Chapter

Functional Attributes and Health Benefits of Novel Prebiotic Oligosaccharides Derived from Xylan, Arabinan, and Mannan

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Abstract

Prebiotic oligosaccharides are produced from many different sources, with substantial differences in chemical structure, bonds between subunits, and degree of polymerization. These structural differences can materially affect microbial utilization and the dose required for efficacy. Most prebiotic oligosaccharides are based on subunits comprised of 6-carbon sugars such as glucose/fructose and alpha bonds. Newer/novel oligosaccharides are derived from 5 carbon sugars and/or connected via beta bonds. Clinical trials with xylooligosaccharides, arabinoxylanoligosaccharides, and mannooligosaccharides have shown improvements in lipids, cholesterol, management of blood glucose, weight management, and laxation, at doses typically ranging from 1 to 4 g per day. Mannooligosaccharides are also showing promise for animal health, with the potential to reduce antibiotic use. These novel prebiotics are showing promise due to greater selectivity and their ability to deliver health benefits at a lower dose compared to conventional prebiotics.

Keywords: xylooligosaccharide, mannooligosaccharide, arabinoxylooligosaccharide, prebiotics, human health, clinical trials, animal studies

1. Introduction

There is increasing recognition of the beneficial role of prebiotics in modulating the microbiome of humans and animals, with the opportunity to beneficially impact health. While the initial focus has been the digestive tract, there has been increasing attention on modification of the microbiome on the skin, in the mouth, and in the urogenital tract. The efficacy of prebiotics is predicated upon selectively feeding beneficial microbes within the target region, with key metabolites, inflammatory and immune markers, etc. driving "distal" health benefits. Within this chapter, we focus on prebiotics that modulate the microbial community in the digestive tract, with a specific focus on novel prebiotics that are based upon 5-carbon sugars as well as 6-carbon sugars with less common beta bonds. When prebiotics are consumed, they are feeding a mixed community of microbes in a dynamic environment in the gastrointestinal tract, where carbohydrates (polymers, oligomers, dimers, monomers) of various types are broken down by digestive enzymes, absorbed into the bloodstream, and utilized by microbes via specific transport and enzyme systems. We elaborate on these concepts in sections 2 and 3, below.

We thus start by describing the environment within the digestive tract – digestive enzymes, systems for carbohydrate absorption, and the microbial communities therein. We then discuss various types of prebiotics, with a particular emphasis on differences in subunits and bond structure. These differences, coupled with differences in microbial enzymes and transport systems, contribute to differences in efficacy, selectivity, and dose between prebiotics. We then focus on prebiotics derived from xylan, arabinan, and mannan, differentiating them from "conventional" prebiotics that rely on subunits of common 6-carbon sugars. Finally, we discuss results from clinical and animal trials with these novel prebiotics, discussing impacts on human and animal health.

2. The gastrointestinal tract, gut microbiota, and carbohydrate absorption

Downstream of the stomach, the digestive tract is comprised of the small intestine and the colon. The colon is often discussed in terms of the proximal and distal regions, which is relevant in the context of prebiotics, considering both rates and locations for bacterial growth. The small intestine is the primary region for drug and nutrient absorption, although some nutrients and metabolites from microbial growth and metabolism are also absorbed from the colon, where the majority of the gastrointestinal bacteria reside. The transit time through the small intestine is very short – only a few hours, whereas the transit time through the colon may be on the order of 30–40 hours or more, depending upon dietary fiber and fluid intake, among other factors [1].

2.1 Enzymes and nutrient absorption in the digestive tract

The small intestine of the human gastrointestinal system contains amylases (from the pancreas) to break down glycogen and starch, a 6-carbon sugar with α-1,4 bonds, and brush border enzymes (lactase, maltase, dextrinase, sucrase) to break down short chain glucooligosaccharides and disaccharides such as sucrose, lactose, and maltose into glucose, fructose, and galactose [2]. Other enzymes aid in the digestion of fats and proteins. The epithelium in the jejunum and ileum of the small intestine is also specifically designed to absorb 6-carbon sugars such as glucose, fructose and galactose via passive, facilitated and active transport systems. Dimers must be broken down into monomers before absorption, and oligomers can persist further into the colon, where they feed microbes that contain enzymes and transport systems to break down complex polymers and oligomers such as xylan and inulin. Short chain fatty acids (SCFAs) can be absorbed from the small intestine, or from the colon if produced as metabolites of bacterial fermentation. It is estimated that >90% of the SCFAs produced in the colon are absorbed, where they can influence, e.g., hepatic regulation of glucose and lipids, and hormones that regulate satiety [3].

2.2 Microbial communities in the digestive tract

The digestive tract is proposed to contain about 10^{13} bacteria, with 100–300 different taxa and thousands of phenotypes [4]. The dominant gut bacteria identified by 16S RNA are *Actinobacteria, Bacteroidetes, Firmicutes, Proteobacteria, Verrucomicrobia*. The small intestine, where simple carbohydrates are abundant, contains about 1–10000 bacteria per gram of intestinal content, primarily *Clostridium, Bacteroides*, and *Streptococcus* species. Zoetendal et al. [5] sampled

the small intestine of four healthy subjects, and observed that *Bacteroidetes*, *Clostridium* (clusters XIVa, IV, XI, and IX) and *Proteobacteria* dominated, with some *Actinobacteria* and *Bacilli* also present. Zoetendal et al. [5] provides evidence that the microbiota, particularly *Streptococcus* species, in the small intestine adapts rapidly to changes in dietary intake, particularly carbohydrates, based upon the presence of transport systems and enzymes that quickly and efficiently utilize simple carbohydrates.

The colon, which survives on complex carbohydrates that are not digested or absorbed in the small intestine, contains about 10^{11} bacteria per gram of intestinal content, mainly *Prevotella, Ruminococcus*, and *Bacteroides*. Mucin degraders such as *Akkermansia mucinophila* are commonly found in the mucous layer. The GI tract, for various reasons, may also contain pathogenic bacteria at levels that may or may not be clinically significant. Furthermore, there is increasing recognition of the impact of the gut microbiota on the efficacy of drugs, since some microbes contain enzymes similar to those in the liver [6, 7], or may initiate breakdown of prodrugs. Such effects have been observed with metformin and L-DOPA, among other drugs, and may account for at least part of the interindividual variation in drug efficacy and side effects.

Most microbes evolved to process conventional 6 carbon sugars, particularly monomeric glucose, fructose and galactose. A smaller fraction is able to use mannose, arabinose, and xylose [8].

3. Background on prebiotics

3.1 Definition, brief description of different types of prebiotics, their structure, and function

Historically, the definition of prebiotics was specific to oligosaccharides affecting the gut microbiome, and health impacts arising therein. Recently, the International Scientific Association for Probiotics and Prebiotics modified the consensus definition to include other types of compounds that may act as prebiotics, and also included prebiotics that could work outside of the digestive tract [9]. Even so, it is essential that that a prebiotic must *selectively* stimulate the *growth* of beneficial bacteria, and that there must be a health benefit arising from the consumption/application of the prebiotic. Molecules such as antibiotics that modify the microbiome by acting as antimicrobial agents against undesirable bacteria would not be considered prebiotics, since they do not act as substrates for beneficial bacteria.

Fructans such as inulin and fructooligosaccharides (FOS) are most common among prebiotics in the marketplace, although galactooligosaccharides (GOS) produced from lactose are also available, and xylooligosaccharides (XOS) were available in Japan since the 1980s [10]. Recently, forms of resistant starch (RS), isomaltooligosaccharides (IMOS), arabinoxylooligosaccharides (AXOS) and mannooligosaccharides (MOS) have become available commercially.

Fundamentally, these prebiotics are all materially different, even though the end goal – promoting growth of beneficial bacteria, is the same. The types of bacteria that can be fed by each of these prebiotics depend upon enzymes and transport systems present in the bacteria, which can vary considerably. The selectivity of a prebiotic is also tied to these enzyme and transport systems; if a high percentage of the bacteria have the necessary enzymes and transport systems, then the prebiotic will feed a diverse array of bacteria, including beneficial bacteria along with undesirable bacteria. Conversely, a highly selective prebiotic may not feed many types of bacteria, because fewer types of microbes have the right microbial "machinery"

to utilize these prebiotics. Such prebiotics are less likely to directly feed undesirable bacteria if these bacteria do not have the right transport systems and enzymes. Below, we describe the different chemical structures of prebiotics, along with the enzymes and transport systems responsible for their utilization.

3.2 Chemical structures of prebiotics

Prebiotics are typically comprised of oligomers of 6-carbon and/or 5-carbon sugars, with different bonding structures, and different chain lengths (see **Figure 1** for examples) [11].

Fructans from inulin are typically linear oligomers primarily made up of fructose monomers connected by β-2,1 bonds. Fructans from agave tend to have a more complex structure with multiple side branches and β-2,6 linkages in addition to β-2,1 bonds [12]. FFn-type fructans such as inulin are comprised entirely of fructose subunits, whereas GFn-type fructans (typically shorter chain oligosaccharides) may have a glucose subunit connected by an α-2,1 bond onto the main fructan chain. These short-chain GFn-type FOS molecules are typically produced by enzymatically adding fructose subunits onto sucrose (glucose-fructose) as the starting substrate. In contrast, short chain FFn FOS would be produced by hydrolysis of inulin (long chain FFn) using endoinulinases [11, 12].

Galactooligosaccharides (GOS) may also be comprised exclusively of galactose subunits, or it may have a glucose terminal subunit arising from use of lactose (glucose-galactose) as the initial substrate to produce GOS using β-galactosidases. The galactose subunits may be connected by β-1,3, β-1,4, or β-1,6 bonds, and may include branched structures, depending upon the type of β-galactosidase [11, 13].

Xylooligosaccharides (XOS) are primarily comprised of xylose subunits connected by β-1,4 bonds, although longer chain XOS may contain branches of arabinose subunits, acetyl groups, or uronic acids (originally present in the xylan source material) that can influence their functionality. XOS that includes arabinose sub-groups are frequently referred to as arabinoxylanoligosaccharides, or AXOS [11, 14].

Mannooligosaccharides are derived from mannan in biomass, and thus are typically made up of mannose subunits connected by β-1,4 bonds [15]. Isomaltooligosaccharides (IMOS) contain glucose subunits, but vary in their bonding structure (affected by manufacturing method). α-1,6 bonds are typical, with either linear or branched structures [11].

Complex starch structures that resist breakdown by pancreatic enzymes into glucose can persist into the colon – thus leading to the concept of "resistant starch" as a prebiotic. Resistant starch (RS) is available in four forms, depending upon method of manufacture and bond structure [16, 17]. Like starch, most of the bonds are of the α-1,4 variety; the presence of other types of bonds may confer "resistance". RS1 is conventional starch that may be trapped within whole grains. RS2 is typically starch with more complex branching or bond structures, rendering it less accessible to amylase. RS3 is produced when starch undergoes retrogradation, i.e., cooked starch is cooled below its gelatinization temperature. RS4 starches have undergone chemical modification, usually by acidification or cross-linking [16].

The microbial ability to utilize specific substrates is dependent upon the enzymes and transporters encoded within the cells. Some microbes can utilize a broad set of substrates, while others are more selective. This also points to the selectivity of the prebiotic; preferably, the prebiotic preferentially feeds beneficial bacteria, with limited growth of undesirable bacteria. **Table 1** summarizes the key hydrolytic enzymes and the corresponding substrates/reactions [19]. These

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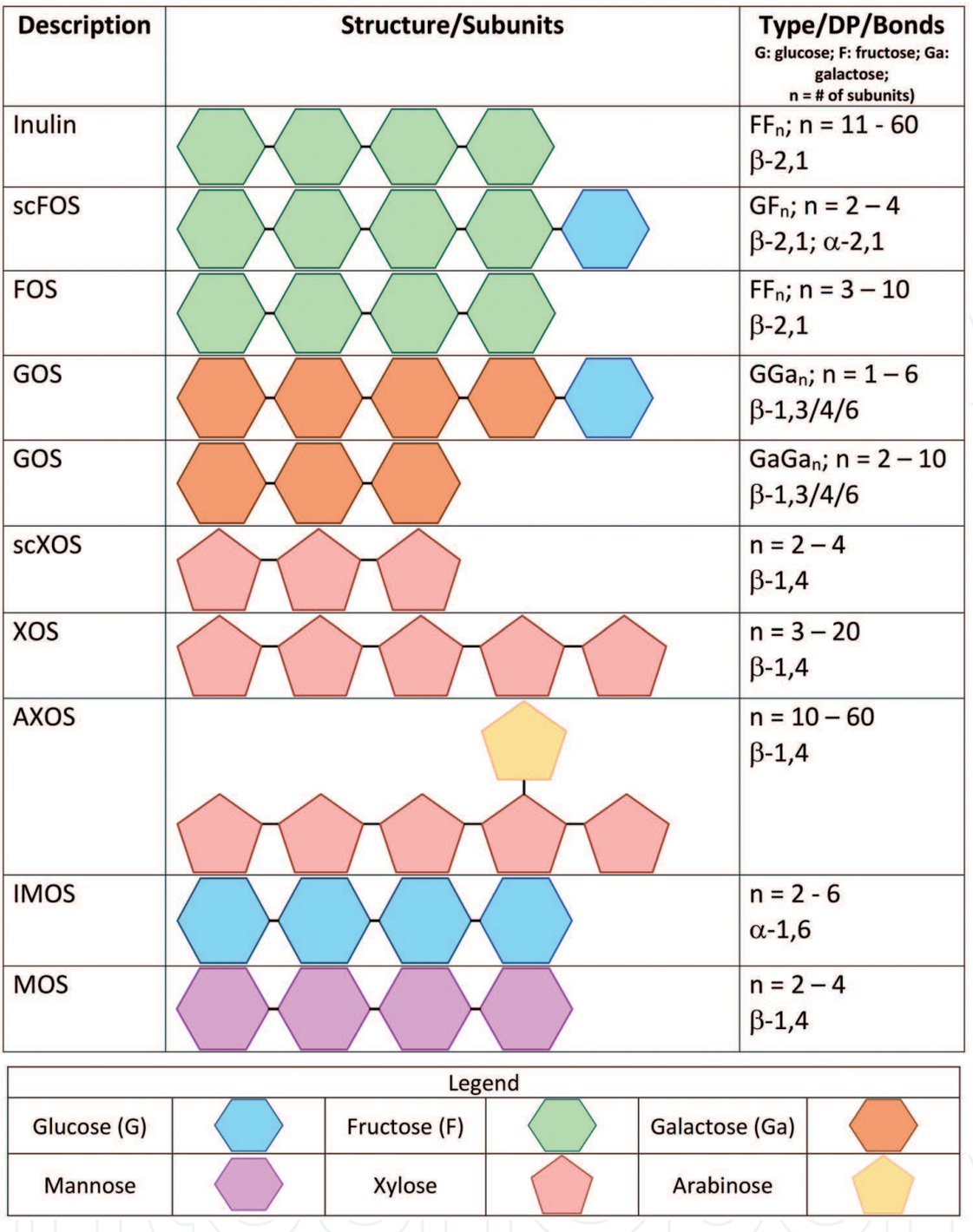

Figure 1.
Chemical and bonding structures of prebiotics.

enzymes may be extracellular, or intracellular, which can influence substrate utilization; intracellular enzymes require a corresponding transporter system.

There are various types of transport systems that move prebiotics into the cell, although microbes are likely to only possess a subset, targeted towards a narrower set of substrates. Some transport systems are specific to (certain) monomers, while others target specific oligosaccharides. Chemical structures must be matched to the structure of the transport system in order to be transported into the cell. **Table 2** summarizes key membrane transport systems involved in utilization of prebiotics and other carbohydrates.

From a selectivity perspective, it is advantageous if the substrate is used intracellularly – thus, the requisite transport system plus intracellular fructofuranosidases

Enzyme	Substrates, reactions
α-amylase, glucoamylase, pullulanase	Converts starch and RS into glucose
Inulinase, β-fructofuranosidase	Converts inulin into FOS, and FOS into fructose/glucose
β-galactosidases	Converts lactose into glucose and galactose; aids individuals with lactose intolerance [18]
β-Endoxylanases, β-xylosidases	Converts XOS into short chain XOS (sc-XOS) and xylobiose, then xylose
β-endoglucanase, cellobiohydrolase, β-glucosidase	Converts cellulose and β-gluco-oligosaccharides into cellobiose, then glucose
β-galactanase, β-galactosidase, α-galactosidase	Converts galactan into β-GOS, then galactose; β-galactosidase can also remove galactose subunits that are present as side chains in xylan/XOS
β-mannanase, β-mannosidase	Converts MOS into mannobiose, then mannose
α-arabinofuranosidase	Releases arabinose from side chains of xylan and AXOS

Table 1.
Key enzymes for carbohydrate utilization.

would have an advantage (i.e., FOS that can be transported intracellularly would have an advantage over FOS/inulin that can only be processed via extracellular enzymes). The restricted capacity of most transporter systems precludes use of long chain fructans by many *Lactobacillus* species [11].

3.3 Types of microbes with enzyme/transport systems of various types

Microbes that have fructofuranosidase encoded extracellularly are able to use long-chain FFN-type FOS, and inulin. Some species of *Lactobacillus* (casei, paracasei) and *Streptococcus* are examples [11]. The resulting short chain fructans may be transported intracellularly for utilization. Mao et al. [23] identified 19 strains from human feces that were capable of metabolizing FOS, including multiple strains of *E. coli*, *Enterobacter cloacae*, *Bifidobacterium* spp., and *Lactobacillus* spp. Additional bacteria also proved capable of growth on FOS. This includes additional strains of *E. coli and Bifidobacteria*, along with several strains of *Streptococcus, Clostridia, Roseburia, Klebsiella*, and *Enterococcus* [23].

According to Rossi et al. [24], virtually all *Bifidobacteria* are able to grow on short-chain FOS. However, most *Bifidobacteria* grew poorly on inulin (only 8 out of 55), because most of the fructofuranosidases are intracellular, and inulin cannot be transported intracellularly. Scott et al. [25] made similar observations when inulin with a DP >25 were fed to *Bifidobacteria*. However, in a mixed culture system, inulin may be broken down into shorter chain FFn-FOS, and then used by *Bifidobacteria*. Thus, growth of *Bifidobacteria* in the presence of inulin is primarily due to cross-feeding in the presence of other microbes that act as primary degraders, rather than direct feeding by inulin.

Different species of *Bifidobacteria* contain ABC transporters, sucrose permeases, fructose PTS transporters, and MFS transporters. The type(s) of available transporters dictate substrate utilization, whether GFn-type short chain FOS, FFn-type FOS, or analogous substrates. Although several strains of *Bifidobacteria* can utilize GOS, there are significant differences between strains [26]. Certain strains of *B. breve* and *B. longum* contain an extracellular galactanase that breaks down long-chain GOS and galactan in plant fiber, producing di- and

Transport system	Target molecules	Examples/implications
ATP-dependent binding cassette (ABC-type) transporter system	There are many variations of the ABC transporter. Transporters in the CUT1 class work on sucrose, lactose, maltose, FOS, maltodextrins, XOS, and other oligosaccharides. Transporters in the CUT2 class generally transport monomers such as arabinose, xylose, ribose, glucose [20, 21]	Such a system is present in *L. acidophilus*, for utilization of short-chain FOS; *Bifidobacteria* have an ABC transporter specific to XOS; various enterobacteria, including *E. coli*, have CUT1 and CUT2 transporters for maltose and various 6-carbon sugars
Sucrose phosphoenolpyruvate phosphotransferase (PTS) transport system	Transport system is highly specific to compounds that incorporate sucrose as part of their structure.	Allows *L. plantarum* to utilize short-chain FOS synthesized from sucrose (GFn-type), but FFn FOS, which lacks the sucrose structure, cannot be used by *L. plantarum*
Major facilitator superfamily (MFS) transporter system	A major transporter system with various types, allowing intracellular transport of glucose, lactose, xylose, oligosaccharides, FFn-type FOS [11, 21]	Present in many bacteria, fungi, yeasts, plants, animals, humans; key for energy homeostasis
Fructose PTS transporters	Transport system targets the fructose component of substrates, thus allowing use of fructose, sucrose, inulin, and both FFn and GFn-type FOS.	*L. rhamnosus GG* contains a fructose PTS transporter, which allows growth on various types of FOS and inulin
Lactose PTS transporters	Transport system targets the lactose component of substrates	*L. gasseri* contains a lactose PTS transporter [22]
LacS and LacY permeases	MFS-type transport systems enabling transport of molecules with lactose module. The LacS transport system allows a microbe to use lactose, GOS (with lactose terminus), and lactitol. LacS and LacY differ based upon source family.	LacS is stated to be the sole transporter for GOS, with specificity for β-galactosides [22].
Sucrose permease	Transport of substrates with a sucrose module, such as GFn-type FOS.	

CUT = Carbohydrate Uptake Transporter; FFn = FOS comprised of "n" fructose (F) subunits and a fructose terminal unit; GFn = FOS containing "n" fructose (F) subunits and a terminal glucose (G); PTS = phosphoenolpyruvate phosphotransferase.

Table 2.
Key Transmembrane transport systems.

tri-saccharides that can be transported into the cell and converted into galactose via intracellular β-galactosidase [27]. *B. lactis* BI-04 contains lactose permease and ABC transport systems, along with β-galactosidase [28], that enable utilization of GOS. *L. acidophilus* has the ability to utilize many different prebiotics, with various monomeric subunits and bond structures [28]. Such broad utilization is due to a multiplicity of molecular transport systems and hydrolytic enzymes, including up to nine different enzymes from the GH13 family that act on α-glucan [11].

Starch, owing to its high DP and complex branched structure, would be degraded in the presence of extracellular enzymes that can act on α-1,4 and α-1,6 linkages between glucose subunits. Certain *Bifidobacteria*, including *B. pseudolongum* and *B. breve* have the necessary enzymes for extracellular starch utilization [29].

Microbes such as the *L. acidophilus* cluster (including *L. johnsonii*, *L. helveticus, L. reuteri* and *L. plantarum*) contain LacS permease and β-galactosidase which allow these microbes to transport GOS into the cell, then break it down into glucose and galactose for metabolism [28].

Several *Bifidobacteria* contain the hydrolytic enzymes needed to break down the β-1,4 linkages present in xylooligosaccharides (XOS) and XOS with arabinose side groups (AXOS). Key enzymes include β-xylosidase and β-xylanase, the latter which breaks down longer chain XOS into shorter chains, ultimately xylobiose, that may be converted into xylose using β-xylosidase. AXOS requires arabinofuranosidase enzymes to process the arabinose side group. Some carbohydrate esterases may also be present to deal with acetyl or feruloyl side groups. The enzymes may be intracellular or extracellular; intracellular enzymes also require transporters such as an ABC transport system to act on the longer chain oligomers. Ejby et al. [30] noted that ABC transporters specific to XOS are exclusive to *Bifidobacteria*. *B. lactis, B. breve*, and *B. bifidum* are among the many species of *Bifidobacteria* that have the requisite enzymes and transport systems for utilization of short and longer chain XOS and AXOS. Crossfeeding of *Bifidobacteria* is aided by *Bacteroides* and *Prevotella*, which act as primary degraders that break down insoluble xylan in plant fiber into soluble oligosaccharides.

The wide variation in structures of prebiotics, along with the different transport and enzyme systems, ultimately dictate the selectivity of the prebiotic in a mixed culture. Monoculture systems provide some useful insights into the utilization of prebiotics by various substrates. Makelainen et al. [31] conducted a thorough study of the growth of >15 microbes, some beneficial, some pathogenic, in the presence of 11 different carbohydrate sources. Aggregate growth over 24 hours was reported as the area under the curve of DP600 measurements. **Figures 2–6** show growth of various probiotics and pathogenic microbes on glucose, FOS, GOS, scXOS, and XOS, respectively. As expected, all bacteria grew well on glucose (**Figure 2**), consistent with the widespread ability of microbes to utilize simple 6-carbon sugars.

Low DP FF_n-FOS proved to be a good substrate for several strains of *Bifidobacteria*, along with *L. paracasei* and *L. acidophilus*, but was also used by *E. coli EHEC*, *S. epidermis*, and *C. perfringens*, among pathogenic bacteria tested. GOS also grew well on *Bifidobacteria* and *Lactobacilli*, but also proved to be an excellent substrate for several pathogenic bacteria, including *E. coli* and *C. perfringens*.

Consistent with the unique chemical structure and enzyme/transporter requirements, there was less microbial growth on scXOS and XOS. Fewer strains of *Bifidobacteria* utilized XOS, along with select strains of Lactobacillus. Li et al., in a study using 29 *Lactobacillus* strains and 35 strains of *Bifidobacterium*, observed that all *Bifidobacterium* strains tested grew on a high dose of XOS, and 30 of 35 strains grew on low dose XOS [32]. They also noted that *Lactobacillus* strains were able to utilize XOS, albeit with fewer strains and at lower efficiency compared to *Bifidobacteria*.

However, importantly, Makelainen et al. [31] noted minimal growth of pathogenic bacteria in the presence of XOS (**Figures 5** and **6**), consistent with a much higher selectivity of XOS for beneficial bacteria. This is a key advantage in a mixed microbial environment such as the GI tract. *Bifidobacteria* and *Lactobacillus* species have to compete with many more bacteria for FOS and GOS, which thus increases the dose required for efficacy. XOS, conversely, is better targeted to *Bifidobacteria*, and in a mixed culture, could be efficacious at a lower dose.

The aggregate area under the curve data reported by Makelainen et al. [31] do not, however, capture changes in growth rates, which can vary over time. Similarly,

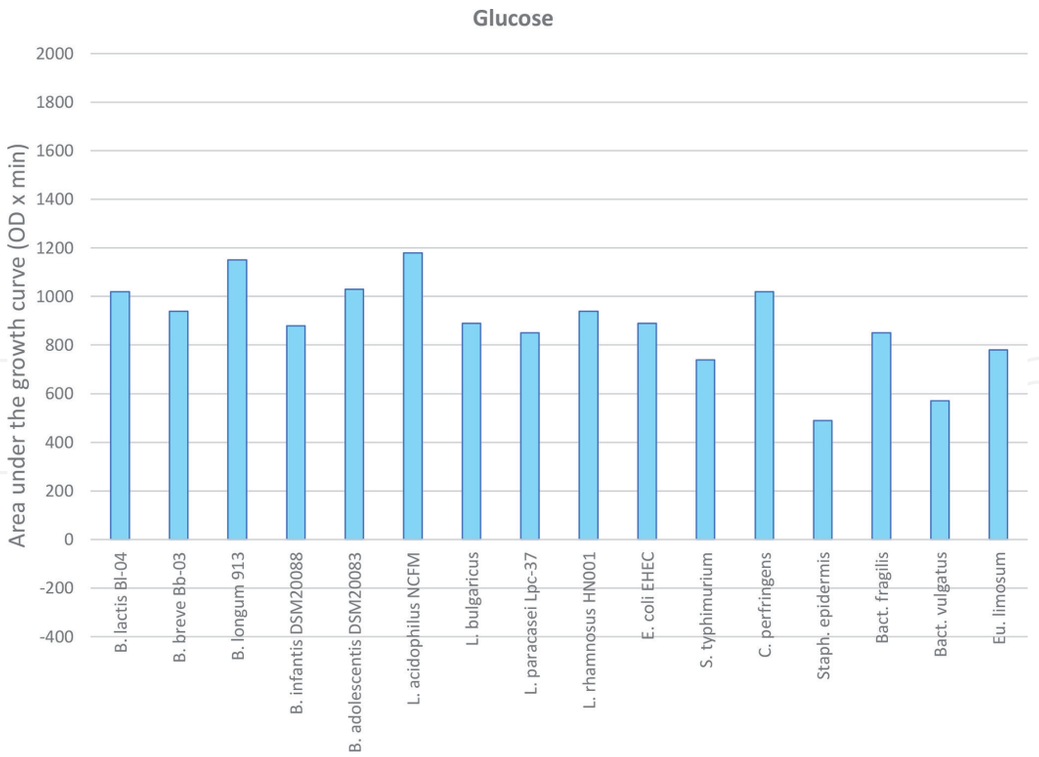

Figure 2.
Microbial growth on glucose (positive control). Data from Makelainen et al. [31].

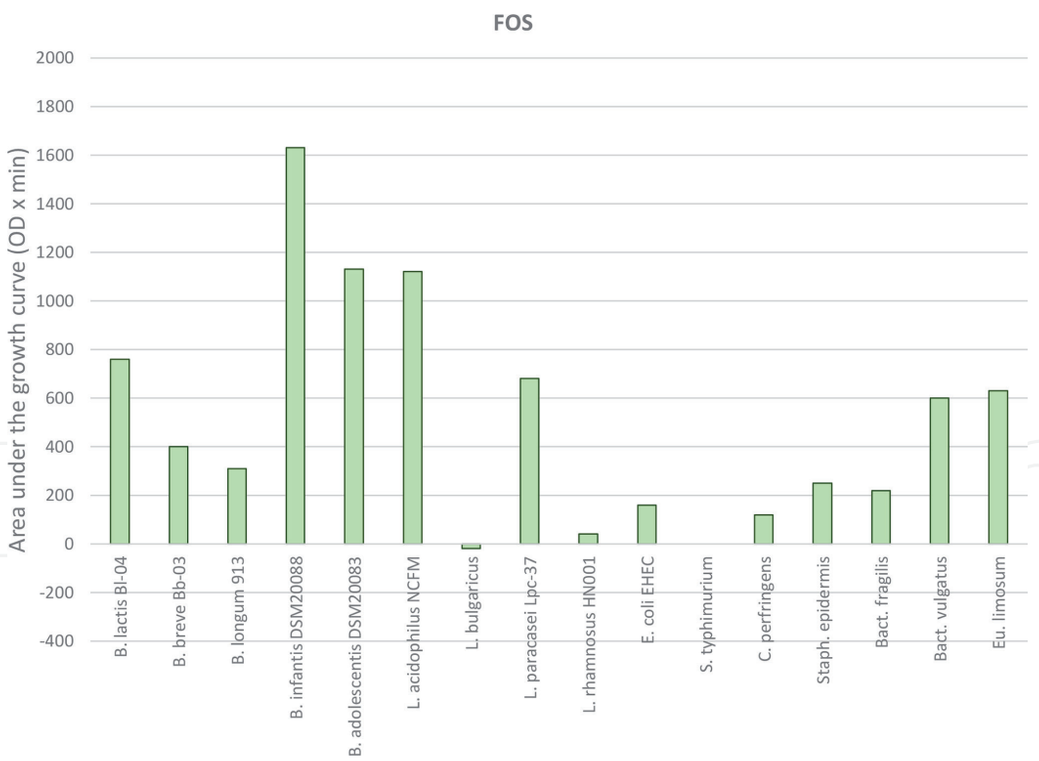

Figure 3.
Microbial growth on FF_n FOS (DP 2–7). FFn = FOS comprised exclusively of fructose (F) subunits. Data from Makelainen et al. [31].

any issues with viability of microbes could be masked by rapid early growth, which may not be sustained. **Figure 7** illustrates growth of a strain of *B. breve* on FOS, XOS, and inulin, showing temporal effects. A noteworthy observation is that viability of *B. breve* decreased significantly after ~12–16 hours if grown on FOS or inulin, whereas growth on XOS sustained *B. breve* for a longer period, even up to 48 hours.

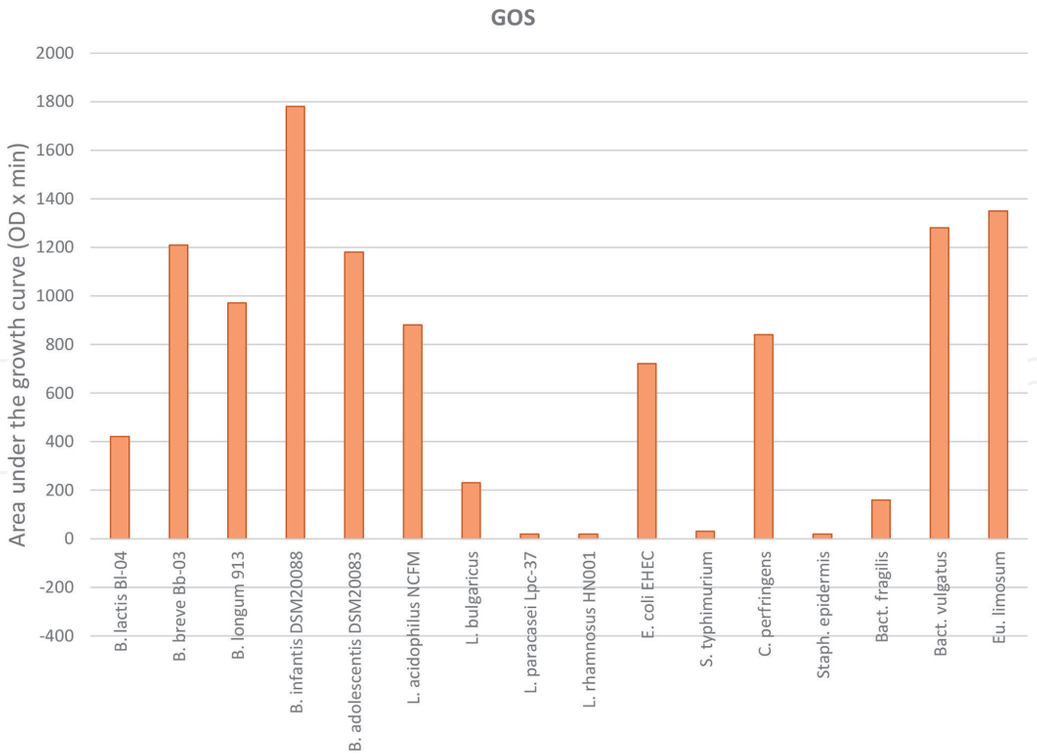

Figure 4.
Microbial growth on GGa_n GOS (DP 3–5). GGa_n GOS is comprised of n subunits of galactose (Ga) with a terminal glucose (G) subunit. Data from Makelainen et al. [31].

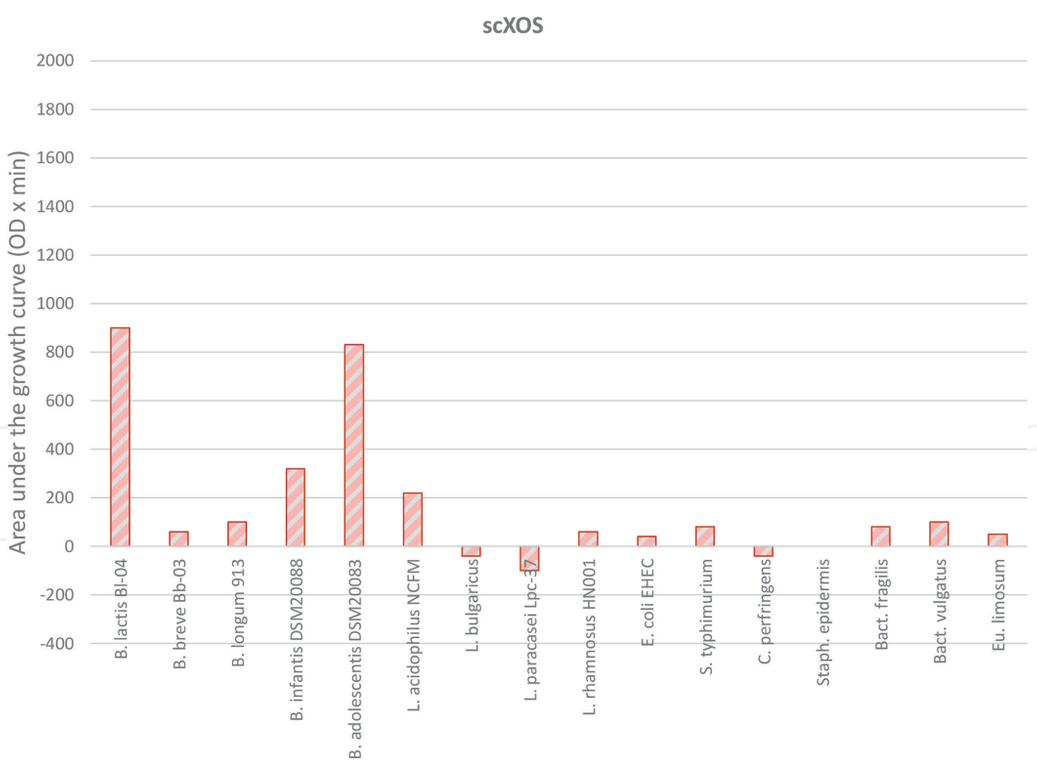

Figure 5.
Microbial growth on short chain XOS (scXOS; DP 2–5). Data from Makelainen et al. [31].

This may have important implications in terms of sustaining key microbes in the digestive tract.

In the next section, we describe health impacts of prebiotics, with a particular emphasis on studies with XOS, AXOS, and MOS, due to their distinct chemical structures and selectivity for beneficial bacteria.

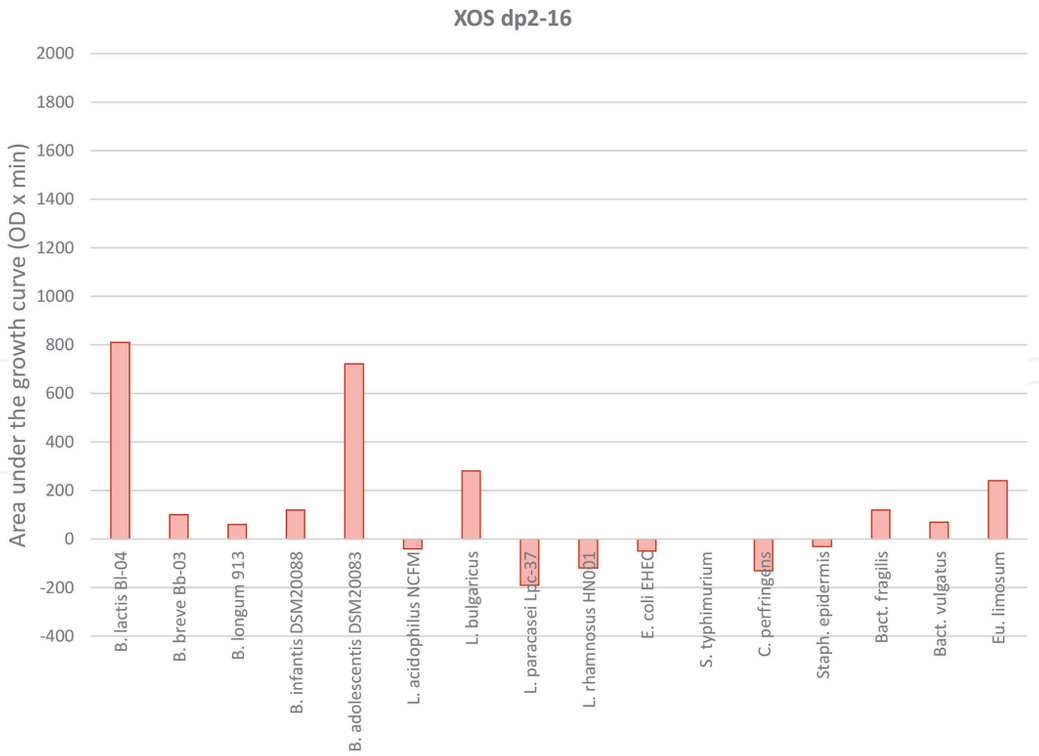

Figure 6.
Microbial growth on XOS (DP 2–10). Data from Makelainen et al. [31].

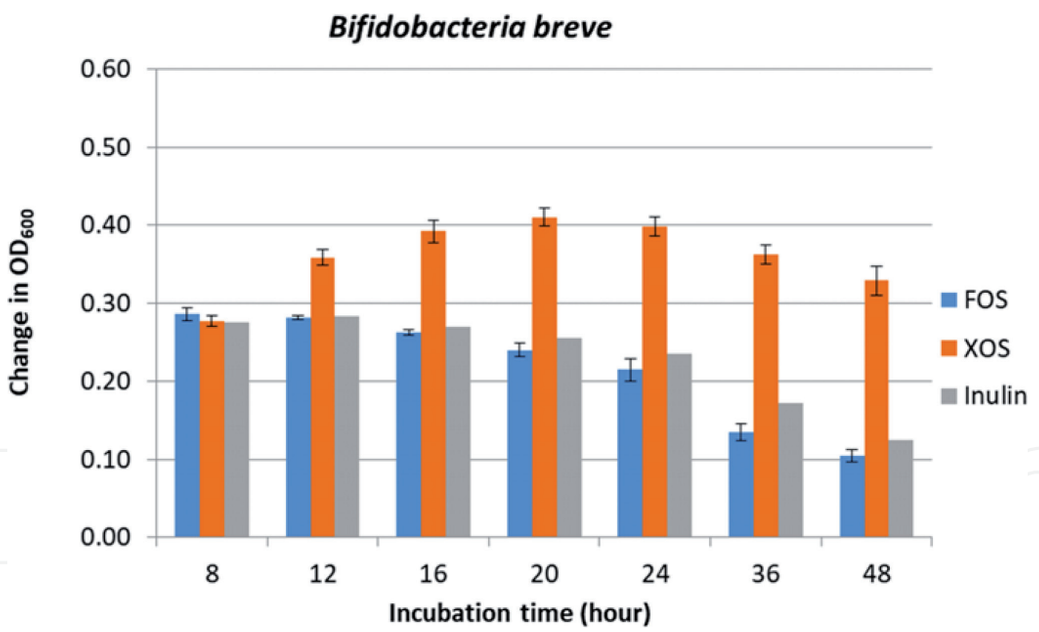

Figure 7.
Comparative growth of B. breve on FOS, XOS, and inulin.

4. Health impacts of prebiotics

Stimulating the growth of beneficial bacteria with prebiotics can lead to a cascade of health effects, as illustrated in **Figure 8**. Much of the historical focus had been on *Bifidobacterium spp*. and *Lactobacillus spp.*, although more recently, beneficial health outcomes have been associated with other microbial species as well. Short chain fatty acids, primarily acetate, propionate, and butyrate, along with lactate, are produced by fermentation of non-digestible fibers and prebiotics, potentially reducing the pH within the colon. This can promote absorption of minerals such as

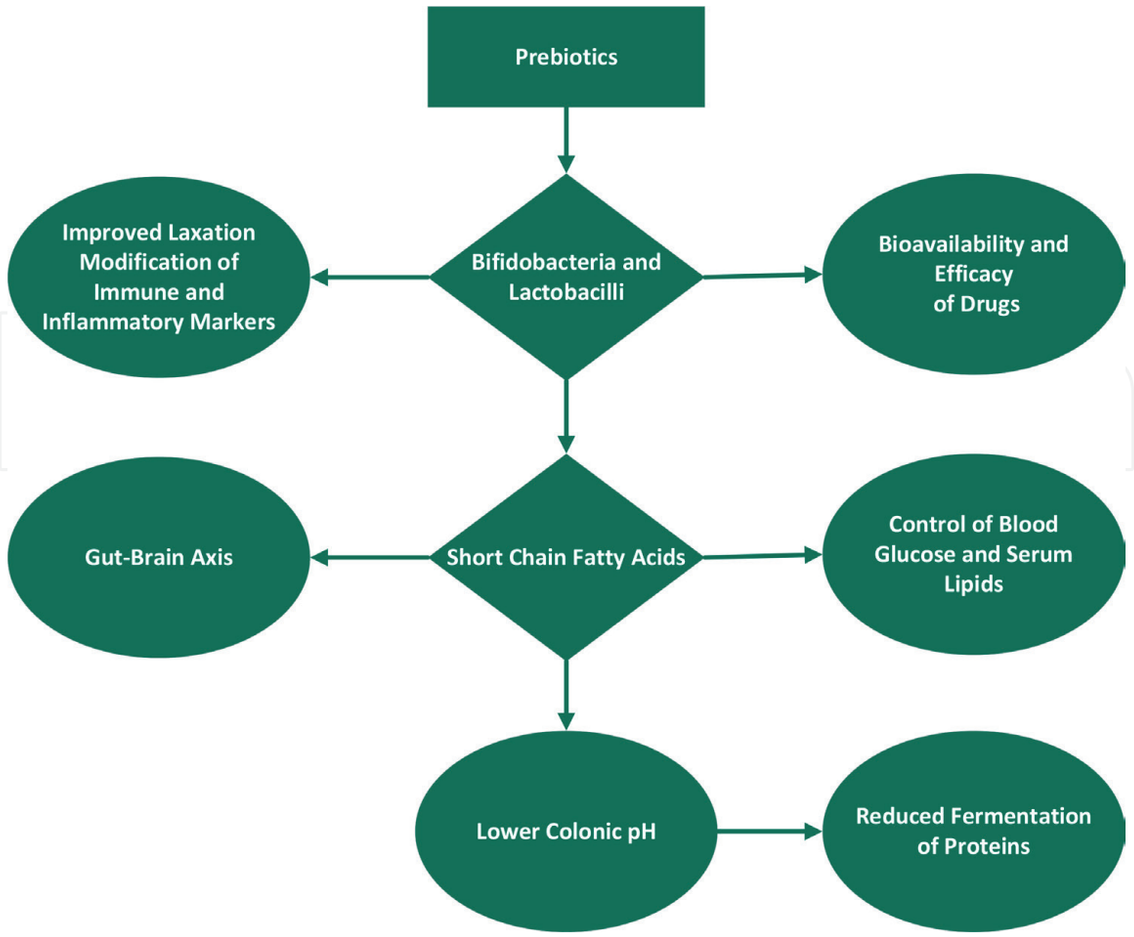

Figure 8.
Health impacts associated with prebiotic intake.

calcium, and limit growth of yeasts, potentially pathogenic bacteria, and microbes responsible for protein fermentation [3]. The extent of such a pH reduction *in vivo* is difficult to establish, however, given that greater than 90% of SCFAs are typically absorbed, and as little as 5% may be excreted [33]. Thus, changes in fecal SCFA levels (or pH) may be difficult to detect, or may not represent what is present in the digestive tract. Autopsy samples indicate SCFA levels are an order of magnitude greater in the cecum than the ileum, and that levels decrease from about 70–140 mM in the proximal colon to 20–70 mM in the distal colon [33].

The effect of increased colonic SCFA production may, however, be observed in the form of distal health impacts mediated by the SCFAs that have been absorbed into the bloodstream. Each SCFA has a different effect on host metabolism, yet the direct effect of a prebiotic on SCFAs can be difficult to establish, due to crossfeeding between microbes that directly consume the prebiotic and other microbes within the digestive tract. Dietary fiber intake also affects SCFAs, further clouding interpretation of fecal SCFA levels. The impacts of prebiotics on SCFA levels are easier to detect via *in vitro* cultures, particularly monocultures, and in gut simulators that contain mixed microbial communities that enable cross-feeding, such as acetate production via one class of microbes, and acetate conversion into butyrate by, e.g., *E. rectale, F. prausnitzii,* or *Roseburia* spp. [3].

SCFAs provide energy for colonocytes, and, after absorption into the portal vein, are metabolized by the liver, modulating cholesterol synthesis, maintaining glucose homeostasis in peripheral tissues, and producing long chain fatty acids. SCFAs can act on leukocytes that modulate immune responses, beneficially impact hormones that influence satiety, and influence neural signals that modulate appetite and food intake via the gut-brain axis [3]. Acetate can influence hormones responsible

for cholesterol production, and may be transported to the muscle and the brain; acetate may thus contribute to effects attributed to the "Gut-Brain Axis". Propionate can play a role in hepatic regulation of glucose and synthesis of cholesterol, while butyrate can promote growth of protective colonocytes via colonic epithelial cells. Consequently, microbes fed directly or indirectly by prebiotics can influence health via SCFAs that act either locally within the digestive tract, or distally, mainly via the liver, and to a lesser extent, in the muscle, kidney, heart, and brain [3].

4.1 XOS/AXOS

As noted previously, XOS and AXOS are oligomers of 5-carbon sugars, connected by β-1,4 bonds. Fewer types of microbes contain the enzymes and transport systems necessary to utilize XOS and AXOS, thus conferring greater selectivity to beneficial bacteria (see **Figures 5** and **6**). Mannooligosaccharides (MOS), although based upon a 6-carbon sugar backbone, are connected via β-1,4 bonds that also render them less susceptible to microbial utilization. In this section, we summarize clinical trial results from these novel prebiotic oligosaccharides.

Tables 3 and **4** summarize clinical trial results with XOS and AXOS, respectively, outlining impacts on the microbiome and various clinical biomarkers. Clinical trials with XOS were conducted with doses ranging from 0.4 to 8 g per day; most were in the range from 1 to 3 g per day. A statistically significant increase in *Bifidobacteria* was observed in most trials with XOS [34–39]. Improvements in laxation were noted by Childs et al. [34], Chung et al. [35], Iino et al. [40], and Tateyama et al. [41]; the latter was a study in constipated pregnant women. Studies by Childs et al. [34], Na and Kim [39], and Sheu et al. [42] observed improvements in triglyceride and cholesterol levels, at doses as low as 2.8 g/d, whereas Yang et al. [43] did not observe changes in triglycerides when dosing XOS at 2 g/d (2.8 g/d of a 70% purity product). Yang et al. [43] observed a tendency towards a reduction in OGTT insulin in prediabetes patients dosed with 2.8 g/d of 70% XOS, but no effect on blood glucose, unlike Na and Kim [39], who noted a reduction in blood glucose at a XOS dose of 2.8 g/day. Sheu et al. [42], in a trial with diabetic patients receiving 4 g/d of XOS, observed a statistically significant reduction in blood glucose and HbA1C.

Studies with AXOS complement studies with XOS, since most AXOS products are comprised of at least ~50% XOS. All studies noted an increase in *Bifidobacteria* [44–48]. Francois et al. [45] and Walton et al. [48] observed increased fecal levels of SCFAs (acetate, propionate, and butyrate). Reductions in cresol, indicative of reduced protein fermentation, were observed by Cloetens et al. [44] and Francois et al. [45] with AXOS, and Lecerf et al. [38] with XOS.

The bifidogenic effect and health benefits observed in the various clinical trials with XOS and AXOS where generally observed at a dose less than 4 g/d. By comparison, approximately 10–20 g/d of FOS is needed to trigger a bifidogenic effect, and the required inulin dose is stated to be at least 15 g/d [49, 50]. Alfa et al. [51] required a dose of 30 g/d of resistant starch to enhance *Bifidobacterium* levels.

Miremadi et al. [52] summarized clinical trials in which various prebiotics were evaluated to assess cardiovascular impacts, particularly reductions in cholesterol, lipids and blood pressure. No improvements to lipid profile were observed with 5.5 g of GOS or 20 g/d of FOS (type not specified), whereas triglyceride levels improved following consumption of 10 and 20 g/d of inulin. Similarly, Alles et al. were unable to detect changes in blood glucose and lipid profiles following administration of 15 g/d FOS [53].

The higher doses required for a bifidogenic effect or clinical efficacy compared to XOS and AXOS is likely due to the greater selectivity of XOS and AXOS for beneficial bacteria, versus the widespread utilization of FOS and GOS [11, 23, 31].

Study authors	XOS purity / dose	Study design and duration	Endpoints
Childs et al. [34]	8 g per day with or without *B. lactis*	Randomized, double-blind, placebo-controlled, factorial cross-over study with 41 healthy adults; 3 weeks dosing, with a 4 week washout between dosing periods	Increased HDL levels by 0.07 mM (P = 0.005) Increase of 0.10 mM is associated with 10% risk reduction in coronary artery disease Increased # of bowel movements per day (P = 0.009) Increased Bifidobacteria (P = 0.008) Reduced IL-10 production (P = 0.049)
Chung et al. [35]	95% pure XOS, 4 g per day	Randomized, placebo-controlled trial with 22 healthy adults (9 control, 13 XOS); includes a 1 week run-in, 3 weeks of dosing, and a 3 week washout	Statistically significant increase in fecal moisture (alleviates constipation) and fecal pH, with return to baseline post-washout (P < 0.05) Statistically significant increase in *Bifidobacteria* content of the stool (P < 0.05)
Finegold et al. [36]	70% pure; 1.4 or 2.8 g per day	Randomized double blind placebo-controlled trial with 32 healthy adults; 2 week run-in period, followed by 8 weeks of treatment and a 2 week washout	Statistically significant increase in *Bifidobacteria* (P = 0.007; 2.4 g per day dose) and *B. fragilis* (P = 0.001; 2.4 g per day dose) relative to placebo/baseline
Iino et al. [40]	0.40 g per day	4 Weeks with 40 healthy adults	Improved stool frequency (P < 0.05)
Kajihara et al. [37]	3 g per day	Baseline data in patient population comprised of 14 adults with cirrhosis, followed by 2 weeks of dosing with 3 g per day of XOS.	Increased *Bifidobacteria* content Reduced *Bacteriodes* Reduced ammonia in serum (statistical data not presented)
Lecerf et al. [38]	5 g per day WitaXOS (80% Pure); 1 g per day XOS + 3 g per day inulin	Randomized, placebo-controlled, doubled blind trial with 60 healthy adults (20 in each of three groups: (i) XOS, (ii) XOS and inulin, (iii) placebo); 4 weeks	Increased *Bifidobacteria* (P = 0.002) Increased butyrate (P = 0.036) Reduced cresol (P = 0.020), acetate (P = 0.011) and fecal pH (P = 0.033)
Na and Kim [39]	1.4 and 2.8 g per day	Randomized dose-dependent trial without a placebo with 14 healthy adults (7 per test group); 4 weeks	Reduced triglycerides, cholesterol and glucose in 2.8 g per day group (all P < 0.05) Increased *Bifidobacteria* content after 14 days (P < 0.05) Increased lactic acid concentration (P < 0.05) While study lacked placebo, subjects saw effect of dose response over multiple doses
Sheu et al. [42]	4 g per day	Randomized, double blind placebo-controlled trial in 26 adults with type II diabetes; 8 weeks	Statistically significant reduction in: Blood glucose (P < 0.05) (described as a point measurement of blood glucose, with the sample obtained after fasting) LDL (P < 0.01) Total cholesterol (P < 0.01) HbA1c (P < 0.05) Apolipoprotein B (P < 0.05)
Tateyama et al. [41]	4.2 g per day in 8 g syrup	29 Adult Women (pregnant with constipation); all received product, at the same dose, for 4 weeks	Statistically significant increase in stool frequency and reduction in constipation Improvement increased every week over the duration of treatment Normalized stool consistency
Yang et al. [43]	2.8 g per day of 70% XOS	8 week trial with 13 healthy adults and 16 prediabetic (increased risk of diabetes) adults	Decreased *Firmicutes* Attenuated changes in *Howardella, Slackia*, and *B. hydrogenotrophica* Tendency to reduce OGTT insulin No effect on blood glucose, triglycerides Limited by small study numbers and lack of statistical analysis

Table 3.
Summary of clinical trials with XOS.

For example, Mao et al. [23] found that 237 out of 453 strains (114 genera) of gut bacteria contained the necessary transporters and enzymes for some type/degree of FOS utilization, suggesting fairly widespread utilization of FOS, which will impact the effective dose required to grow the targeted beneficial bacteria. Similarly, Goh et al. [11] note the widespread ability of microbes to utilize lactose, suggesting the presence of LacS (or similar) transporters and enzymes in many microbes that

Study Authors	AXOS Product / Purity / Dose	Study Design and Duration	Endpoints
Cloetens, L. et al. [44]	AXOS, 10 grams per day (63% XOS, 17% arabinan, 12% glucan)	Randomized, placebo-controlled cross-over study with 20 healthy adults; 3 weeks with AXOS or placebo, with 4-week washout between treatments	Increased *Bifidobacteria* (P = 0.012) Increased *B. adolescentis* (P = 0.013) After 3 weeks, reduced urinary p-cresol (P = 0.011)
Francois, I. et al. [45]	Fugeia wheat bran extract, 3 grams per day, 10 grams per day (comprised of 79% AXOS, which, in turn, consists of 49% XOS and 10% glucan) [1]	Double blind randomized placebo controlled cross-over trial with 20 healthy adults; 3-week treatment periods interspersed with 2-week washout periods	At 10 grams per day: Increased acetate, propionate, and butyrate (P = 0.009, P = 0.05, P = 0.001, respectively) Reduced p-cresol (P = 0.039) Lower fecal pH (P = 0.039) Increased *Bifidobacteria* (P < 0.001) Increased stool frequency (P = 0.258) Reduced LDL (P = 0.168)
Francois, I. et al. [46]	Fugeia Wheat Bran Extract, 5 grams per day (comprised of 79% AXOS, which, in turn, consists of 49% XOS and 10% glucan)	Double blind randomized placebo controlled cross-over trial with 29 healthy children; 3 weeks	Increased *Bifidobacteria* (P = 0.069) Reduced isovaleric and isobutyric acid (P < 0.01) No impact on stool frequency or consistency
Maki, K.C., et al. [47]	2.2 grams or 4.8 grams AXOS per day added to cereal	Randomized, double blind, placebo-controlled cross-over trial with 65 healthy adults; 3 weeks treatment with 2 weeks washout between treatments	Dose-dependent increase in Bifidobacteria observed; statistically significant increase in the 4.8 grams per day AXOS group (P<0.001) No statistically significant impact on fasting blood glucose, total cholesterol, HDL, or triglycerides
Walton, G. et al. [48]	180 grams per day of Bread, with or without enrichment with 2.2g AXOS	Double-blind, randomized, placebo-controlled cross-over trial with 40 healthy adults; five periods of 3 weeks each	Increased *Bifidobacteria* relative to baseline (P = 0.0011) Increased butyrate (P = 0.041) and propionate (P = 0.045) Other results confounded by XOS/fructans in the placebo bread, which increased *Bifidobacteria*, short chain fatty acids, and bowel frequency relative to the baseline. The trend to further improve these characteristics via the AXOS-enriched bread were thus masked.

Table 4.
Summary of clinical trials with AXOS.

would allow use of the GGa_n type of GOS. This is consistent with the observations of Makelainen et al. [31], who also observed excellent growth of many species, beneficial and pathogenic, on GOS (see also **Figure 4**). Ultimately, increased sharing of prebiotic substrates among bacteria means that a higher dose is needed to trigger sufficient growth of the targeted beneficial bacteria that produce the desired health benefits.

4.2 MOS

Manno-oligosaccharides (MOS), extracted from yeast cell walls, coffee or biomass rich in mannan, are typically comprised of up to 6 mannose subunits connected by β-1,4 bonds. MOS have not been as extensively evaluated in humans, but have been recognized as a nutritional supplement for livestock.

Salinardi et al. [54] observed a reduction in body volume and adipose tissue in men consuming 4 g/d MOS, attributed to an increase in fat excretion in the feces or inhibition of hepatic lipogenesis. Kumao et al. [55] observed a reduction in fat utilization and an increase in fecal fat excretion in people consuming 3 g/d of MOS for 7 days. St.-Onge et al. [56] also observed a reduction in body weight and adipose tissue following consumption of 4 g/d MOS for 12 weeks. Umemura et al. evaluated

the impact of 1 g/d of MOS consumption on fecal microbiota and laxation [57]. They noted an increased ratio of *Bifidobacterium* spp., and enhanced defecation frequency/volume, reducing constipation. A study in mice by Zheng et al. [58] suggested that MOS acted synergistically with metformin, altering the gut microbiota in a manner that would decrease clinical diabetic parameters, including a reduction in blood glucose. This may have promise for future clinical trials and application of MOS plus metformin for management of type II diabetes.

Jahromi et al. [59] examined MOS supplementation (1 g/kg) to broiler chicks, and observed increased levels of *Lactobacillus spp*. and *Bifidobacteria spp*., while reducing levels of *E. coli* and *Enterobacter* by >50%. Navidshad et al. [60] compared MOS derived from yeast cell walls with MOS from palm kernel expeller, assessing their efficacy as a supplement (2 g/kg) to the diet of broiler chicks. The yeast-derived MOS improved weight gain, while reducing the intestinal percentage of *E. coli* in the birds. MOS has been reported to have receptors for fimbriae on *E. coli*, which can help to control or limit colonization within the digestive tract [61]. Jahromi et al. [62] also evaluated *In Ovo* injection of MOS, and feeding of MOS to chicks. The single *In Ovo* injection had some short term but limited long term effects, other than an increase in *Bifidobacterium* spp. at 14 days. Feeding MOS in the diet markedly improved levels of *Lactobacillus* and *Bifidobacteria*, while reducing levels of pathogenic strains of *Salmonella*, *E. coli*, and *Campylobacter*. Adding MOS to the diet also improved levels of serum immunoglobulins IgA, IgM, and IgG. The immunomodulatory effect of MOS in chicks was stated to be a significant benefit that could help improve productivity, and reduce disease (thereby reducing use of antibiotics). Zhao et al. [63] studied the impact of supplementing 0.1 wt% MOS or 0.1 wt% FOS in weanling pigs over 28 days. They observed greater average daily weight gain and average daily feed intake in pigs consuming MOS compared to controls. Nutrient digestibility also improved, along with diarrhea score (potentially by inhibiting *E. coli*). Collectively, the authors concluded that MOS could enhance piglet growth and health.

5. Summary

Increased understanding of enzyme and transporter expression in various microbes, and key differences between the various classes of enzymes and types of transporters, are enhancing our knowledge about microbial selectivity for substrates, including prebiotics. Differences in chemical structure, degree of polymerization, and bonds between subunits affect microbial utilization of prebiotics. Novel prebiotics derived from xylan, arabinan, and mannan are comprised of less common subunits based on 5 carbon sugars (xylose, arabinose), and/or are connected via β-bonds. The unique types of subunits and bond structures confer greater selectivity for beneficial bacteria, and health benefits at a lower dose compared to conventional prebiotics comprised of glucose, fructose and galactose subunits.

Clinical trials with XOS and AXOS led to improvements in laxation, triglycerides, cholesterol, and blood glucose, typically at doses from 1 to 3 g per day, far less than the 10–30 g per day required with FOS, GOS, inulin, and resistant starch. Preliminary clinical trials with MOS suggested the potential for weight management and reductions in adipose tissue at doses in the range of 4 g per day. Furthermore, MOS seems to have a unique capacity to inhibit proliferation of *E. coli*, and its addition to livestock feed has improved livestock health with a concurrent reduction in antibiotic use. Ultimately, these novel prebiotics may usher in a new era of prebiotic utilization, driven by their greater selectivity for beneficial bacteria, and easier product formulation and efficacy at a lower dose.

Disclosures and conflicts of interest

The co-authors are both affiliated with Prenexus Health, a company that produces xylooligosaccharide prebiotics.

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