Fermentation of Plant Cell Wall Derived Polysaccharides and Their Corresponding Oligosaccharides by Intestinal Bacteria

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New types of nondigestible oligosaccharides were produced from plant cell wall polysaccharides, and the fermentation of these oligosaccharides and their parental polysaccharides by relevant individual intestinal species of bacteria was studied. Oligosaccharides were produced from soy arabinogalactan, sugar beet arabinan, wheat flour arabinoxylan, polygalacturonan, and rhamnogalacturonan fraction from apple. All of the tested substrates were fermented to some extent by one or more of the individual species of bacteria tested. *Bacteroides* spp. are able to utilize plant cell wall derived oligosaccharides besides their reported activity toward plant polysaccharides. *Bifidobacterium* spp. are also able to utilize the rather complex plant cell wall derived oligosaccharides in addition to the bifidogenic fructooligosaccharides. *Clostridium* spp., *Klebsiella* spp., and *Escherichia coli* fermented some of the selected substrates in vitro. These studies do not allow prediction of the fermentation in vivo but give valuable information on the fermentative capability of the tested intestinal strains.

Keywords: *Oligosaccharides; intestinal bacteria; plant cell wall; prebiotics*

INTRODUCTION

Nondigestible oligosaccharides (NDOs) are oligosaccharides which escape digestion in the upper gastrointestinal tract. According to the IUB−IUPAC nomenclature, oligosaccharides are defined as saccharides containing between 3 and 10 sugar moieties (Voragen, 1998). The oligosaccharides, fructooligosaccharides, and transgalactooligosaccharides belong to the group of prebiotics, meaning that they are nondigestible food ingredients, that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon and thus improve health (Gibson and Roberfroid, 1995). Mixtures of oligosaccharides which combine the effect of stimulating the body's own bacteria and those added (probiotics) are considered as synbiotics (Knorr, 1998).

Some NDOs occur naturally in various plants (Campbell et al., 1997), processed foods, and human breast milk (Thurl et al., 1996). Nondigestible oligosaccharides, as well as non-starch-polysaccharides (NSP), resistant starch, and mucin can all be regarded as substrate for the intestinal species of bacteria (Vercellotti et al., 1977). NDOs are commercially produced by extraction, chemical condensation (Dendene et al., 1994), transglycosylation reactions (Smart, 1993; Barthomeuf and Pourrat, 1995), or controlled hydrolysis of polysaccharides (Coussement, 1995; Yun et al., 1997). The various types of dietary carbohydrates might influence the growth of specific bacteria species, their metabolic activity, short-chain fatty acid (SCFA) production, and rate of fermentation in different ways dependent on their structures.

Species often involved in the breakdown of carbohydrates belong to genera *Bacteroides*, *Bifidobacterium*, *Ruminococcus*, *Eubacterium*, *Lactobacillus*, and *Clostridium*. Some species (mainly *Bacteroides*) are regarded as utilizing mainly NSP, while others grow by crossfeeding on smaller fragments (often oligosaccharides) produced by primary NSP degraders (Macfarlane and Cummings, 1991). Bifidobacteria have been reported to be mainly oligosaccharide utilizers, although some of them are also able to ferment polysaccharides.

Products formed during fermentation in the colon from NSP have been the subject of many studies, whereas almost no data are available on the fermentation of oligosaccharides derived from NSP. Most of the research has focused on the influence of fructooligosaccharides and galactooligosaccharides on the intestinal flora (Ito et al., 1993; Alles et al., 1996; Buddington et al., 1996; Garleb et al., 1996). However, plant cell wall polysaccharides are also an interesting potential source for the production of oligosaccharides covering a wide range of different structures. Plant cell wall polysaccharides are present in large amounts as fiber-rich byproducts, e.g., cereal bran, fruit pomace, sugar beet pulp, potato fiber, and press cake of oleaginous seeds. Using specific extraction methods and hydrolysis using glycanases, a variety of oligosaccharides can be produced. Little attention is paid to the possible use of plant cell wall derived oligosaccharides as prebiotic substrate. As these types of oligosaccharides can be formed during production and/or processing of food (e.g., formation of arabinoxylooligosaccharide during bread making in case *endo*-xylanases are added as bread-improvers, in fruit juices, purees and nectars if enzymes are used, isomaltooligosaccharides in beer) they might be an important prebiotic substrate. Also adding these types of oligosaccharides to the diet might have interesting effects on the composition and/or activity of the bacterial flora.

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Fermentation of complex carbohydrates is assumed to be a result of a combined action of several bacteria; however, knowledge on how individual intestinal species of bacteria utilize complex poly- and oligosaccharides is important to better understand their fermentation in the colon.

The aim of this study was to produce various structurally different oligosaccharides from plant cell wall polysaccharides and to study the influence of the chemical structure of these oligosaccharides on the fermentation behavior by various intestinal bacteria. Further, this behavior was compared to the fermentation behavior of parental polysaccharides and commercially available oligosaccharides. To obtain more information on the fermentation capabilities of various intestinal strains, this study was performed with pure cultures and well-defined substrates.

MATERIALS AND METHODS

Materials. Wheat flour arabinoxylan was obtained from Megazyme (International Ireland Ltd., Wicklow, Ireland). Sorgum arabinoxylan was obtained as described by Verbruggen et al. (1995). A rhamnogalacturonan-enriched fraction was isolated from apple liquefaction juice and was subsequently saponified according to the method of Schols et al. (1990). Soy arabinogalactan was obtained on a small scale as described by Huisman et al. (1998), and a large amount of soy arabinogalactan was a gift from Novo Nordisk Ferment AG (Dittingen, Switzerland). Sugar beet arabinan was a gift from British Sugar (Peterborough, U.K.). Polygalacturonic acid was obtained from ICN (Costa Mesa, CA). Xylooligosaccharides were a gift from Suntory Ltd. (Japan). Fructooligosaccharides obtained from inuline by controlled hydrolysis (Raftilose P95) were kindly provided by ORAFTI (Tienen, Belgium).

Enzymes. Polygalacturonase was purified from *Kluyveromyces fragilis* (Versteeg, 1979), rhamnogalacturonan hydrolase was purified from the commercial preparation Ultra-sp produced with *Aspergillus aculeatus* (Novo Nordisk), an *endo*-xylanase was purified from *Aspergillus tubigensis* (Graaf et al., 1994), and *endo*-galactanase and *endo*-arabinanase were cloned from *A. aculeatus* and kindly provided by Novo Nordisk A/S (Copenhagen, Denmark). The cloned enzymes may not be completely pure since the host microorganism produces low amounts of its own enzymes in addition to the genetically introduced enzyme.

Preparation of Oligosaccharides from Plant Cell Wall Polysaccharides. A solution of polymer (1% w/v) was incubated with an appropriate amount of suitable *endo*-glycanase, and the degradation was followed by high-performance anion-exchange chromatography (HPAEC) and high-performance size-exclusion chromatography (HPSEC) (see Analytical Methods). After inactivation of the enzyme (10 min, 100 °C), the solution was concentrated under reduced pressure. Mostly, the concentrated digest required centrifugation (14 000 × g, 5 min) to remove insoluble material. The supernatants were fractionated on a Sephadex G10 column (600 × 50 mm or 600 × 100 mm, Pharmacia LKB Biotechnology, Uppsala, Sweden), or two columns (600 × 26 mm) were packed in series with fractogel TSK HW-40(S) (25−40 μm, Merck, Darmstadt, Germany) thermostated at 60 °C, or a Biogel P-2 column (1000 × 26 mm) (200−400 mesh, Bio-Rad) thermostated at 60 °C. Samples up to 5 mL were applied on the column and eluted with distilled water (5, 2.5, 0.5 mL/min, respectively, for G10, TSK, and BioGel material) using a Pharmacia Whiled system equipped with a Pharmacia P50 pump. A Shodex RI-72 detector was used to monitor the refractive index. The fractions were analyzed for oligosaccharides by HPAEC or HPSEC, and appropriate fractions were pooled.

Bacterial Strains. Eighteen bacterial strains were selected from the culture collection in our department. Most strains were of human origin; some strains originated from porcine feces (Hartemink et al., 1996).

Oligosaccharide and Polysaccharide Fermentation. The selected strains were screened for their ability to ferment structurally different oligosaccharide and polysaccharide fractions. Strains were pregrown in thioglycollate broth (Oxoid, Unipath LTD, Basingstoke, Hampshire, U.K.). The sugar-free thioglycollate medium as well as the oligosaccharide or polysaccharide solutions were sterilized separately for 15 min at 121 °C. Thioglycollate supplemented with 0.5% oligosaccharides or polysaccharides (w/v) (1 mL) was inoculated with 10% (w/v) of an overnight full-grown strain for 48 h at 37 °C in an anaerobic chamber with an atmosphere consisting of CO_2 (10%), H_2 (10%), and N_2 (10%). After anaerobic incubation, the pH was measured using a micro-pH meter (Sentron, Roden, The Netherlands). The experiments were performed in duplicate. The changes in content of residual polysaccharides and oligosaccharides and formation of reaction products were measured by HPAEC and HPSEC. For HPAEC and HPSEC analyses, the cultures were centrifuged and the supernatant was diluted 10 times with H_2O and boiled for 5 min to inactivate enzymatic activity. The strains were checked microscopically for purity before and after fermentation.

Analytical Methods. The HPAEC system consisted of a Dionex Bio-LC GPM-II quaternary gradient module (Dionex Corporation, Sunnyvale, CA) equipped with a Dionex Carbopac PA-100 column (4 × 250 mm) in combination with a Carbopac PA-100 guard column (3 × 25 mm). Samples (20 μL) were injected using a Spectra Physics SP8880 autosampler. The oligomers were analyzed using a gradient of sodium acetate in 100 mmol L^{-1} NaOH. For RGAOS, the gradient was as follows: 0−5 min, 0 mM; 5−35 min, 0−430 mmol L^{-1}; 35−40 min, 430−1000 mmol L^{-1}. For AOS, the elution involved a linear gradient of 0−500 mmol L^{-1} acetate in 100 mmol L^{-1} NaOH for 40 min. For GAOS, the elution involved a linear gradient of 200−700 mmol L^{-1} sodium acetate in 100 mmol L^{-1} NaOH for 40 min. For AGOS, the elution involved a linear gradient of 0−400 mmol L^{-1} sodium acetate in 100 mmol L^{-1} NaOH for 40 min. Elution of AXOS was done with a linear gradient of 0−100 mmol L^{-1} sodiumacetate in 100 mmol L^{-1} NaOH for 5 min, followed by a linear gradient of 100−400 mmol L^{-1} sodium acetate in 100 mmol L^{-1} NaOH for 40 min. The elution of XOS involved a linear gradient of 0−250 mmol L^{-1} sodium acetate for 30 min. All gradients ended by 5 min with 1000 mmol L^{-1} sodium acetate and a reequilibration with 100 mmol L^{-1} NaOH for 15 min. The eluant (1 mL/min) was monitored using a Dionex PED detector in the pulsed amperometric detection (PAD) mode.

HPSEC was performed on a SP8800 HPLC (Spectra Physics) equipped with three Bio-Gel TSK-columns (each 300 × 7.5 mm) in series (40XL, 30XL, and 20XL; Bio-Rad Labs) in combination with a TSK XL guard column (40 × 6 mm) and elution at 30 °C with 0.4 M acetic acid/sodium acetate (pH 3) at 0.8 mL/min. The eluate was monitored using a Shodex SE-61 refractive index detector. The system was calibrated with pectins with molecular weights in the range of 10000−100000 Da (as determined by viscosimetry).

The polymeric material was analyzed for sugar composition. Uronic acid was determined by the colorimetric *m*-hydroxybiphenyl assay (Ahmed and Labavitch, 1977). Neutral sugars were determined by GLC after pretreatment (1 h, 30 °C) with aqueous 72% H_2SO_4, followed by hydrolysis with 1 M H_2SO_4 (3 h, 100 °C) and conversion of the products into alditol acetates (Englyst and Cummings, 1984). The alditol acetates were analyzed on a glass column (3 m × 2 mm i.d.), packed with Chrom WAW 80−100 mesh coated with 3% OV275 in a Carbo Erba Fractovap 2300 GC.

RESULTS

Production of New NDOs. *Production of (Arabino)-galactooligosaccharides (AGOS) from Soy*. Dehulled soy beans were defatted, deproteinated, destarched, and treated with NaOH according to Huisman et al. (1998) to obtain an arabinogalactan-enriched extract. The sugar composition of the arabinogalactan-enriched

Table 1. Sugar Composition (mol %) of Various Cell Wall Polysaccharides and Extracts[a]

	AGPS[1]	AGPS[2]	APS	RGAPS	AXWPS	AXSPS
rhamnose	2	2	5	16	0	0
fucose	3	1	0	0	0	0
arabinose	27	38	60	20	30	46
xylose	7	2	0	11	69	40
mannose	0	0	0	0	0	0
galactose	39	52	15	18	0	2
glucose	2	0	6	2	1	2
uronic acid	20	4	16	33	0	10

[a] AGPS: arabinogalactan enriched polysaccharide fraction. APS: arabinan enriched polysaccharide fraction. RGPS: rhamnogalacturonan enriched polysaccharide fraction. AXWPS: arabinoxylan polysaccharide from wheat flour. AXSPS: glucuronoarabinoxylan polysaccharide from sorghum.

polysaccharide fraction (AGPS[1]) obtained is given in Table 1. This fraction was mainly composed of galactose, arabinose, and galacturonic acid residues, but also some other sugar residues were present. This arabinogalactan extract was treated with various *endo*-glycanases for 1 and 24 h, and the degradation of the polymer and subsequent oligosaccharide formation was analyzed with HPSEC and HPAEC. Treating the extract for 24 h with a purified *endo*-galactanase resulted in the production of oligosaccharides with a degree of polymerization (DP) of 2−5. An incubation time of 1 h resulted in the formation of oligomers with a DP ranging from 2 to 9.

The arabinogalactan-enriched polysaccharide fraction (AGPS[2]) consisting of 52% galactose and 38% arabinose (Table 1) was used for the large-scale production of AGOS. AGPS[2] (15 g; 1% w/v) was incubated with the cloned *endo*-galactanase. The oligosaccharides formed eluted similar on HPAEC as those formed after incubation of the soy extract with the purified *endo*-galactanase. The reaction products were applied onto a Sephadex G-10 column to remove the monomers and the remaining polymeric fraction. The mixture was fractionated using the Biogel P-2 column in fractions containing di-, tri-, tetra-, penta-, hexa-, hepta-, octa-, and nonasaccharides representing 22%, 26%, 16%, 12%, 9%, 9%, 3%, and 3%, respectively, of the oligosaccharide mixture. HPAEC analysis revealed that the different DP fractions consisted of several types of oligosaccharides. The oligosaccharides present at the highest concentration in the various fractions were identified as galactooligosaccharides which were β1−4 connected to each other. The proposed structure of the oligosaccharides present in the mixture is given in Table 2.

Production of Arabinooligosaccharides (AOS) from Sugar Beet. Arabinan can be hydrolyzed toward oligosaccharides by action of *endo*-arabinanases (Beldman et al., 1993). Linear α-1,5-arabinan (3 g) extracted from sugar beet consisting mainly of arabinose (Table 1) was incubated with an *endo*-arabinanase. AOS with a DP of 2−10 were formed. The digest was applied onto a Biogel P-2 column, and the sugars eluting in the dimeric, trimeric, tetrameric, and pentameric fraction were pooled. The AOS were identified using standards present in the laboratory as α-1−5 linked arabinose oligomers. Due to the presence of some polygalacturonidase activity in the *endo*-arabinanase preparation, also some galacturonic acid (5% w/w) was present in the pooled mixture.

Production of Galacturonooligosaccharides (GAOS). Linear GAOS were obtained by incubating 3 g of polygalacturonic acid with a purified polygalacturonase from *Kluyveromyces fragilis* according to Versteeg (1979). Using HPSEC, the degradation of the polygalacturonic acid was monitored. When the polymer was completely degraded toward oligomeric material, the reaction was stopped. The digest was dialyzed against Millipore deionized water to remove most of the monogalacturonic acid and digalacturonic acid and some trigalacturonic acid. The mixture obtained contained linear galacturonooligosaccharides with a DP ranging from 2 to 10 (Table 2).

Production of Rhamnogalacturonooligosaccharides (RGAOS) from Apple. From apple a highly branched rhamnogalacturonan-enriched polysaccharide fraction (RGPS) was obtained as described by Schols et al. (1990). This fraction was degraded by rhamnogalacturonan hydrolase. The digest was fractionated on a Sephadex G-50 column according to Schols et al. (1994). Fractions containing sufficient amounts of oligosaccharides were pooled, and the structures of the oligosaccharides present in these pools were determined by comparing their elution pattern with the elution pattern of oligosaccharides with known structure (Schols et al., 1994). In Table 2 the structures of the RGAOS present in the mixture are given.

Production of Arabinoxylooligosaccharides (AXOS) from Wheat Flour. Arabinoxylan-enriched fractions were obtained from wheat flour and degraded with an *endo*-xylanase from *A. tubigensis*. The resulting digest was subsequently fractionated on Fractogel TSK. The fractions were analyzed by HPAEC, and the oligomers present were identified using standards present in the laboratory (Gruppen et al., 1992). The fraction containing mainly double-branched oligosaccharides (Table 2) was used for further fermentation experiments.

Linear xylooligosaccharides (XOS) were obtained after partial purification of commercially available xylooligosaccharides. The commercial mixture contained 22% monosaccharides, which were removed by size-exclusion on a BioGel P-2 column. The di-, tri-, and tetrameric fractions were pooled and contained, besides the linear β1−4 linked xylooligosaccharides, also some other oligosaccharides (most likely glucooligosaccharides).

Behavior in the Presence of Isolated Strains. Various plant cell wall polysaccharides and oligosaccharides were fermented by selected bacterial strains isolated from human and porcine feces. After anaerobic fermentation for 48 h, the pH was measured (Table 3). The amount of residual soluble polysaccharides or oligosaccharides was measured with HPSEC and HPAEC. The degree of fermentation of the polymers could be estimated by HPSEC from the shift in molecular weight or from the lowered amount of polymer left in the supernatant after fermentation. The presence of monomeric and oligomeric material after fermentation of the polymer was determined with HPAEC. The fermentation of the oligosaccharides was judged by comparing the HPAEC elution patterns of the oligosaccharides before and after fermentation. The thioglycolate broth and the oligosaccharide mixtures were also analyzed separately to determine the origin of the observed peaks. This allowed specific determination of the degree of fermentation of the substrate by the various intestinal bacteria. In Table 4 the degradation of the polymers or oligomers by the various strains is given.

Arabinogalactan-Enriched Polysaccharide Fraction (AGPS[1]) and (Arabino)galactooligosaccharides (AGOS).

Table 2. Structure of the Oligosaccharides Derived from Plant Cell Wall Polysaccharides[a]

GOS	ß-D-Gal*p*-(1,4)-[ß-D-Gal*p*-(1,4)]$_n$-D-Gal*p* with n= 0-5
AGOS	unidentified branched arabinogalactan derived oligosaccharides
AOS	α-L-Ara*f*-(1,5)-[α-L-Ara*f*-(1,5)]$_n$-L-Ara*f* with n=0-3
GAOS	α-D-GalA*p*-(1,4)-[α-D-GalA*p*-(1,4)]$_n$-D-GalA*p* with n=0-7
RGOS	
RG_1	α-Rha-(1,4)-α-GalA-(1,2)-α-Rha-(1,4)-GalA
RG_2	α-Rha-(1,4)-α-GalA-(1,2)-α-Rha-(1,4)-GalA ↓ ↓ β-Gal-(1,4) β-Gal-(1,4)
RG_3	α-Rha-(1,4)-α-GalA-(1,2)-α-Rha-(1,4)-α-GalA-(1,2)-α-Rha-(1,4)-GalA ↓ ↓ ↓ β-Gal-(1,4) β-Gal-(1,4)$_n$ β-Gal-(1,4)$_m$ with either n=1 and m=0, or n=0 and m=1.
(Xyl)$_n$	ß-Xyl*p*-(1,4)-[ß-Xyl*p*-(1,4)]$_n$-ß-Xyl*p* n=0-4
AXOS	
5.5	ß-Xyl*p*-(1,4)-ß-Xyl*p*-(1,4)-ß-Xyl*p*-(1,4)-Xyl*p* α-Ara*f*(1,2)
5.1	ß-Xyl*p*-(1,4)-ß-Xyl*p*-(1,4)-Xyl*p* α-Ara*f*(1,2) α-Ara*f*(1,3)
6.1	ß-Xyl*p*-(1,4)-ß-Xyl*p*-(1,4)-ß-Xyl*p*-(1,4)-Xyl*p* α-Ara*f*(1,2) α-Ara*f*(1,3)
8.1	ß-Xyl*p*-(1,4)-ß-Xyl*p*-(1,4)-ß-Xyl*p*-(1,4)-ß-Xyl*p*-(1,4)-Xyl*p* α-Ara*f*(1,2) α-Ara*f*(1,3) α-Ara*f*(1,3)
9.1	ß-Xyl*p*-(1,4)-ß-Xyl*p*-(1,4)-ß-Xyl*p*-(1,4)-ß-Xyl*p*-(1,4)-Xyl*p* α-Ara*f*(1,2) α-Ara*f*(1,2) α-Ara*f*(1,3) α-Ara*f*(1,3)
10.1	ß-Xyl*p*-(1,4)-ß-Xyl*p*-(1,4)-ß-Xyl*p*-(1,4)-ß-Xyl*p*-(1,4)-ß-Xyl*p*-(1,4)-Xyl*p* α-Ara*f*(1,2) α-Ara*f*(1,2) α-Ara*f*(1,3) α-Ara*f*(1,3)

[a] (Gal)$_n$: galactooligosaccharides. (Gal)$_n$(Ara)$_n$: branched (arabino)galactooligosaccharides. (Ara)$_n$: arabinooligosaccharides. (GalA)$_n$: galacturonooligosaccharides. RGOS: rhamnogalacturonooligosaccharides., (Xyl)$_n$: xylooligosaccharides. AXOS: arabinoxylooligosaccharides.

Species belonging to the different bacterial groups tested were able to ferment AGPS[1]. Using HPSEC, it was shown that 12 species of bacteria degraded the AGPS[1] to some extent (Table 4); however, the degree of fermentation differed. In Figure 1, some HPSEC elution patterns obtained for various fermentation liquids are given. *Bacteroides ovatus* (*B. ovatus*) (Figure 1E) fermented the extract almost completely within the incubation time as can be seen when the pattern is compared with the HPSEC elution pattern of the untreated polymer (Figure 1A). *E. coli* (Figure 1D) degraded the polymer only to some extent; the molecular weight

Table 3. Acidification of the Medium after Fermentation of Various Polysaccharides and Oligosaccharides by Several Strains[a]

		arabinogalactan		arabinan		(rhamno)galacturonan			(arabino)xylan				
bacteria	origin	AGPS	AGOS	APS	AOS	RGAPS	RGAOS	GAOS	AXWPS	AXSPS	AXOS	XOS	FOS
	blanc	6.6	6.6	6.6	6.6	6.6	6.8	5.5	6.6	6.6	6.6	6.3	6.6
Bi. breve	ATCC 15700	6.1	4.7	5.8	5	5.9	6.5	nd	6.3	5.7	5.9	nd	4.5
Bi. longum	ATCC 15707	5.6	4.5	5.4	4.8	5.8	6.6	5.1	5.6	5.3	5.0	4.6	4.9
Bi. infantis	ATCC 15697	6.0	4.4	6.0	6.1	6.0	6.6	5.2	6.1	6.0	5.9	5.1	4.5
Bi. adolescentis	ATCC 15703	5.9	4.6	5.9	4.9	5.9	7.6	5.1	5.9	5.9	4.5	4.6	4.6
C. beijerinckii	human feces	6.8	nd	6.8	5.3	6.8	7.1	5.5	6.8	6.8	7.1	5.1	6.6
C. clostridiiforme	human feces	5.4	6.0	5.7	5.4	6.6	7.1	5.6	6.4	6.5	6.9	nd	5.5
C. ramosum	human feces	6.9	5.4	6.8	6.6	6.8	7.1	5.8	7.0	6.9	6.9	6.4	6.8
C. sporogenes	human feces	6.8	>6	6.9	6.5	6.8	6.9	5.2	6.9	6.8	6.9	5.5	6.7
C. sartagoformum	human feces	6.6	>6	7.2	5.7	6.8	6.8	5.4	6.8	6.8	6.9	5.1	6.6
C. perfringens	human feces	6.4	>6	6.4	6.4	6.4	6.6	6.2	6.6	6.4	5.9	5.3	5.4
B. vulgatus	ATCC 8482	6.1	5.2	5.7	6.1	5.9	4.9	5.1	6.4	6.1	5.2	5.6	5.1
B. ovatus	ATCC 8483	5.6	5.2	5.7	6.2	5.6	5.7	5.1	5.6	5.6	5.0	5.3	5.4
B. thetaiotaomicron	ATCC 29741	5.6	4.5	5.5	6.3	5.6	5.6	5.0	6.1	5.8	5.9	5.3	5.5
L. casei	Yakult	6.3	5.7	6.3	6.3	6.6	6.5	5.3	6.3	6.4	6.2	5.5	5.8
L. acidophilus	swine feces	6.5	5.5	6.6	6.3	6.6	6.5	5.3	6.7	6.5	6.7	4.9	6.4
L. fermentum	swine feces	6.7	5.2	6.2	6.2	6.7	5.9	5.1	6.7	6.7	6.3	4.5	6.6
E. coli	human feces	6.4	6.2	6.7	5.3	6.7	7.1	5.1	6.6	6.7	6.8	6.8	6.5
K. pneumoniae	human feces	6.6	4.8	6.8	7.1	6.8	6.9	5.6	6.7	6.8	6.0	6.0	5.8

[a] AGPS: arabinogalactan enriched polysaccharide fraction. AGOS: (arabino)galactooligosaccharides. APS: arabinan enriched polysaccharide fraction. AOS: arabinooligosaccharides. RGPS: rhamnogalacturonan enriched polysaccharide fraction. RGAOS: rhamnogalacturonooligosaccharides. GAOS: galacturonooligosaccharides. AXWPS: arabinoxylan polysaccharide from wheat flour. AXSPS: glucuronoarabinoxylan polysaccharide from sorghum. AXOS: arabinoxylooligosaccharides. XOS: xylooligosaccharides. FOS: fructooligosaccharides. nd: not determined.

Table 4. Fermentative Degradation of Various Polysaccharides and Oligosaccharides by Several Bacteria As Determined with HPSEC or HPAEC[a]

		arabinogalactan		arabinan		(rhamno)galacturonan			(arabino)xylan				fructan
bacteria	origin	AGPS	AGOS	APS	AOS	RGAPS	RGAOS	GAOS	AXWPS	AXSPS	AXOS	XOS	FOS
	blanc												
Bi. breve	ATCC 15700	+−	+	−	+−	−	−	nd	−	−	−	nd	+
Bi. longum	ATCC 15707	+−	+	+−	+	−	−	−	+	−	+−	+	+
Bi. infantis	ATCC 15697	−	+	−	−	−	−	−	−	−	−	−	+
Bi. adolescentis	ATCC 15703	+−	+	+−	+−	−	−	−	−	−	+	+	+
C. beijerinckii	human feces	−	nd	+−	+−	−	−	−	−	−	−	+	−
C. clostridiiforme	human feces	+−	+−	+−	+	−	−	+	−	−	−	nd	+−
C. ramosum	human feces	−	+	+−	−	+−	−	+	−	−	−	−	−
C. sporogenes	human feces	−	−	−	−	−	−	−	−	−	−	−	−
C. sartagoformum	human feces	+−	−	−	+−	−	−	−	−	−	−	−	−
C. perfringens	human feces	−	−	−	−	−	−	−	−	−	−	−	+
B. vulgatus	ATCC 8482	+−	+−	+	+−	+−	+	−	−	+−	+	+	+−
B. ovatus	ATCC 8483	+−	+	−	−	+	+	+	+	+	+−	+	+−
B. thetaiotaomicron	ATCC 29741	+−	+	+	−	+−	+	−	−	−	−	−	+−
L. casei	Yakult	−	+−	−	−	−	−	−	−	−	−	−	−
L. acidophilus	swine feces	+−	+	−	−	−	−	−	−	−	−	+	+−
L. fermentum	swine feces	+−	+	−	−	−	−	−	−	−	−	−	+−
E. coli	human feces	+−	−	−	+−	−	−	−	−	−	−	−	−
K. pneumoniae	human feces	+−	+	−	−	−	−	−	−	−	−	+	+−

[a] AGPS: arabinogalactan enriched polysaccharide fraction. AGOS: (arabino)galactooligosaccharides. APS: arabinan enriched polysaccharide fraction. AOS: arabinooligosaccharides. RGPS: rhamnogalacturonan enriched polysaccharide fraction. RGAOS: rhamnogalacturonooligosaccharides. GAOS: galacturonooligosaccharides. AXWPS: arabinoxylan polysaccharide from wheat flour. AXSPS: glucuronoarabinoxylan polysaccharide from sorghum. AXOS: arabinoxylooligosaccharides. XOS: xylooligosaccharides. FOS: fructooligosaccharide. + complete degradation. +− partial degradation. − no degradation. nd: not detected.

shifted from approximately 65 to 20 kDa. *Lactobacilus acidophilus* (Figure 1C) and *Bifidobacterium breve* (*Bi. breve*) (Figure 1B) changed the elution behavior of the AGPS[1] only slightly. After 48 h of fermentation, no formed monomeric or oligomeric material could be detected in any of the AGPS[1] fermentations.

A decrease in pH (see Table 3) of the medium was observed for almost all strains tested when grown on a mixture of (arabino)galactooligosaccharides, consisting of linear β1−4 galactooligosaccharides and some branched (arabino)galactooligosaccharides. In Figure 2 the HPAEC elution pattern of the AGOS before (Figure 2A) and after fermentation is given. Some strains showed degradation of oligosaccharides as observed with HPAEC, but this degradation was not always accompanied with a drop in pH (Tables 3 and 4). Most of the tested strains showing a decrease in pH fermented the mixture completely (Figure 2C), although for *B. vulgatus* only partial fermentation of the oligomer mixture was observed after 48 h (Figure 2B). It should be stated that the peaks still present in Figure 2C originate from the medium and not from the AGOS.

Arabinan-Enriched Polysaccharide Fraction (APS) and the Arabinooligosaccharides (AOS). The APS from sugar beet was only slightly degraded by *Bi. longum*, *Bi. adolescentis*, *Clostridium beijerinckii* (*C. beijerinckii*), *C. clostridiiforme*, *C. ramosum*, *B. ovatus*, and *B. thetoatoamicron* as measured by HPSEC (shift in M_w from 32 to 22 kDa). The relative amount of polymer did not decrease, showing that these species hydrolyzed the

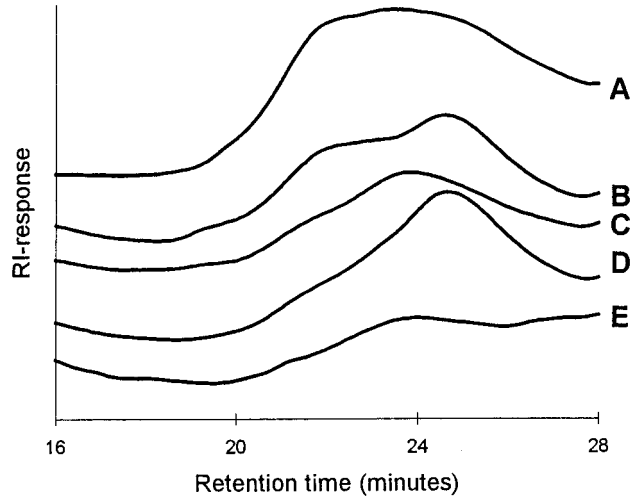

Figure 1. HPSEC of arabinogalactan enriched polysaccharide fraction from soy before and after fermentation by various intestinal bacteria: (A) blank, (B) *Bi. breve*, (C) *Lb. acidophilus*, (D) *E. coli*, (E) *B. ovatus*.

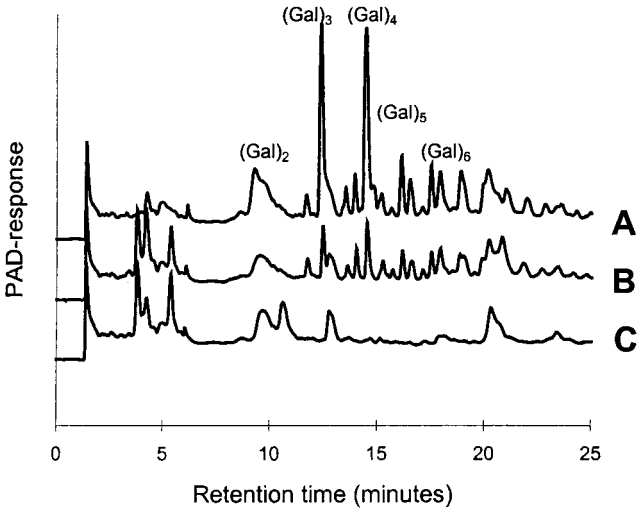

Figure 2. HPAEC analysis of (arabino)galactooligosaccharides before (A) and after fermentatation *B. vulgatus* (B) and *L. acidophilus* (C).

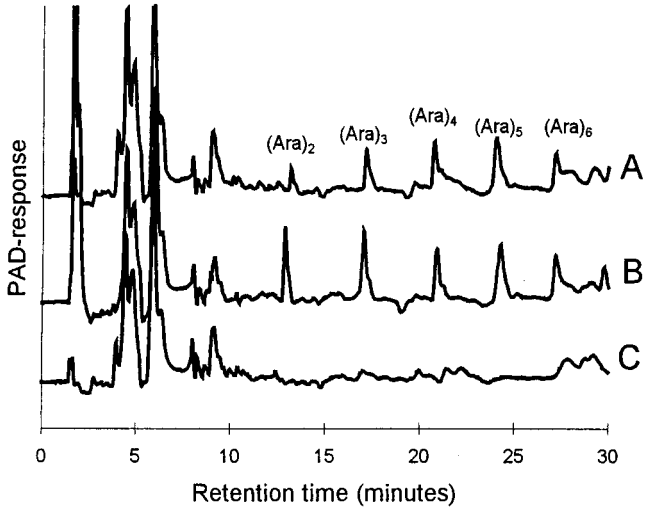

Figure 3. HPAEC elution pattern of arabinooligosaccharides formed in the supernatant before (C) and after fermentation of *B. thetaiotoamicron* (B) and *B. vulgatus* (A).

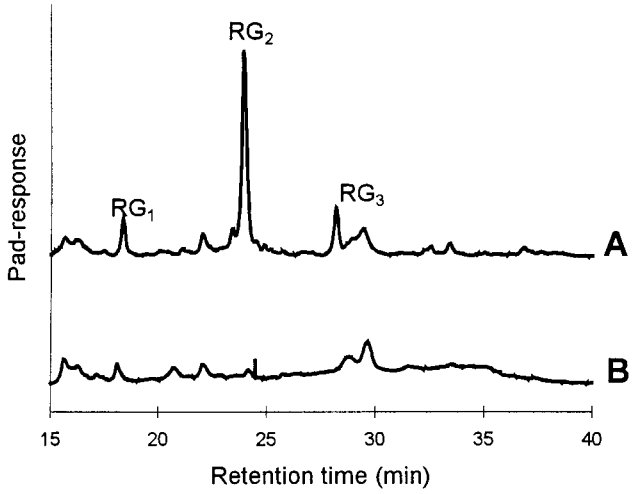

Figure 4. HPAEC elution pattern of rhamnogalacturonanoligosaccharides before (A) and after (B) fermentation by *B. ovatus*.

polymer only slightly but did not utilize it. *Bi. longum*, *C. clostridiiforme*, *B. vulgatus*, and *B. thetaiotaomicron* degraded this polymer but also decreased the amount of polymer, as measured by the change of area under the curve and a drop in pH. For the latter two species, arabinooligosaccharides still could be detected in the supernatant (Figure 3), suggesting that they could not utilize the oligosaccharides formed within the selected fermentation time. *Bi. longum* and *C. clostridiiforme* appeared to possess the enzyme system to fully utilize arabinan.

AOS of DP 2−6 were completely fermented by *Bi. longum* and *C. clostridiiforme*. *E. coli* and *Bi. adolescentis* fermented only arabinotriose completely, while *C. beijerinckii* and *C. sartagoformum* only degraded the arabinotriose and arabinotetraose to some extent. *C. butyricum* degraded only arabinotetraose within the time of incubation. The other species tested (Table 4) were unable to degrade the oligosaccharides.

Rhamnogalacturonan-Enriched Polysaccharide Fraction (RGAPS) and (Rhamno)galacturonooligosaccharides ((R)GAOS). RGAPS was fermented by a limited number of bacterial species only (Table 4). *B. ovatus* completely degraded the polymers, while the other species tested were only able to degrade the population with the highest molecular weight.

RGAOS were only completely fermented by the *Bacteroides* strains tested: *B. vulgatus*, *B. ovatus*, and *B. thetaiotaomicron*. In Figure 4 the elution profile is given of the RGAOS before and after incubation with *B. ovatus*. These species therefore probably produce galacturonidases, galactosidases, and rhamnosidases when grown on these oligosaccharides. These species also lowered the pH of the supernatant after fermentation. The other species tested showed no activity toward RGAOS.

C. clostridiiforme, *C. ramosum*, and *B. ovatus* only fermented linear GAOS. The other species tested did not show any degradation of the oligosaccharides. Since our substrates were only slightly buffered, the GAOS resulted in a pH-drop, and this complicates comparison with other substrates.

Arabinoxylan Polysaccharide from Wheat Flour (AX-WPS), Glucuronoarabinoxylan Polysaccharide from Sorghum (AXSPS), and (Arabino)xylooligosaccharides (AXOS and XOS). Two arabinoxylans originating from different sources (wheat and sorghum) were selected for

studying the fermentation of branched xylans. Wheat flour arabinoxylan consisted of arabinose and xylose, while the polymer from sorghum (glucuronoarabinoxylan) contained glucuronic acids as substituents as well (Table 1); the polymer form sorghum was only partially water-soluble.

Bi. longum and *B. ovatus* fermented AXWPS completely, as judged from HPSEC (results not shown). However, HPAEC analysis of the supernant showed that after 48 h of fermentation of the arabinoxylan polymer by *B. ovatus* the supernatant still contained significant amounts of oligosaccharides.

AXSPS was partially fermented by *B. vulgatus*, and this resulted in a small drop in pH. *B. ovatus* fermented the soluble glucuronoarabinoxylan completely over a period of 48 h, and no oligomers could be detected in the supernatant.

HPAEC analysis revealed that *Bi. longum*, *Bi. adolescentis*, *L. acidophilus*, *Klebsiella pneumoniae* (*K. pneumoniae*), *C. beijerinckii*, *B. vulgatus*, and *B. ovatus* were able to ferment xylobiose, -triose, and -tetraose.

The more branched arabinoxylooligosaccharides (Table 2) could only be fermented completely by *Bi. adolescentis*, *Bi. longum*, and *B. vulgatus*. *B. ovatus* showed only β-xylosidase activity toward the xylose residue present at the nonreducing end of the double-substituted oligomer 6.1, resulting in the formation of the oligosaccharide 5.1 (Table 2). The other strains tested showed no degradation (Table 4).

Fructooligosaccharides (FOS). All *Bifidobacterium* spp. and *Bacteroides* spp. tested and some *Clostridia* spp., *Lactobacillus casei* (*L. casei*), and *K. pneumoniae* showed acidification of the FOS-containing media after 48 h of fermentation. Except for *L. casei* and some *Clostridium* spp., all of them were able to ferment the FOS mixture to some extent. *L. acidophilus* and *L. fermentum* fermented only 1-kestose.

DISCUSSION

The oligosaccharides at present commercially available are produced by extraction from natural plant foods, by acidic or enzymatic hydrolyses of fructans or by transglycosylation starting from sucrose (Hang and Woodams, 1996), lactose (Zarate and Lopez, 1990), maltose (Hayashi et al., 1994), and starch derivatives by using specific bacterial enzymes (Monsan et al., 1989). Here we describe a new class of fermentable oligosaccharides produced by controlled hydrolysis from various naturally occurring plant cell wall polysaccharides. Soy flour, sugar beet pulp, apple, and wheat flour were selected as raw materials for the production of, respectively, (arabino)galacto-, arabino-, rhamnogalacturono-, and arabinoxylooligosaccharides after extraction of the polysaccharide of interest. The oligosaccharides derived from cell wall polysaccharides are structurally different from the oligosaccharides, which are currently commercially available as prebiotics (Playne and Crittenden, 1996). Little research so far has focused on the production of plant cell wall derived oligosaccharides as potential prebiotics, although these types of oligosaccharides might be formed in the colon due to the action of NSP-fermenting bacteria.

All the soluble polysaccharides and oligosaccharides tested in this study were fermented in vitro by faecal inocula (data not shown). Depending on the type of poly- and oligosaccharides, different strains were able to degrade these substrates. All substrates could be completely fermented by at least one of the tested bacteria.

Within the group of *Bacteroides*, the species tested fermented all or most of the substrates to some extent, showing that *Bacteroides* spp. have a wide variety of glycanases and glycosidases. The rhamnogalacturonooligosaccharides were the only type of oligosaccharides that were selectively fermented by them. The *Bacteroides* spp. are known to degrade xylans containing low levels of arabinose (Salyers et al., 1981; Cooper et al., 1985). In addition, our study showed that highly branched xylans from wheat flour and sorghum and oligosaccharides derived from them were also fully fermented by some *Bacteroides* spp. In addition to the type II arabinogalactan (β1−3, 1−6 arabinogalactans) (Salyers et al., 1981), our study showed that bacteroides were also able to ferment type I arabinogalactan (β1−4 arabinogalactans) and galactooligosaccharides.

Clostridium spp. were also able to ferment most of the substrates to some extent, although they showed low activity toward the highly branched xylans and rhamnogalacturans. The fermentation capabilities of intestinal *Clostridium* spp. are not often reported as these species of bacteria are normally not dominant in the colon (Finegold et al., 1983). However, several studies showed high saccharolytic activity of *Clostridium* spp. occurring in nature (Henrissat, 1997).

Our studies show that bifidobacteria are able to ferment (arabino)galactooligosaccharides, arabinooligosaccharides, arabinoxylooligosaccharides, xylooligosaccharides, and fructooligosaccharides but are not able to utilize rhamno- and galacturonooligosaccharides. Bifidobacteria possess high glycosidase activity toward linear substrates such as fructooligosaccharides and galactooligosaccharides; however, this study shows that bifidobacteria also produce enzymes, which degrade plant cell wall derived oligosaccharides. Van Laere et al. (1997) showed that *Bi. adolescentis* when grown on arabinoxylooligosaccharides produced three different enzymes able to degrade the double-substituted arabinoxylooligosaccharides. The arabinoxylan polysaccharides (AXWPS by *Bi. longum*) were also fermented to some extent by some bifidobacteria; however, the oligosaccharides derived from the related polysaccharides were generally fermented to a higher extent. Yamada et al. (1993) already reported that bifidobacteria fermented arabinoxylooligosaccharides obtained by degradation of wheat bran in vitro. Our experiments, however, provide no information on the growth rate. Further in vitro and in vivo studies will be needed to evaluate whether these types of oligosaccharides selectively stimulate the growth and/or activity of a selected number of beneficial bacteria.

Fermentation of a specific substrate was not always accompanied with a drop in pH, and this supports the conclusions of Barry et al. (1995) that a pH drop should not be used as an index for fermentation. HPAEC and HPSEC are better methods to specifically follow fermentation of oligo- and polysaccharides, but complete depolymerization does not always mean full utilization.

Our experiments give information on the capability of intestinal species of bacteria to utilize the polysaccharides and oligosaccharides. Our studies, however, do not allow predictions about the fermentation in vivo, since this will depend on various factors such as the availability of other substrates, growth factors, intes-

tinal pH, actual number of bacteria, and the interactions between the different species of bacteria present.

It can be concluded that the *Bacteroides* spp., predominant intestinal bacteria, are also able to utilize plant cell wall derived oligosaccharides besides their reported activity toward plant polysaccharides. Bifidobacteria are able to utilize the structurally rather complex oligosaccharides derived from plant cell wall polysaccharides in addition to the bifidogenic oligosaccharides such as fructooligosaccharides and xylooligosaccharides, showing their broad range of glycosidases. The degradation of oligosaccharides and polysaccharides by *E. coli* might be important in humans which host high numbers of these organisms in the small intestine. The ability of the lactobacilli and bifidobacteria to ferment specific oligosaccharides and polysaccharides might be important in the development of synbiotics.

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