and Smalla 1998). A whole community PCR is carried out and partial 16S rDNA sequences 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 and 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 characterized and this relatively rapid technique also offers the potential of monitoring gut flora over time (Zoetendal et al. 1998).

### Review of Candidate Prebiotics

For each candidate a brief description of the chemistry and manufacturing process is given followed by a review of data available to fulfill the three criteria for prebiotic classification described above, that is

1. Resistance to digestive processes in the upper part of the GI tract
2. Fermentation by intestinal microbiota
3. Selective stimulation of growth and/or activity of a limited number of the health-promoting bacteria in that microbiota

#### Inulin-Type Fructans

**Chemistry, Nomenclature, and Manufacture**

Inulin-type fructans are linear fructans in which the fructosyl–fructose linkages are all β-(1−2) and the linear chain is either a α-d-glucopyranosyl-[β-d-fructofuranosyl]₀⁻¹β-d-fructofuranoside (GpyFnᵢ) or a β-d-fructopyranosyl-[β-d-fructofuranosyl]₀⁻¹β-d-fructofuranoside (FpyFnᵢ). When present, the fructosyl–glucose linkage is always β-(2−1) as in sucrose.

The most common inulin-type fructan presently produced and used by the food industry is chicory inulin. It is a mixture of oligo- and polymers in which the DP (degree of polymerization that defines the number of fructosyl monomers) varies from 2 to approximately 60 units with an average value (DPav) = 12. About 10% of the fructan chains in native chicory inulin have a DP ranging between 2 (F₂) and 5 (GF₄). The partial enzymatic hydrolysis of inulin using an endoinulinase (EC 3.2.1.7) produces oligofructose, which is a mixture of both GpyFnᵢ and FpyFnᵢ molecules, with the DP varying from 2 to 7 and a DPav = 4. Oligofructose can also be obtained by enzymatic synthesis (transfructosylation) using the fungal (*Aspergillus niger*) β-fructosidase (EC 3.2.1.7). In this synthetic compound, all oligomers are of GpyFnᵢ-type, the DP varies from 2 to 4 and DPav = 3.6. By applying specific separation technologies the food industry also produces a long chain inulin known as inulin HP (DP 10 to 60 and DPav = 25). Finally, by mixing oligofructose and long chain
inulin, specific products known as Synergy® have also developed. The different industrial products, derived from chicory inulin, vary in DP\textsubscript{av}, DP\textsubscript{max}, and DP distribution and they have miscellaneous technological but rather common biological properties (Franck 2002).

Inulin-type fructans and inulin are generic terms that cover all β-(1→2) linear fructan molecules. In any circumstances that justify identification of the oligomers versus polymers, the terms oligofructose and/or inulin can be used respectively. Even though the inulin hydrolysate and the synthetic compound (usually identified as fructooligosaccharide, FOS, or short chain fructooligosaccharide, scFOS) have a slightly different DP\textsubscript{av} (4 and 3.6 respectively), the term oligofructose must be used to identify both. Indeed, oligofructose and FOS are considered to be synonyms for the mixture of small inulin oligomers with DP\textsubscript{max} < 10 (Quemener 1994; Roberfroid et al. 1998; Coussement 1999; Roberfroid 2002).

**Inulin-Type Fructans and Criteria for Classification as Prebiotic**

**Criterion 1: Resistance to gastric acidity, hydrolysis by mammalian enzymes, and gastrointestinal absorption**

The resistance of inulin-type fructans to digestive processes has been extensively studied and demonstrated by applying all the methods (both \textit{in vitro} and \textit{in vivo}) described in the section on Testing Methodologies. Inulin-type fructans are nondigestible oligosaccharides that, moreover and for nutritional labeling, classify as dietary fiber (Roberfroid 1993).

**Criteria 2 and 3: Fermentation by intestinal microflora and selective stimulation of the growth and/or activity of intestinal bacteria**

Numerous \textit{in vitro} studies, summarized in Table 3.3, support the selective stimulation of bacterial growth by inulin. This has been carried out in defined pure culture fermentation and using a mixed fecal inoculum in both batch and continuous culture (Wang and Gibson 1993; Gibson and Wang 1994a; Roberfroid et al. 1998).

As well as \textit{in vitro} work, \textit{in vivo} studies have also demonstrated that in germ-free rats associated with a human fecal flora, feeding oligofructose, inulin, or a mixture of both, selectively stimulated the growth of bifidobacteria as well as lactobacilli while reducing the number of clostridia. Such treatments also increased the relative proportion of butyrate indicating a change in bacterial activity (Levrat et al. 1991; Campbell et al. 1997; Kleessen et al. 2001; Poulsen et al. 2002).

Human trials to demonstrate a prebiotic effect of oligofructose and inulin include those with a controlled diet, and cross-over feeding trials although the dose, substrate, duration, and age of volunteers vary (Mitsouka et al. 1987; Gibson et al. 1995; Buddington et al. 1996; Bouhnik et al. 1996, 1999; Kleessen et al. 1997; Kruse et al. 1999; Menne et al. 2000; Rao 2001; Tuohy et al. 2001; Guigoz et al. 2002; Williams et al. 1994; Harmsen et al. 2002) (Table 3.4).

The efficacy of inulin has also been evaluated with a view to its administration to formula-fed infants (Coppa et al. 2002). Moro et al. (2002)
TABLE 3.3
Summary Description of Studies Carried out to Demonstrate the In Vitro Selectivity of Inulin-Type Fructans in Both Pure Culture, Mixed Batch Culture, and Mixed Continuous Culture Fermentation

<table>
<thead>
<tr>
<th>Aims of Study</th>
<th>Observations</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Batch culture using fecal inocula to study fermentation of inulin-type fructans, starch, polydextrose, fructose, and pectin</td>
<td>Bifidobacteria most increased with inulin-type fructans while populations of E. coli and clostridia were maintained at relatively low levels</td>
<td>Wang and Gibson (1993)</td>
</tr>
<tr>
<td>Examining the growth of bifidobacteria on different types of oligofructose in pure culture. Eight species tested as well as species of clostridia, bacteroides, enterococci, and E. coli</td>
<td>Linear oligofructose had more of a bifidogenic effect than larger MW molecules and branched chain varieties. Bifidobacteria species showed a preference for inulin-type fructans compared to glucose</td>
<td>Gibson and Wang (1994b)</td>
</tr>
<tr>
<td>Continuous culture fermentation to study fermentation of oligofructose</td>
<td>Selective culturing showed bifidobacteria, and to a lesser extent lactobacilli, preferred oligofructose to inulin and sucrose. Bacteroides could not grow on oligofructose</td>
<td>Gibson and Wang (1994b)</td>
</tr>
<tr>
<td>Species of bifidobacteria (longum, breve, pseudocatenulatum, adolescentis) were tested in pure culture for their ability to ferment inulin-type fructans</td>
<td>B. adolescentis was seen to grow best and was able to metabolize all types of inulin-type fructans</td>
<td>Marx et al. (2000)</td>
</tr>
<tr>
<td>Batch culture using fecal inocula to study fermentation of oligofructose, branched fructan, levan, maltodextrin</td>
<td>FISH revealed that branched fructan had the best prebiotic effect, followed by oligofructose</td>
<td>Probert and Gibson (2002)</td>
</tr>
<tr>
<td>The ability of bifidobacteria and lactobacilli to grow on MRS agar containing oligofructose was investigated.</td>
<td>7/8 bifidobacteria and 12/16 lactobacilli were able to grow on agar containing oligofructose</td>
<td>Kaplan and Hutkins (2000)</td>
</tr>
</tbody>
</table>

observed an increase in bifidobacteria and lactobacilli in infants who received formula milk supplemented with a mixture of long chain inulin and galactooligosaccharides, 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 support the classification of inulin-type fructans as prebiotic,
### TABLE 3.4
Summary Presentation of Major Studies Demonstrating the Selective Stimulation of Bacterial Growth by Inulin-Type Fructans in Healthy Human Feeding Trials

<table>
<thead>
<tr>
<th>Study Design</th>
<th>Observations</th>
<th>Investigators</th>
</tr>
</thead>
<tbody>
<tr>
<td>23 subjects fed oligofructose (8 g/day) for 2 weeks</td>
<td>Selective increase in fecal bifidobacteria</td>
<td>Mitsouka et al. (1987)</td>
</tr>
<tr>
<td>10 subjects fed oligofructose (4 g/day) for 2 weeks</td>
<td>Selective increase in fecal bifidobacteria</td>
<td>Williams et al. (1994)</td>
</tr>
<tr>
<td>12 subjects fed oligofructose (4 g/day) for 25 days</td>
<td>Selective increase in fecal bifidobacteria</td>
<td>Buddington et al. (1994)</td>
</tr>
<tr>
<td>8 subjects on a controlled diet were fed oligofructose (15 g/day) for 15 days Subsequently 4 of these subjects were fed inulin (15 g/day) for 15 days</td>
<td>Oligofructose selectively increased fecal bifidobacteria and decreased bacteroides, clostridia, and fusobacteria. Inulin selectively increased bifidobacteria and decreased Gram positive cocci</td>
<td>Gibson et al. (1995)</td>
</tr>
<tr>
<td>20 subjects were fed 12.5 g/day oligofructose for 12 days</td>
<td>Significant increase in fecal bifidobacteria</td>
<td>Bouhnik et al. (1996)</td>
</tr>
<tr>
<td>10 female elderly subjects were given inulin (20 and 40 g/day) for 19 days</td>
<td>Selective increase in fecal bifidobacteria and significant decrease in bacteroides</td>
<td>Kleessen et al. (1997a)</td>
</tr>
<tr>
<td>40 subjects fed 2.5, 10, and 20 g/day oligofructose for 7 days</td>
<td>Selective agars showed that bifidobacteria were most increased by 10 and 20 g doses of oligofructose compared to 2.5 g and that the optimum dose of oligofructose was found to be 10 g/day</td>
<td>Bouhnik et al. (1999)</td>
</tr>
<tr>
<td>Chicory inulin hydrosylate fed to 8 subjects in a controlled feeding study, 8 g/day</td>
<td>Selective agars showed an increase in fecal bifidobacteria</td>
<td>Menne et al. (2000)</td>
</tr>
<tr>
<td>8 subjects fed up to 34 g/day inulin for a period of 2 months</td>
<td>Selective increase in fecal bifidobacteria that lasted for the whole 2 months period</td>
<td>Kruse et al. (1999)</td>
</tr>
<tr>
<td>8 young volunteers fed oligofructose (5 g/day) for 3 weeks</td>
<td>Selective increase in fecal bifidobacteria</td>
<td>Rao et al. (2001)</td>
</tr>
<tr>
<td>8 subjects fed biscuits containing high molecular weight inulin 21 days</td>
<td>FISH revealed a selective increase in fecal bifidobacteria</td>
<td>Touhy et al. (2001)</td>
</tr>
<tr>
<td>19 elderly patients fed oligofructose (8 g/day) for 3 weeks</td>
<td>Selective increase in fecal bifidobacteria</td>
<td>Guigoz et al. (2002)</td>
</tr>
<tr>
<td>12 adult volunteers were given long chain inulin (9 g/day) for 2 weeks</td>
<td>Quantification of all bacteria, bifidobacteria, the <em>Eubacterium rectale–Clostridium coccoides</em> group (Erec group), Bacteroides, and eubacteria were counted with FISH probes. A significant increase in bifidobacteria and a significant decrease in Erec group was observed</td>
<td>Hamsen et al. (2002)</td>
</tr>
</tbody>
</table>
since they fulfil all the three criteria. These compounds are now considered as the model prebiotics.

**Transgalactooligosaccharides**

**Chemistry and Manufacture of Transgalactooligosaccharides**

Enzymatic transglycosylation of lactose produces a mixture of oligosaccharides known as transgalactooligosaccharides (TOS) (Crittenden 1996). The composition of the mixture depends upon the enzyme used and the reaction conditions. They generally consist of oligosaccharides from tri- to pentasaccharide with $\beta(1\rightarrow6)$, $\beta(1\rightarrow3)$ and $\beta(1\rightarrow4)$ 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 exact composition of the mixture be given in reports of the studies.

**Criterion 1: Resistance to gastric acidity, hydrolysis by mammalian enzymes, and gastrointestinal absorption**

The data on nondigestibility do not fully match the criteria. However, there are suggestions that TOS do reach the colon intact (Tomomatsu 1994).

**Criteria 2 and 3: Fermentation by intestinal microflora and selective stimulation of the growth and/or activity of intestinal bacteria**

In an early study (Minami 1983), testing the fermentation of “isogalactobiose” of unknown linkage, it was reported that one strain of each of *B. infantis, B. longum, B. adolescentis,* and *L. acidophilus* metabolized it, while one strain each of *S. fecalis* and *E. coli* did not. However, in a more extensive study (Tanaka 1983), it was found that many strains of enteric bacteria could not metabolize the isogalactobiose. Testing enzymatically synthesized TOS in a pure culture study, these authors found that all of the bifidobacteria, most lactobacilli and enterobacteria, and some streptococci fermented the TOS with bifidobacteria displaying the most vigorous growth.

In a study by Rowland and Tanaka (1993) gnotobiotic rats inoculated with human fecal flora were fed a TOS-containing diet before being sacrificed. Cecal contents analyzed 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 also found significant decreases in nitrate reductase and $\beta$-glucuronidase activities as indicative of changes in microflora activity. This was followed by an *in vivo* volunteer feeding study that showed significant increases in fecal bifidobacteria. This study, however, only fed subjects for one week per dose and there was no reported washout period between treatments.

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

Adding a mixture of oligosaccharides (90% GOS and 10% long chain inulin) to infant formula milk has been shown to increase fecal bifidobacteria in both preterm and term infants (Dubey and Mistry 1996; Knol 2001; Rivero-Urgell and Santamaria-Orleans 2001; Boehm et al. 2002; Moro et al. 2002; Vandenplas 2002).

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

Lactulose

Chemistry and Manufacture of Lactulose

Lactulose is manufactured by the isomerization of lactose to generate the disaccharide galactosyl-β-(1→4) fructose. It is widely prescribed as a laxative (Tamura 1993) but has hitherto not been used for food applications.

Criterion 1: Resistance to gastric acidity, hydrolysis by mammalian enzymes, and gastrointestinal absorption

Investigations of the enzymatic degradation of lactulose have found that human and calf intestinal β-galactosidases did not degrade lactulose (Gibson and Angus 2000).

Criteria 2 and 3: Fermentation by intestinal microflora and selective stimulation of the growth and/or activity of intestinal bacteria

One of the earliest studies on lactulose fermentation was that of Sahota et al. (1982) who used 37 species of bacteria in pure culture. They found that Bacteroides oralis, Bact. vulgatus, B. bifidum, C. perfringens, Lact. casei sub. casei, and four other strains of Lactobacillus spp. fermented lactulose. However, the in vitro data presently available do not demonstrate a selective stimulation of bacterial growth in mixed populations of microorganisms.

Tomoda (1991) fed yoghurt supplemented with lactulose to healthy volunteers and reported a significant increase in fecal 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, subsequently performed by Terada et al. (1993), found a selective and significant increase in fecal bifidobacteria and decreases in C. perfringens, streptococci, bacteroides, and lactobacilli. In a parallel group, randomized, double blind, placebo-controlled trial, Ballongue et al. (1997) provided more evidence that lactulose significantly increased Bifidobacterium, Lactobacillus, and Streptococcus, concomitant with significant decreases in Bacteroides, Clostridium, coliforms, and Eubacterium. Concentrations of acetate and lactate were increased while
butyrate, propionate, and valerate concentrations decreased. All of the bacterial enzyme activities measured were significantly lowered (25–45%). More recently, using fluorescent in situ hybridization, Tuohy et al. (2002) have also demonstrated, a statistically significant and selective increase in bifidobacteria 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 in human studies. However, up to now, that compound has not been used as a food ingredient or as a food supplement.

### Isomaltooligosaccharides

**Chemistry and Manufacture of Isomaltooligosaccharides**

Manufacture of isomaltooligosaccharides (IMO) includes hydrolysis of starch by the combined action of α-amylase and pullulanase followed by isomerization of the resultant maltooligosaccharides by α-glucosidase (Kohmoto et al. 1988, 1991) that catalyzes a transfer reaction converting the α(1→4) linked maltooligosaccharides into α(1→6) linked IMO with different molecular weights.

**Criterion 1: Resistance to gastric acidity, hydrolysis by mammalian enzymes, and gastrointestinal absorption**

In rats, Kaneko et al. (1995) have demonstrated that IMO is slowly digested in the jejunum, that components with a higher DP are less digestible, and that the hydrogenated derivative of IMO is nondigestible. As such, it can only enter the colon in variable amounts. No human data are yet available and it cannot presently be concluded that IMO are nondigestible or only partly so.

**Criteria 2 and 3: Fermentation by intestinal microflora and selective stimulation of the growth and/or activity of intestinal bacteria**

The fermentation properties of IMO have been tested by a combination of pure culture studies and human volunteer trials.

In a pure culture study, Kohmoto et al. (1988) have tested isomaltose, isomaltotriose, panose, and the commercial product Isomalto-9000 and reported that *B. adolescentis*, *B. longum*, *B. breve*, and *B. infantis* (not *B. bifidum*) metabolize the test sugars. Isomaltooligosaccharides were also metabolized by *Bacteroides*, *Enterococcus fecalis*, and *Clostridium ramosum* but not by a range of other enteric bacteria. At present, there appears to be no continuous culture fermentation work carried out with IMO. The *in vitro* data presently available do not demonstrate a selective stimulation of bacterial growth. *In vivo*, the same authors carried out a volunteer trial that involved feeding IMO and found a significant increases in bifidobacteria.

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. (1995). However, since these authors only determined the counts of bifidobacteria and the total microflora and no other bacterial groups, the data do not hitherto 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.

### Lactosucrose

**Chemistry and Manufacture of Lactosucrose**

Lactosucrose is produced from a mixture of lactose and sucrose using the enzyme β-fructofuranosidase (Playne and Crittenden 1996). The fructosyl residue is transferred from sucrose to the C1-position of the glucose moiety in the lactose, producing a nonreducing oligosaccharide (Hara et al. 1994).

**Criterion 1: Resistance to gastric acidity, hydrolysis by mammalian enzymes, and gastrointestinal absorption**

No data are available on this criterion.

**Criteria 2 and 3: Fermentation by intestinal microflora and selective stimulation of the growth and/or activity of intestinal bacteria**

In chronically constipated patients receiving lactosucrose, Kumemura (1992) found a significant increase in bifidobacteria and a significant decrease in clostridia. Fecal bacteria were enumerated on agars, although the follow-up characterization procedures are not clear.

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

**Conclusion:** The evidence for prebiotic status of lactosucrose is still not sufficient. In conclusion, lactosucrose cannot, at present, be classified as prebiotic.

### Xylooligosaccharides

**Chemistry and Manufacture of Xylooligosaccharides**

Xylooligosaccharides (XOS) are manufactured by enzymatic hydrolysis of xylan from corn cobs. 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 recognized as a dietary fiber indicating that it 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

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 metabolized by the majority of bifidobacteria and lactobacilli tested but by few other bacteria, notable exceptions being Bacteroides and Clostridium butyricum. A recent pure culture study by Jaskari (1998) has shown that XOS from oat spelt xylan was metabolized by bifidobacteria but also by bacteroides, Clostridium difficile, and E. coli. Lactobacilli did not metabolize the XOS. Although this study appears to show a lack of selectivity in the fermentation of XOS in contrast to the studies reported above, studies relying on pure cultures do not represent the situation in the colon. Crittenden and Playne (2002) suggested that bifidobacteria were able to utilize xylooligosaccharides 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 fecal and cecal 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 analyzed for short-chain fatty acids (SCFA). Significant increases were found in bifidobacteria and Megasphaera. There was also a significant increase in the concentration of organic acids in the feces.

Conclusion: The evidence for prebiotic status of XOS is still not sufficient. In conclusion, therefore, XOS cannot at present be classified as prebiotic.

Soybean Oligosaccharides

Chemistry and Manufacture of Soybean Oligosaccharides

Soybean oligosaccharides (SOS) are α-galactosyl sucrose derivatives (raffinose, stachyose). They are isolated from soybeans 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, but not really demonstrated, to reach the colon after feeding to humans (Oku 1994).

Criteria 2 and 3: Fermentation by intestinal microflora and selective stimulation of the growth and/or activity of intestinal bacteria

The fermentation properties of these oligosaccharides have been studied either as mixtures of oligosaccharides or as individual components. In an early study Minami (1983) studied the fermentation of raffinose in pure cultures and found it to be metabolized by bifidobacteria and a range of enteric organisms whereas L. acidophilus, S. fecalis, and E. coli could not. Hayakawa
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et al. (1990) compared pure raffinose and stachyose with refined SOS. In a pure culture study, bifidobacteria (with the exception of B. bifidum) and lactobacilli (with the exception of L. casei) metabolized the test sugars while a range of other enteric bacteria did not or did so poorly. A pure culture study by Jaskari (1998) found that Lact. acidophilus, B. infantis, B. bifidum, B. longum, Bacteroides thetaiotamicron, and Bact. fragilis grew well on raffinose, E. coli grew poorly, while Clostridium difficile did not. 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 prebiotic status of SOS is still not sufficient. In conclusion, and mostly because of the unreliable microbial methods, SOS cannot, at present, be classified as prebiotic.

Glucoooligosaccharides

Chemistry and Manufacture of Glucoooligosaccharides

Glucoooligosaccharides are synthesized 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 tetrasaccharides:

- Glucosyl α(1→2)Glucosyl, α(1→6)Glucosyl α(1→4)Glucose.
- Gluco-oligosaccharides can also be produced via fermentation in the presence of 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 1993).

Criteria 2 and 3: Fermentation by intestinal microflora and selective stimulation of the growth and/or activity of intestinal bacteria

Branched chain oligomers produced using Leuconostoc mesenteroides B-742 have been shown to be readily utilized by bifidobacteria and lactobacilli in a pure culture study by Chung and Day (2002) but not by Salmonella spp. or E. coli.

Djouzi et al. (1995) found that glucoooligosaccharides were utilized by Bifidobacterium breve, B. pseudocatenulatum, B. longum, not by B. bifidum but or lactobacilli but well by Bacteroides spp. and Clostridium spp. Fed to germ-free rats inoculated with the artificial mixed culture composed of Bact. thetaiotaomicron, B. breve, and C. butyricum, glucoooligosaccharides had no effect on bacterial populations (Djouzi et al. 1995).

Conclusion: The evidence for prebiotic status of gluco-oligosaccharides is still not sufficient. In conclusion, gluco-oligosaccharides cannot, at present, be classified as prebiotic.
Miscellaneous Carbohydrates

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

- Germinated barley foodstuffs (Kanauchi et al. 1998a,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 (Szlágyi 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-acetylchitooligosaccharides (Chen et al. 2002)
- Polydextrose (Murphy 2001)
- Sugar alcohols (Piva et al. 1996)

Prebiotic Responses

Concerning the quantitative aspects of the prebiotic effect two questions have attracted (too much!) attention (mostly for marketing purposes!):

- Can a dose–effect relationship be established?
- Are the different prebiotics equally effective?

In spite of the large number of studies available, the only data available today to discuss these issues have been obtained with inulin-type fructans. As discussed previously (Roberfroid 2005, 2007), these data show that the daily dose of a prebiotic (i.e., inulin) does not correlate with the absolute numbers of “new” bacterial cells that have appeared as a consequence of the prebiotic consumption ($r = 0.06$ and $-0.09$ respectively; NS). The daily dose is thus, by itself, not a determinant of its prebiotic effect, even if, in one group of volunteers with relatively similar initial counts of fecal bifidobacteria, a limited dose–effect relationship can be established (Bouhnik et al. 1999).
reason is that a key parameter, that is, the initial number of fecal bifidobacteria, before the administration of the prebiotic, is usually not taken into account. In the first report of a prebiotic effect and after observing an inverse correlation between these numbers and their “crude” increases after oligofructose feeding, Hidaka already argued that the initial numbers of bifidobacteria influence the prebiotic effect (1986). Roberfroid et al. (1998), Rao (2001) and Rycroft et al. (2001) have reached essentially the same conclusion.

At the population level, it is the fecal flora composition (e.g., the number of fecal bifidobacteria before the prebiotic treatment), characteristic to each individual, that determines the efficacy of a prebiotic and not necessarily the dose itself. The ingested prebiotic stimulates the whole indigenous population of bifidobacteria to grow, and the larger that population the larger the number of new bacterial cells appearing in feces. The “dose argument” (often used as a marketing argument!) is thus not straightforward and cannot be generalized because, as supported by the scientific data, the factors controlling the prebiotic effect are multiple. The “dose argument” can thus be misleading for the consumers and should not be allowed. As a consequence, 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 can also not be made.

In addition, the biological significance of changes in numbers of bacteria is limited if these changes are expressed in logarithmic values alone. Indeed and again, the initial counts of, for example, bifidobacteria determine the significance of the changes induced by the consumption of the prebiotic. In absolute numbers (decimal values), even a small logarithmic increase (e.g., +0.1 log₁₀) can still represent a large increase in bacterial cell population (if the initial log₁₀ number is 7 or 9, such an increase corresponds to +10⁶ and +10⁸ respectively or 100x greater in the latter than in the former) and this can have important consequences in terms of biological activity of the microflora. Expressing changes in fecal microflora compositions in log values without reference to the initial number of, for example, bifidobacteria is thus of low, if any, value.

Future Perspectives and Conclusions

Prebiotics have great potential as agents to improve or maintain a balanced intestinal microflora to enhance health and well-being. They can be incorporated into many foodstuffs. (For more details, see Chapter 22.) There are, however, several questions that still need to be answered. For example, this review has based 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 to function...
relationship accrues, as well as on individual metabolic profiles of target bacteria and identification, isolation, and characterization of all dominant bacterial groups/genera or species in the colonic microbiota, 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 regular intake of prebiotics. With the new generation of molecular microbiological techniques now becoming available, it will be possible to gain definitive information on 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 antiadhesive approaches (Gibson 2000).

The current most popular choices for prebiotic use are lactobacilli and bifidobacteria. This is largely based upon their success in the probiotic area (Fuller 1997; Majamaa 1997; Flourié 1998; Roberfroid 1998; Gibson 2000; Kazuhiro Hirayama 2000; Capurso 2001; Fooks and Gibson 2002; Tannock 2002). However, as our knowledge of the gut flora diversity improves (through using the molecular procedures described earlier), then it may become apparent that other microorganisms could be fortified through their use. One example may be the eubacteria (Eubacterium–Clostridium cocoides cluster) which 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/lactobacilli may be more desirable than others. It is also important for colonic function, to identify molecules that can be fermented distally—the principal site of chronic gut disorders like bowel cancer and ulcerative colitis.

At the end of the present chapter aimed at updating the prebiotic definition and introducing the Handbook of Prebiotics, it must be underlined that only three carbohydrates, essentially nondigestible oligosaccharides, today fulfil the criteria for prebiotic classification (Table 3.5). For the other candidates, either data are promising but more studies are still required. In particular, it must be stressed that data regarding the fulfilment of Criterion 1, namely, “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 real drive is the nutritional, physiological, and microbial benefits of prebiotics that have been published so far and are extensively reviewed by
TABLE 3.5
Summary on the Prebiotic Status of Various Oligosaccharides

<table>
<thead>
<tr>
<th>Carbohydrate</th>
<th>Nondigestibility</th>
<th>Fermentation</th>
<th>Selectivity of Fermentation</th>
<th>Prebiotic Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inulin-type fructans</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Transgalactooligosaccharides (TOS)</td>
<td>Probable</td>
<td>????</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Lactulose</td>
<td>Probable</td>
<td>????</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Isomaltooooligosaccharides (IMO)</td>
<td>Partly</td>
<td>Yes</td>
<td>Promising</td>
<td>No</td>
</tr>
<tr>
<td>Lactosucrose</td>
<td>NA</td>
<td>NA</td>
<td>Promising</td>
<td>No</td>
</tr>
<tr>
<td>Xylooligosaccharides</td>
<td>NA</td>
<td>NA</td>
<td>Promising</td>
<td>No</td>
</tr>
<tr>
<td>Soybean oligosaccharides</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>No</td>
</tr>
<tr>
<td>Glucooligosaccharides</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>No</td>
</tr>
</tbody>
</table>

???? preliminary data, but further research is needed.  
NA = data not available.

experts in the different chapters of this handbook. Furthermore, the challenge of the future exploitation of these benefits into authentic health issues remains.

References


Prebiotics: Concept, Definition, Criteria, Methodologies, and Products


Handbook of Prebiotics


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