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Microbial Utilization and Selectivity of Pectin Fractions with Various Structures[▽]

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To evaluate the fermentation properties of oligosaccharides derived from pectins and their parent polysaccharides, a 5-ml-working-volume, pH- and temperature-controlled fermentor was tested. Six pectic oligosaccharides representing specific substructures found within pectins were prepared. These consisted of oligogalacturonides (average degrees of polymerization [DP] of 5 and 9), methylated oligogalacturonides (average DP of 5), oligorhamnogalacturonides (average DP of 10 as a disaccharide unit of galacturonic acid and rhamnose), oligogalactosides (average DP of 5), and oligoarabinosides (average DP of 6). The influence of these carbohydrates on the human fecal microbiota was evaluated. Use of neutral sugar fractions resulted in an increase in *Bifidobacterium* populations and gave higher organic acid yields. The *Bacteroides-Prevotella* group significantly increased on all oligosaccharides except oligogalacturonides with an average DP of 5. The most selective substrates for bifidobacteria were arabinan, galactan, oligoarabinosides, and oligogalactosides.

Pectins are complex plant heteropolysaccharides consisting in the main of four types of carbohydrate structure. These are partially methyl and/or acetyl esterified polygalacturonic acid (PGalA), rhamnogalacturonan type I (RG I), xylogalacturonan, and rhamnogalacturonan type II (RG II). PGalA is a linear homopolymer of α-(1,4)-galacturonic acid. Xylogalacturonan consists of a PGalA backbone with xylose bound to galactopyranosyluronic acid (Gal*p*A) at C-3. RG I and RG II comprise the so-called "hairy regions" due to their branched structures. The -4-α-D-GalA*p*-1,2-α-L-Rhap-1- disaccharide sequence constitutes the main chain of RG I, with substitutions at position 4 of the rhamnose residues as well as acetylation on C-2 or C-3 of the galacturonic acid. Side chains are α-(1,5)-arabinan, β-(1,4)-galactan, and arabinogalactan type I. RG II is very complex, but it is believed to be the most conserved part of pectin. This molecule has PGalA as a backbone and is branched with rhamnose and other rare sugars such as apiose, 2-*O*-methylxylose, and 2-*O*-methylfucose (31).

The biological effects of pectins have been studied extensively, and they are reported to be highly fermentable dietary fibers (5). Apart from their complexity and heterogeneity, both pectins and their derivatives have been investigated for many other potential health benefits. Pectins are found in oriental medicinal herbs and are thought to be responsible for their therapeutic effects (18, 27). Studies to date indicate that different regions of pectins may mediate the different health effects. For example, modified citrus pectin may control the progress of malignancy (12) or induce apoptosis in cancer cell lines (20, 29). In terms of the selectivity of fermentation by fecal bacteria, pectic oligosaccharides had greater selectivity toward bifidobacteria in batch cultures than the parent pectin (24, 25, 30).

Many plant by-products are rich in pectins, which could possibly be sources of active pectic compounds and may prove useful as alternative prebiotics for human consumption. As pectic molecules are complex and heterogeneous, different regions of pectins may exert different prebiotic potential and/or other health-supporting functions. Therefore, the aim of this study is to obtain specific carbohydrate structures from pectins and identify their fermentation properties. Selectivity can be studied using *in vitro* fecal batch cultures with analysis of the microbiota changes and metabolic end product production (34). However, the conventional fermentation scale needs gram quantities of the test substances. The *in vitro*, 5-ml-working-volume, pH- and temperature-controlled fermentation system used here was developed and validated for assessment of the fermentation properties of pectic oligosaccharides that are only available in milligram amounts.

MATERIALS AND METHODS

Validation of the miniature fermentor. The miniature fermentor (5-ml working volume) was validated against the conventional 100-ml vessel (70-ml working volume) (35). Batch culture fermentations were set up in parallel, each using fecal slurries from three healthy donors (average age, 34 ± 3.5 years; one female, two males) who had taken neither prebiotics nor probiotics and who had not had antibiotic treatment within 3 months before the study to inoculate both types of fermentors. One miniature vessel and two 100-ml vessels were set up per donor. Culture media, substrate concentrations, pH, and temperature conditions were identical in the two different fermentors. Fermentors were maintained at pH 6.70 to 6.90 and 37°C under an anaerobic atmosphere by continuously feeding N_2 gas. All chemicals and reagents were obtained from Sigma-Aldrich (Poole, United Kingdom). The biological growth media were from Oxoid Ltd. (Basingstoke, United Kingdom). Basal medium ingredients (per liter) were 2.00 g peptone-water, 2.00 g yeast extract, 0.10 g NaCl, 0.04 g K_2HPO_4, 0.04 g KH_2PO_4, 0.01 g $MgSO_4 \cdot 7H_2O$, 0.01 g $CaCl_2 \cdot 6H_2O$, 2.00 g $NaHCO_3$, 2 ml Tween 80, 0.05 g hemin, 10 μl vitamin K_1, 0.50 g L-cysteine–HCl, 0.50 g bile salts, and 4 ml resazurin (0.05 g/liter). Medium was sterilized at 120°C for 20 min before being aseptically added into the sterile fermentors.

Fructo-oligosaccharides (Beneo P95; Orafti, Tienen, Belgium) were used at 1% (wt/vol) as the sole carbon source. Fecal samples were obtained *in situ* from

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TABLE 1. Concentration of bacteria in fecal inocula and during 24 h in batch fermentation of fructo-oligosaccharides in the 70-ml- and 5-ml-working-volume fermentors

Vessel and time (h)	Mean concn ($\log_{10}$ cells/ml) ± SD (n = 3) of bacteria detected with probe[a]:						
	DAPI	Bac303	Bif164	Lab158	Erec482	Chis150	Ato291
100 ml							
0 (inocula)	8.88 ± 0.14	8.07 ± 0.17	7.76 ± 0.18	7.11 ± 0.21	8.19 ± 0.22	6.39 ± 0.24	7.63 ± 0.18
5	9.41 ± 0.15	8.34 ± 0.44	8.69 ± 0.44	7.44 ± 0.22	8.09 ± 0.75	6.37 ± 0.30	8.01 ± 0.41
10	9.58 ± 0.11	8.62 ± 0.45	8.89 ± 0.40	7.41 ± 0.10	7.99 ± 0.86	6.70 ± 0.07	8.14 ± 0.50
24	9.60 ± 0.16	8.69 ± 0.27	8.86 ± 0.40	7.51 ± 0.21	7.95 ± 0.71	6.58 ± 0.11	7.92 ± 0.55
5 ml							
0 (inocula)	8.88 ± 0.14	8.07 ± 0.17	7.76 ± 0.18	7.11 ± 0.21	8.19 ± 0.22	6.39 ± 0.24	7.63 ± 0.18
5	9.51 ± 0.11	8.39 ± 0.24	8.72 ± 0.41	7.55 ± 0.19	7.90 ± 0.70	6.35 ± 0.28	7.99 ± 0.53
10	9.59 ± 0.18	8.68 ± 0.52	8.91 ± 0.53	7.57 ± 0.12	7.66 ± 1.04	6.70 ± 0.22	8.16 ± 0.49
24	9.62 ± 0.19	8.72 ± 0.29	8.87 ± 0.47	7.70 ± 0.16	7.79 ± 1.15	6.69 ± 0.05	7.93 ± 0.58

[a] DAPI, total cells; Bac303, *Bacteroides-Prevotella*; Bif164, *Bifidobacterium*; Lab158, *Lactobacillus*/*Enterococcus*; Erec482, *E. rectale*/*C. coccoides*; Chis150, *C. histolyticum*; Ato291, *Atopobium*.

the Department of Food and Nutritional Sciences, University of Reading. Samples were kept in an anaerobic cabinet and processed within 10 min. Fecal slurries (10% [wt/wt]) in 0.17 M phosphate-buffered saline, pH 7.3 (Oxoid), were homogenized in a stomacher (Stomacher 400; Seward, United Kingdom) at normal speed for 2 min. The inoculum size was 10% (vol/vol). pH was regulated with a pH controller (Fermac 260; Electrolab, Tewkesbury, United Kingdom), which added acid and alkali as required (0.5 M HCl and 0.5 M NaOH for the 70-ml fermentor and 0.25 M HCl and 0.25 M NaOH for the miniature vessel). Fermentation samples were taken at 0, 12, and 24 h. Samples at 0 h were taken directly from the 10% (wt/wt) fecal slurry. Samples were analyzed for bacterial populations and concentration of short-chain fatty acids (SCFA).

Preparation of the pectin fractions. The five parent polymers, polygalacturonic acid (PGalA; Sigma, St. Louis, MO), 34.5% methylated citrus pectin (MPec; Danisco A/S, Copenhagen, Denmark), potato galactan (galactan) and beet arabinan (arabinan) (British Sugar, Peterborough, United Kingdom), and RG I from *Arabidopsis thaliana* seed mucilage, were used as raw materials for the production of six oligosaccharide structures representing pectin subunits.

OGalA. Oligogalacturonides (OGalA) were prepared through the autolysis of PGalA as previously described (32). PGalA solution (1% [wt/vol]) in deionized water was incubated at 100°C for 25 h. The solution was then precipitated with ethanol (64% [vol/vol] final concentration) at 4°C for 18 h. The precipitate and supernatant were harvested by centrifugation at 9,816 × *g* for 15 min. The supernatant containing alcohol-soluble oligogalacturonides was evaporated at 40°C to concentrate before dialysis against deionized water (molecular mass cutoff, 6,000 to 8,000 Da; Cellusep) and then freeze-drying. The final product (called OGalA DP5) contained oligogalacturonides with degrees of polymerization (DP) of 1 to 10. The precipitant (alcohol-insoluble oligogalacturonides) was dissolved in 100 ml deionized water before evaporation at 40°C to eliminate ethanol. The solution was then freeze-dried, and the product (oligogalacturonides with DP of 1 to 23) was called OGalA DP9.

Methylated oligogalacturonides (MOGalA). MPecs (40 mg) were dissolved in 15 ml acetate buffer (50 mM, pH 4.0) and mixed on a wheel agitator overnight at 4°C (30 tubes; total amount of raw material = 1,200 mg). MPec then was partially hydrolyzed by purified endopolygalacturonase (3) at 40°C. Enzyme activity (4.544 nanokatals) was added to the tubes periodically at 0, 24, and 48 h (1 nanokatal is the amount of enzyme that releases 1 nmol galacturonic acid per second at pH 4.0, 30°C). After 72 h of hydrolysis, the tubes were boiled for 10 min to stop enzyme activity. Tubes were pooled, and the solution was dialyzed against deionized water (1.5 liters) using dialysis tubing with a molecular mass cutoff of 6,000 to 8,000 Da. The sample was then concentrated 10-fold (by evaporation at 40°C under reduced pressure) and centrifuged at 22,360 × *g* for 10 min. The supernatant was filtered through a Vivaspin centrifugal concentrator (molecular mass cutoff of 10,000 Da; Sartorius), and the filtrate was freeze-dried.

ORham. Oligorhamnogalacturonides (ORham) were prepared as described by Ralet et al. (33). Two grams of *Arabidopsis thaliana* seeds was placed in 20 ml deionized water in a screw-cap tube (8 tubes; total amount of raw material = 16 g) and agitated overnight at 4°C before centrifuging at 33,540 × *g* for 10 min. The supernatant was filtered through G2 sintered filter glass before dialysis (molecular mass cutoff, 6,000 to 8,000 Da) against deionized water for 65 h, and the solution was freeze-dried. Then 100 mg of dried RG I was dissolved in 4 tubes containing 20 ml of 100 mM acetate buffer, pH 4.0. Rhamnogalacturonase (Novozymes, Bagsvaerd, Denmark) was prepared to 0.2 mg/ml, and 120 μl was added to each tube for 3 h of incubation at 40°C. Tubes were boiled for 10 min to stop the enzymatic activity. Ethanol (96% [vol/vol]) was added to the solution to a final concentration of 46% (vol/vol). The solution was precipitated for 18 h before centrifugation at 9,816 × *g* for 15 min. The insoluble fraction was washed as before with 46% (vol/vol) ethanol and again centrifuged. The supernatants were pooled and concentrated at 40°C under reduced pressure. The sample was desalted by loading onto a Sephadex G10 column (1.6 by 100 cm) eluted with deionized water at flow rate of 3.9 ml/min, and fractions of 15.6 ml were recovered. The profile was monitored by refractometry (Knauer, Germany) and conductometry (Radiometer Analytical, France). The quantity of sugar in the desalted fraction was analyzed as described below, and then the sugar was concentrated at 40°C under reduced pressure. The concentrate was then freeze-dried.

PGOS. Oligogalactosides (PGOS) were obtained by acidic hydrolysis of galactan (4). Eight hundred milligrams of galactan was hydrolyzed in 20 mM HCl (1% [wt/vol]) and incubated at 110°C for 5 h. Then the pH was adjusted to 7.00

TABLE 2. Parent polymers, pectic oligosaccharide products, DP analyzed by HPAEC, yield obtained, and sugar composition of oligosaccharide products analyzed by gas chromatography

Parent polymer	Oligomer	DP	% yield	% of[a]:			
				GalA	Rham	Ara	Gal
PGalA	OGalA DP5	1–10	29.18	90.93	3.39	0.70	3.99
PGalA	OGalA DP9	4–23	6.53	97.52	0.62	0.45	1.23
MPec (34.5% DM)	MOGalA (29% DM)	1–10	25.34	95.95	0.47	0.47	2.28
Rhamnogalacturonide from *Arabidopsis* seed mucilage	ORham	2–19 (repeating unit of GalA and Rham)	1.28	46.20	52.25	0	0
Potato pulp galactan (galactan)	PGOS	1–10	36.55	0	0	2.02	94.49
Beet arabinan (arabinan)	OAr	1–11	11.73	1.30	0.92	93.4	4.38

[a] GalA, galacturonic acid; Rham, rhamnose; Ara, arabinose; Gal, galactose.

using 100 mM NaOH. The sample was loaded on the Sephadex G10 column as above, except that the desalted product was separated into 4 fractions. These fractions were analyzed by high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD; see below), and those containing the highest-DP oligosaccharides were pooled, evaporated at 40°C, and freeze-dried.

Oligoarabinosides (OAr). Arabinan (1% [wt/vol]) was hydrolyzed in 50 mM H_2SO_4 at 85°C for 3 h 45 min. The pH of the solution was then adjusted to 7.00 using 100 mM NaOH immediately after hydrolysis. Na_2SO_4 was removed by adding 9.5 volumes of cold 96% (vol/vol) ethanol to the concentrated hydrolyzed sample (20 ml) and centrifuging at 14,880 × *g* for 15 min. The supernatant was concentrated and loaded on to the Sephadex G10 column (1.6 by 100 cm) eluted with deionized water at flow rate of 4 ml/min (8 ml/fraction). Fractions containing oligomers detected by HPAEC-PAD (described below) were pooled, evaporated at 40°C, and freeze-dried.

Oligosaccharide assays. Quantities of neutral sugars and galacturonic acids were analyzed by the orcinol-sulfuric acid (41) and the metahydroxybiphenyl methods, respectively, using an automated analyzer (40). The DP of oligosaccharides in the product were characterized by HPAEC-PAD. The column was a Dionex CarboPac PA1 column, 2 by 250 mm, eluted at room temperature. The mobile phase was a gradient of water (A), 1 M sodium acetate (B), and 0.5 M NaOH (C) at a flow rate of 0.25 ml/min. The DP of oligosaccharides was defined by comparison with known purified standards. For the analysis of OGalA DP5, OGalA DP9, MOGalA, and ORham, linear gradients composed of the following were used: 0 to 20 min, 55 to 30% A, 25 to 50% B, 20% C; 20 to 40 min, 30 to 20% A, 50 to 60% B, 20% C; 40 to 60 min, 20 to 10% A, 60 to 70% B, 20% C; 60 to 66 min, 10 to 0% A, 70 to 80% B, 20% C; 66 to 67 min, 0 to 55% A, 80 to 25% B, 20% C; 67 to 90 min, 55% A, 25% B, 20% C. The gradient of the liquid phase for the analysis of PGOS and OAr was the linear gradient composed as follows: 0 to 40 min, 70 to 30% A, 10 to 50% B, 20% C; 40 to 42 min, 30 to 80% A, 50 to 20% B, 20 to 0% C; 42 to 49 min, 80 to 70% A, 20 to 10% B, 0 to 20% C; 49 to 70 min, 70% A, 10% B, 20% C. Neutral sugar composition was determined from analysis of the alditol acetate derivatives (9) by gas-liquid chromatography on a DB-225 fused-silica capillary column (J&W Scientific, Courtaboeuf, France; 30 m by 0.32-mm inside diameter [i.d.]). The degree of methylation (DM) was determined by measuring alcohol oxidase and *N*-methylbenzothiazolinone-2-hydrazone (2).

Fermentation selectivity of the pectin fractions and parent polymers. The four parent polymers (PGalA, MPec, galactan, and arabinan) and six oligomers (OGalA DP5, OGalA DP9, MOGalA, ORham, PGOS, and OAr) were evaluated for their effects on human fecal microbial populations and activity in parallel to inulin (BeneoST; Orafti, Tienen, Belgium) as the positive control. The *in vitro* fermentation was performed using the 5-ml-working-volume, pH- and temperature-controlled fermentor as described above. Fermentations were performed in quadruplicate using fecal samples from four healthy donors (average age, 29.5 ± 4.4 years; three females, one male) who had taken neither prebiotics nor probiotics and who had not had antibiotic treatment within 3 months before the study. Samples (0.7 ml) from each fermentor were collected at 0, 12, 24, and 36 h after inoculation for fluorescence *in situ* hybridization (FISH) and high-performance liquid chromatography (HPLC) analysis.

Bacterial enumeration. Enumeration of the target fecal bacterial groups was performed by FISH with 16S rRNA probes (45). The 16S rRNA-targeted oligonucleotide probes used were Lab158 (17), Ato291 (16), Bif164 (23), Bac303 (26), Chis150 (11), Fprau645 (38), and Erec482 (11) for *Lactobacillus*/*Enterococcus*, *Atopobium* cluster, *Bifidobacterium* spp., *Bacteroides*/*Prevotella*, *Clostridium histolyticum* group, *Faecalibacterium prausnitzii* cluster, and *Eubacterium rectale*-*Clostridium coccoides*, respectively. Total cell counts were achieved by using 4′,6-diamidino-2-phenylindole (DAPI). The concentration of cells obtained was expressed as $\log_{10}$ cells/ml.

Organic acid analysis. Fermentation samples were centrifuged at 13,000 × *g* for 5 min, and the supernatant was kept at −20°C until analysis. Prior to HPLC analysis, all samples were centrifuged at 13,000 × *g* for 10 min and filtered through a 0.2-μm filter. An ion-exclusion Aminex HPX-87H column (7.8 by 300 mm; Bio-Rad, Watford, United Kingdom) was used. Chromatography was performed at 65°C using 0.005 mM H_2SO_4 as a mobile phase at a flow rate of 0.6 ml/min. 2-Ethyl butyric acid (Sigma-Aldrich, United Kingdom) at a final concentration of 20 mM was the internal standard. Concentrations of the organic acids were calculated using calibration curves of acetic, propionic, butyric, and lactic acids in concentrations between 4 and 128 mM, and results were expressed in mM.

Formic acid was determined using a formate dehydrogenase-based formic acid assay kit (Megazyme, Ireland).

TABLE 3. Concentrations of *Bifidobacterium*, *Lactobacillus/Enterococcus*, *E. rectale/C. coccoides*, and *C. histolyticum* in fecal inocula and during 36 h of batch fermentation of the pectins and pectic oligosaccharides using the 5-ml-working-volume fermentor

Probe[a]	Time (h)	Mean concn[b] ($\log_{10}$ cells/ml) ± SD (n = 4) on substrate:									
		PGalA	OGalA DP5	OGalA DP9	MPec	MOGalA	ORham	Arabinan	OAr	Galactan	PGOS	Inulin
Bif164 (7.82 ± 0.20)	12	7.76 ± 0.27^{B}	7.70 ± 0.25^{B}	7.55 ± 0.06^{B}	7.78 ± 0.36^{B}	7.81 ± 0.19^{B}	7.90 ± 0.23^{B**}	8.60 ± 0.19^{A**}	8.57 ± 0.21^{A**}	8.64 ± 0.22^{A**}	8.34 ± 0.10^{A**}	8.41 ± 0.43^{A**}
	24	7.68 ± 0.26^{C}	7.49 ± 0.14^{C*}	7.70 ± 0.24^{BC}	7.50 ± 0.46^{C}	7.78 ± 0.17^{C}	7.80 ± 0.11^{C}	8.84 ± 0.09^{A**}	8.63 ± 0.06^{AB**}	8.70 ± 0.17^{AB**}	8.61 ± 0.23^{AB**}	8.49 ± 0.34^{B**}
	36	7.62 ± 0.28^{C}	7.53 ± 0.23^{C}	7.54 ± 0.19^{C*}	7.56 ± 0.43^{C}	7.65 ± 0.14^{C}	7.69 ± 0.09^{C}	8.62 ± 0.14^{A**}	8.70 ± 0.21^{A**}	8.54 ± 0.35^{A}	8.61 ± 0.28^{A**}	8.29 ± 0.23^{A**}
Lab158 (6.62 ± 0.07)	12	6.89 ± 0.31	6.88 ± 0.13**	6.89 ± 0.28	6.51 ± 0.24	6.99 ± 0.26	6.88 ± 0.08**	7.02 ± 0.34	6.99 ± 0.39	6.90 ± 0.22	6.98 ± 0.38	7.22 ± 0.23**
	24	7.10 ± 0.47	6.90 ± 0.26	7.20 ± 0.56	7.05 ± 0.74	7.01 ± 0.43	7.11 ± 0.37	7.09 ± 0.53	7.11 ± 0.54	7.02 ± 0.45	7.31 ± 0.41**	7.43 ± 0.39**
	36	6.98 ± 0.53	6.77 ± 0.15	6.98 ± 0.67	6.87 ± 0.52	6.74 ± 0.42	6.94 ± 0.28	6.90 ± 0.65	7.09 ± 0.58	6.94 ± 0.62	7.04 ± 0.33	7.34 ± 0.21**
Erec482 (7.28 ± 0.24)	12	6.84 ± 0.44	7.03 ± 0.21	6.62 ± 0.13*	7.16 ± 0.64	7.01 ± 0.43	7.33 ± 0.54	7.05 ± 0.34	6.91 ± 0.57	7.01 ± 0.64	6.82 ± 0.46	6.93 ± 0.41
	24	7.76 ± 0.50	6.84 ± 0.29	7.12 ± 0.62	7.58 ± 0.51	7.62 ± 0.58	7.53 ± 0.39	7.46 ± 0.52	7.73 ± 0.75	7.43 ± 0.68	7.28 ± 0.70	7.60 ± 0.21
	36	7.98 ± 0.62^{A}	6.84 ± 0.42^{B}	7.56 ± 0.45^{A}	7.79 ± 1.00^{A}	7.57 ± 0.50^{A}	7.75 ± 0.46^{A}	7.70 ± 0.30^{A}	7.71 ± 0.42^{A**}	7.45 ± 0.56^{A}	7.50 ± 0.29^{A}	7.76 ± 0.37^{A}
Chis150 (6.31 ± 0.14)	12	7.08 ± 0.48^{AB**}	6.12 ± 0.22^{C}	6.51 ± 0.36^{BC}	6.43 ± 0.30^{BC}	7.31 ± 0.80^{A}	6.65 ± 0.26^{ABC}	7.01 ± 0.43^{AB}	6.57 ± 0.65^{ABC}	6.57 ± 0.34^{ABC}	6.67 ± 0.62^{ABC}	7.00 ± 0.45^{AB}
	24	7.05 ± 0.34**	6.19 ± 0.69	6.63 ± 0.59	6.22 ± 0.74	6.80 ± 0.45	6.56 ± 0.29	7.09 ± 0.51	6.77 ± 0.60	6.85 ± 0.66	6.81 ± 0.60	6.95 ± 0.63
	36	6.72 ± 0.43	6.43 ± 0.69	6.44 ± 0.47	5.93 ± 0.64	6.55 ± 0.63	6.50 ± 0.24	6.88 ± 0.47	6.48 ± 0.61	6.62 ± 0.77	6.53 ± 0.83	6.70 ± 0.91

[a] Bif164, probe for *Bifidobacterium*; Lab158, probe for *Lactobacillus/Enterococcus*; Erec482, probe for *E. rectale/C. coccoides*; Chis150, probe for *C. histolyticum*. Values in parentheses are concentrations ($\log_{10}$ cells/ml) at 0 h.

[b] Uppercase letters indicate significant differences between treatments at the same time point. *, significant decrease from 0 h; **, significant increase from 0 h.

TABLE 4. Concentrations of *Bacteroides*/*Prevotella*, *F. prausnitzii* cluster, and total cell number in fecal inocula and during 36 h of batch fermentation of the pectins and pectic oligosaccharides using the 5-ml-working-volume fermentor

Probe[a]	Time (h)	Mean concn[b] ($\log_{10}$ cells/ml) ± SD (n = 4) on substrate:				
		PGalA	OGalA DP5	OGalA DP9	MPec	MOGalA
Bac303 (7.85 ± 0.30)	12	$8.63 \pm 0.54^{A**}$	7.62 ± 0.46^{B}	8.54 ± 0.85^{A}	8.19 ± 0.85^{AB}	$8.78 \pm 0.43^{A**}$
	24	$8.92 \pm 0.41^{A**}$	7.86 ± 0.66^{B}	$8.97 \pm 0.07^{A**}$	8.50 ± 0.68^{A}	$8.98 \pm 0.13^{A**}$
	36	$9.25 \pm 0.17^{A**}$	8.40 ± 0.63^{CD}	$9.01 \pm 0.44^{AB**}$	$9.07 \pm 0.12^{AB**}$	$8.97 \pm 0.31^{AB**}$
Fprau645 (8.11 ± 0.22)	12	7.62 ± 0.72^{ABC}	$7.13 \pm 0.48^{C*}$	7.16 ± 0.72^{C}	7.29 ± 0.87^{BC}	7.85 ± 0.84^{AB}
	24	7.82 ± 0.34^{AB}	$7.00 \pm 0.31^{E*}$	7.24 ± 0.50^{DE}	7.63 ± 0.37^{BCD}	$8.43 \pm 0.09^{A**}$
	36	7.62 ± 0.42^{BC}	$6.88 \pm 0.61^{DE*}$	7.54 ± 0.38^{C}	8.16 ± 0.33^{AB}	8.20 ± 0.13^{A}
DAPI (9.24 ± 0.10)	12	9.41 ± 0.23	9.42 ± 0.11**	9.50 ± 0.05**	9.29 ± 0.30	9.55 ± 0.05**
	24	9.58 ± 0.21**	9.36 ± 0.08**	9.53 ± 0.15**	9.34 ± 0.26	9.67 ± 0.05**
	36	9.64 ± 0.09**	9.37 ± 0.22	9.56 ± 0.20**	9.56 ± 0.25	9.61 ± 0.02**

[a] Bac303, probe for *Bacteroides*/*Prevotella*; Fprau645, probe for *F. prausnitzii* cluster; DAPI, probe for total cells. Values in parentheses are concentrations ($\log_{10}$ cells/ml) at 0 h.
[b] Uppercase letters indicate significant differences between treatments at the same time point. *, significant decrease from 0 h; **, significant increase from 0 h.

Statistical analysis. Statistical analysis was performed using SPSS for windows, version 15.0. Validation of the miniature fermentor against the conventional fermentor was done by paired *t* test. For the fermentation of pectin fractions, univariate analysis of variance (ANOVA) and Duncan's test were used. A two-sample *t* test was also performed in order to see the significant changes between concentrations at inoculation and subsequent sampling time points within the same treatment.

RESULTS

Bacterial population and SCFA data are presented as average concentrations ± standard deviations (SD). Data from individual donors are not presented.

Validation of the miniature vessel. There were no statistically significant differences in the SCFA concentrations between the two vessel types ($P < 0.05$). Amounts and patterns of organic acids varied between donors. Acetate was the main fermentation product, followed by lactate, formate, butyrate, and propionate. Lactate accumulated only in fecal cultures from one donor, and these cultures had low acetate concentrations. Generally, total organic acid concentrations increased throughout fermentation and reached maximum levels after 24 h.

Microbial profiles are presented in Table 1. Bacterial concentrations in samples collected from the fermentors showed no statistically significant differences between the two types of vessel ($P \leq 0.05$). Concentrations of *Bacteroides*/*Prevotella*, *Bifidobacterium* spp., *Lactobacillus*/*Enterococcus*, *C. histolyticum* subgroup, and *Atopobium* cluster started to increase after 5 h of fermentation. When the bacterial population at 10 h was compared with its baseline (0 h), it was found that the magnitude of changes varied among donors. Levels of the *E. rectale*/*C. coccoides* group remained stable, which correlated with the small concentrations of butyric acid.

Preparation of the pectic oligosaccharides. The DP, product yields, and sugar compositions of oligosaccharides are summarized in Table 2. OGalA DP5 and OGalA DP9 were found in alcohol-soluble and alcohol-insoluble fractions, respectively. The average DP of those two fractions were 5 and 9. Sugar composition data showed that the major component of both fractions was galacturonic acid, with trace amounts of neutral sugars. The MOGalA products had a degree of methylation (DM) of 29%, slightly lower than that of the parent polymer (34.5%). The oligosaccharide product had DP of 1 to 10 and had only small amounts of neutral sugars (4.05% [wt/wt]). Data from gas chromatography indicated that the ORham product contained galacturonic acid (46.2%) and rhamnose (52.25%) in a molar ratio of 1.21:1 (DP of 2 to 19), which corresponds to a repeating unit of rhamnose and galacturonic acid. β-(1,4)-PGOS prepared by acid hydrolysis of potato galactan mainly consisted of galactose (94.49%) of DP 1 to 10 and small amounts of arabinose, mannose, and glucose. Arabinose was the main component of OAr (93.4%) of DP 1 to 11, with lesser amounts of galacturonic acid, rhamnose, and galactose. It was, therefore, possible that some of the OAr material was not a linear chain.

Fermentation of the pectins and pectic oligosaccharides. Tables 3 and 4 show the changes in bacterial populations during the *in vitro* fermentation of different substrates. Results from four donors exhibited responses of different magnitudes but with similar overall patterns.

With the exception of ORham, fractions containing galacturonic acid (PGalA, OGalA DP5, OGalA DP9, MPec, and MOGalA) did not promote *Bifidobacterium* spp., as they either remained at stable levels or tended to decrease during fermentation. Galactan, PGOS, arabinan, and OAr showed a behavior similar to that of inulin. Significant increases ($P \leq 0.05$) were found with PGOS, arabinan, and OAr between 12 and 36 h of fermentation. Galactan increased bifidobacteria between 12 and 24 h (Table 3). Only a transient bifidogenic effect was observed from ORham, which exhibited a significant increase in *Bifidobacterium* spp. at 12 h of fermentation.

F. prausnitzii did not grow on most substrates, including inulin, but higher numbers were seen on MOGalA and MPec. A statistically significant increase ($P \leq 0.05$) was seen only on MOGalA (Table 4). No difference in *Lactobacillus*/*Enterococcus*, *E. rectale*/*C. coccoides* group, and total cell concentrations between substrates having neutral and acid sugars was seen. Significant increases in *Bacteroides*/*Prevotella* were seen on all substrates from 12 h of fermentation. *Bacteroides*/*Prevotella* populations did not change on OGalA DP5 but increased at 36 h on MPec. A significant increase in *C. histolyticum* was

TABLE 4—*Continued*

Mean concn[b] ($\log_{10}$ cells/ml) ± SD (n = 4) on substrate:					
ORham	Arabinan	OAr	Galactan	PGOS	Inulin
$8.82 \pm 0.57^{A**}$	$9.00 \pm 0.44^{A**}$	$8.71 \pm 0.47^{A**}$	$8.28 \pm 0.30^{AB**}$	$8.42 \pm 0.30^{AB**}$	$8.46 \pm 0.23^{A**}$
$8.63 \pm 0.31^{A**}$	$9.03 \pm 0.20^{A**}$	$8.59 \pm 0.16^{A**}$	$8.72 \pm 0.16^{A**}$	$8.77 \pm 0.14^{A**}$	$9.00 \pm 0.39^{A**}$
8.56 ± 0.40^{BCD}	$8.85 \pm 0.21^{ABCD**}$	$8.60 \pm 0.22^{BCD**}$	$8.35 \pm 0.09^{D**}$	$8.57 \pm 0.31^{BCD**}$	$8.90 \pm 0.28^{ABC**}$
7.59 ± 0.74^{ABC}	7.93 ± 0.48^{A}	7.47 ± 0.42^{ABC}	7.62 ± 0.54^{ABC}	7.65 ± 0.50^{ABC}	7.34 ± 0.62^{BC}
7.45 ± 0.53^{BCDE}	7.96 ± 0.28^{AB}	$7.44 \pm 0.28^{BCDE*}$	$7.28 \pm 0.41^{CDE*}$	$6.99 \pm 0.37^{E*}$	$7.60 \pm 0.25^{BCD*}$
$7.41 \pm 0.29^{CD*}$	7.86 ± 0.18^{ABC}	$7.38 \pm 0.29^{CD*}$	$7.37 \pm 0.25^{CD*}$	$6.73 \pm 0.36^{E*}$	7.50 ± 0.37^{C}
9.43 ± 0.19	$9.55 \pm 0.18^{**}$	$9.48 \pm 0.13^{**}$	$9.42 \pm 0.14^{**}$	$9.55 \pm 0.09^{**}$	$9.41 \pm 0.18^{**}$
$9.40 \pm 0.13^{**}$	$9.63 \pm 0.05^{**}$	$9.54 \pm 0.11^{**}$	$9.54 \pm 0.10^{**}$	$9.53 \pm 0.04^{**}$	$9.49 \pm 0.21^{**}$
9.54 ± 0.31	$9.58 \pm 0.08^{**}$	$9.61 \pm 0.14^{**}$	9.36 ± 0.15	$9.45 \pm 0.11^{**}$	$9.49 \pm 0.10^{**}$

found on PGalA fermentation. An increase in total bacterial levels occurred on all substrates except for MPec compared to baseline; however, there were no significant differences among substrates.

Table 5 shows the concentrations of organic acids during 36 h of fermentation of pectic oligosaccharides and parent polymers. All substrates were fermentable, as shown by an increase in total SCFA concentrations. Formate and lactate were transient metabolites, seen only at 12 h of fermentation. Butyrate levels were low and did not exceed 10% of total SCFA, while propionate concentrations were higher on all substrates. Acetate was a major SCFA product. The total SCFA data show that substrates were fermented at different rates. PGalA, OGalA DP5, MPec, and ORham were fermented at lower rates than inulin. OGalA DP5 and MPec produced lower acetate yields than inulin at 12 h. Propionate levels varied among the different substrates. Substrates which were more slowly fermented than inulin (PGalA, OGalA DP5, OGalA DP9, and MPec at 12 h and MPec and OGalA DP5 at 24 h) also produced lower levels of propionate. ORham gave the highest propionate levels (23 to 31% of total SCFA from 12 to 36 h of fermentation).

Variation between individual donors was seen. Donor 1 produced more propionate than the other three donors at 24 to 36 h but had the lowest acetate levels between 12 and 24 h. Donors 2, 3, and 4 were generally similar with respect to SCFA production, except that donor 2 produced higher propionate than all other donors at 12 h and also gave the highest butyrate level at 12 to 36 h.

DISCUSSION

The smallest-scale study on human fecal fermentation published to date used 0.7 ml with 7 mg substrate (36). This was a simple model, developed to evaluate changes in the microbiota and SCFA profiles on fecal fermentation of scarce carbohydrates. However, the method was not pH controlled and was not reliable beyond 12 h. Up to now the minimum amount of test substrate required per fermentation run in pH- and temperature-controlled anaerobic cultures was 0.5 g (in a 50-ml-working-volume fermentor) (35). This has hindered the evaluation of substrates that could be obtained only in extremely small quantities. As judged by the microbiota changes and SCFA concentrations, the 5-ml pH-controlled vessel developed here generally displayed the same fermentation profiles as the larger vessels. However, the low culture volume does pose a limitation on the number of samples that can be obtained throughout fermentation, and as such careful selection of the sampling time points is required.

Here, we have used the validated 5-ml fermentor to investigate the fermentation profiles of a selection of pectic oligosaccharides and parent pectins. FISH was used to enumerate numerically predominant and functionally significant members of the fecal flora. As this method does not differentiate between metabolically active and inactive cells, SCFA profiles were used in combination with microbial data to characterize the fecal microbiota behavior on each test substrate.

The fermentation selectivity of specific regions of the complex pectin structure has not been reported before. Oligosaccharides (DP of 3 to 7) from Bergamot with 63% galacturonic acid (24) and pectic oligosaccharides extracted from orange albedo containing 6.29% galacturonic acid (25) have been evaluated for their prebiotic potential in *in vitro* pH- and temperature-controlled human fecal cultures. Those two pectic oligosaccharides exhibited comparable prebiotic potential for fructo-oligosaccharides (Beneo P95; Orafti, Tienen, Belgium). Composition, molecular weight, and structure might all affect the degree and pattern of microbial utilization, and it is not possible to identify factors rendering pectic oligosaccharides more selectively fermentable by beneficial bacteria than pectin.

This study aimed to increase our understanding of the relationship between structure and human fecal fermentation characteristics of pectin subunits. Fractions representing pectin subunits were produced from pectic polysaccharides. In more detail, some fractions were acidic (OGalA and MOGalA) while others were neutral (OAr and PGOS); some were fully acidic (OGalA), while others were methylated (MOGalA). The two fractions of OGalA differed by their DP; ORham had alternating residues of rhamnose and GalA, while the others were homogenous oligosaccharides; finally, OGalA, MOGalA, and ORham had linear structures, while of OAr and PGOS could contain branching.

In this study, PGalA, OGalA, MOGlaA, and MPec exhibited no bifidogenic effect. This was in agreement with a previous study reporting that none of a selection of *Bifidobacterium* and

TABLE 5. SCFA concentrations in fecal inocula and during 36 h of batch fermentation of pectins and pectic oligosaccharides using the 5-ml-working-volume fermentor

SCFA[a]	Time (h)	Mean SCFA concn[b] (mM) ± SD (n = 4) on substrate:				
		PGalA	OGalA DP5	OGalA DP9	MPec	MOGalA
Lactate (0)	12	1.4 ± 1.1^{C}	2.4 ± 0.5^{C}	2.6 ± 2.6^{C}	1.4 ± 1.2^{C}	0.1 ± 0.7^{C}
	24	0.0^{B}	2.1 ± 0.3^{B}	1.1 ± 1.3^{B}	0.7 ± 1.2^{B}	0.0^{B}
	36	0.0	1.3 ± 1.1	0.6 ± 0.7	1.0 ± 1.7	0.0
Formate (0)	12	2.5 ± 2.2	9.6 ± 2.2	8.9 ± 2.4	6.9 ± 3.6	6.9 ± 5.7
	24	1.2 ± 1.4	4.0 ± 2.6	0.5 ± 0.9	3.5 ± 5.2	1.4 ± 0.8
	36	0.0	1.0 ± 2.0	1.3 ± 1.4	0.6 ± 0.4	0.5 ± 0.7
Acetate (1.2 ± 1.1)	12	19.0 ± 11.2^{BCD}	14.7 ± 7.2^{D}	31.5 ± 16.5^{AB}	17.1 ± 10.9^{CD}	32.6 ± 14.4^{A}
	24	31.7 ± 10.8^{ABC}	23.6 ± 7.9^{C}	32.3 ± 10.9^{ABC}	23.5 ± 9.5^{C}	41.6 ± 6.3^{A}
	36	34.7 ± 6.2^{ABC}	32.9 ± 11.2^{ABC}	33.3 ± 5.4^{ABC}	30.0 ± 5.7^{BC}	40.8 ± 5.4^{A}
Propionate (0)	12	3.6 ± 3.8^{BC}	1.4 ± 1.0^{C}	3.2 ± 3.6^{BC}	3.8 ± 4.42^{BC}	4.8 ± 2.9^{ABC}
	24	8.1 ± 2.7^{AB}	2.5 ± 3.8^{C}	7.4 ± 2.9^{AB}	4.8 ± 2.2^{BC}	10.5 ± 4.6^{A}
	36	8.2 ± 2.5^{BCD}	6.6 ± 4.3^{CD}	8.2 ± 2.4^{BCD}	4.7 ± 4.1^{C}	12.6 ± 8.9^{ABC}
Butyrate (0.2 ± 0.4)	12	0.7 ± 1.3	0 ± 0.1	0.5 ± 1.1	0.7 ± 1.2	1.0 ± 2.0
	24	1.3 ± 1.8	0.0	1.4 ± 2.8	0.7 ± 1.1	1.4 ± 1.4
	36	1.0 ± 0.9^{BC}	0.0^{C}	1.7 ± 3.4^{BC}	0.0^{C}	1.4 ± 0.2^{BC}
Total (1.4 ± 1.5)	12	26.8 ± 14.3^{D}	28.3 ± 10.0^{D}	46.8 ± 18.2^{BCD}	25.8 ± 20.7^{D}	46.1 ± 10.8^{BCD}
	24	42.4 ± 9.5^{AB}	32.3 ± 3.5^{BC}	42.7 ± 14.8^{AB}	25.6 ± 11.5^{C}	54.2 ± 7.6^{A}
	36	44.0 ± 7.7^{BC}	41.9 ± 11.5^{BC}	45.0 ± 10.6^{BC}	36.5 ± 5.4^{C}	55.4 ± 12.6^{AB}

[a] Values in parentheses are concentrations (mM) at 0 h.
[b] Uppercase letters indicate significant differences between treatments at the same time point.

Lactobacillus strains could degrade unbranched rhamno-oligogalacturonides, rhamno-oligogalacturonides carrying galactosyl branches, or OGalA with DP of 3 to 9 but that *Clostridium* and *Bacteroides* could do so (43). A strong bifidogenic effect was obtained with inulin, arabinan, OAr, galactan, and PGOS. Bifidobacteria can degrade a variety of arabinose and galactose polysaccharides, oligosaccharides, and monosaccharides but poorly utilize ORham, PGalA, and pectin (19, 37, 42, 43, 44). The *Bacteroides-Prevotella* group significantly increased on all oligosaccharides except OGalA DP5. *Bacteroides* species are major carbohydrate-degrading organisms in the gut and can degrade pectins (6, 21, 43). Bifidogenic effects, at least *in vitro*, are also influenced by the molecular weight of carbohydrate, with low-molecular-weight OAr (DP of 1 or 2) producing higher bifidobacterial numbers than higher-DP OAr and the parent, arabinan (1). In this study, no such effect between arabinan and OAr with DP of 1 to 10 was seen. Similarly, there was no significant difference in *Bifidobacterium* counts at 36 h between galactan and PGOS. The high bifidogenic effect of arabinan, OAr, galactan, PGOS, and inulin was seen early in the fermentation (from 12 h) and lasted until 36 h of fermentation. However, no significant increase in bifidobacteria on galactan was seen at 36 h.

The carbohydrate source is known to regulate the rate of fermentation and profiles of end products (19). All substrates tested in this study were fermentable to different extents. The total SCFA data suggest that neutral sugars were more readily fermented than acidic sugars. Citrus pectin was more rapidly degraded than larch arabinogalactan, although the SCFA concentration on citrus pectin was 0.35 mg/mg pectin compared to 0.43 mg/mg larch arabinogalactan (10). Fermentation of rhamnose gave relatively higher propionate than fermentation of some monosaccharides, disaccharides, and pectin (19). Arabinose-rich apple pectin gave a higher SCFA yield with more propionate than complex apple pectin (15). These reports agreed with the high propionate proportion from ORham, arabinan, and OAr seen in the present study.

The main constituent of pectin is galacturonic acid. In non-pH-controlled fermentations, pectins with various DMs were hydrolyzed by a human fecal inoculum into mainly unsaturated oligogalacturonides and the final products of the fermentation were SCFA, mainly acetate (6, 7). It was suggested that degradation of PGalA and pectin was due to pectinolytic enzymes of the lyase type and pectin methylesterase (28), while hydrolase type activity was shown to be very low (6, 7, 10, 28). Methylation of galacturonic acid may also play a role in microbial fermentation. Citrus pectins with lower DMs were fermented faster than pectins with higher DMs in studies comparing methylation levels from 0 to 94.7% (6, 7). The opposite results have also been reported, however: SCFA yield from 5% DM apple pectin fermentation was lower than that from 36 and 56% DM apple pectins (13, 14). No influence of methylation was seen in this study except that MOGalA showed faster fermentation and gave higher total SCFA, acetate, and propionate than OGalA DP5 at 24 h. Inconsistency in the literature may at least in part be due to the use of different models, pectin sources, and fecal inocula. MOGalA produced higher total SCFA, acetate (12 to 36 h), and propionate (24 h) than MPec, possibly as a result of molecular size.

At present, increasing the populations of *Bifidobacterium* spp. and *Lactobacillus* spp. in the human gut is considered to be a health benefit (22). *F. prausnitzii* may be considered a

TABLE 5—*Continued*

Mean SCFA concn[b] (mM) ± SD (n = 4) on substrate:					
ORham	Arabinan	OAr	Galactan	PGOS	Inulin
0.7 ± 0.8^{C}	6.3 ± 3.1^{BC}	12.7 ± 9.0^{AB}	12.5 ± 3.0^{AB}	15.3 ± 9.1^{A}	6.7 ± 2.4^{BC}
0.3 ± 0.5^{B}	0.8 ± 1.7^{B}	2.2 ± 4.4^{B}	2.4 ± 4.0^{B}	7.7 ± 6.3^{A}	3.1 ± 2.3^{B}
0.2 ± 0.4	0.0	0.0	0.00	2.5 ± 5.0	0.0
2.9 ± 3.9	8.5 ± 5.2	5.8 ± 5.9	5.5 ± 8.7	4.4 ± 4.6	5.7 ± 8.9
0.5 ± 0.3	0.4 ± 0.4	0.4 ± 0.4	1.0 ± 0.6	1.1 ± 1.1	0.8 ± 0.6
0.7 ± 1.1	1.0 ± 1.5	0.4 ± 0.5	0.7 ± 0.5	0.4 ± 0.5	0.4 ± 0.2
29.2 ± 16.1^{ABC}	31.9 ± 3.2^{AB}	31.4 ± 4.8^{AB}	39.9 ± 6.4^{A}	42.0 ± 3.4^{A}	31.0 ± 7.8^{AB}
27.3 ± 13.9^{BC}	34.7 ± 2.6^{BC}	29.4 ± 3.9^{BC}	34.8 ± 2.0^{ABC}	37.2 ± 7.5^{AB}	27.5 ± 7.3^{BC}
32.1 ± 5.4^{ABC}	38.1 ± 4.6^{ABC}	33.5 ± 6.2^{ABC}	39.6 ± 5.9^{AB}	42.3 ± 6.2^{A}	28.5 ± 6.1^{C}
9.2 ± 8.29^{A}	8.7 ± 4.8^{A}	6.3 ± 4.9^{AB}	6.6 ± 4.3^{AB}	6.2 ± 5.8^{AB}	8.8 ± 5.1^{A}
11.7 ± 6.0^{A}	11.7 ± 2.7^{A}	10.2 ± 2.56^{A}	9.0 ± 2.0^{AB}	9.4 ± 3.2^{AB}	10.9 ± 3.2^{A}
16.2 ± 8.9^{A}	15.9 ± 8.0^{A}	14.5 ± 10.1^{AB}	12.6 ± 8.0^{ABC}	12.4 ± 6.8^{ABC}	12.6 ± 5.5^{ABC}
0.6 ± 0.7	0.6 ± 1.3	1.0 ± 2.0	0.6 ± 2.6	1.5 ± 3.0	2.8 ± 2.7
1.0 ± 1.2	2.0 ± 1.2	1.3 ± 2.1	3.5 ± 3.4	2.8 ± 4.7	3.3 ± 2.1
1.0 ± 0.7^{BC}	1.5 ± 1.2^{BC}	2.2 ± 2.1^{BC}	3.5 ± 2.4^{AB}	3.2 ± 4.2^{AB}	5.1 ± 2.9^{A}
36.6 ± 29.0^{CD}	57.2 ± 2.8^{ABC}	59.0 ± 11.3^{ABC}	67.4 ± 10.1^{AB}	71.8 ± 9.7^{A}	56.0 ± 12.6^{ABC}
40.7 ± 20.8^{ABC}	49.5 ± 4.2^{AB}	43.7 ± 7.6^{AB}	50.4 ± 3.5^{A}	58.1 ± 11.0^{A}	45.5 ± 9.8^{AB}
50.4 ± 14.1^{ABC}	56.7 ± 13.7^{AB}	50.8 ± 15.3^{ABC}	56.3 ± 11.4^{AB}	62.3 ± 15.5^{A}	46.7 ± 9.2^{BC}

beneficial bacterial species, as it is a predominant butyrate-producing organism in the human colon (8, 39). It was previously demonstrated that low *F. prausnitzii* numbers correlated with the recurrence of inflammatory bowel disease. Moreover, this bacterium has been shown to have anti-inflammatory properties in mice (38). MOGalA supported significantly increased populations of *F. prausnitzii* compared to the parent polysaccharide and to other pectic fractions.

This study has shown that the structures of oligosaccharides fractionated from pectins had a significant impact on fermentation by human fecal bacteria, with greatest bifidogenic activity seen with the low-molecular-weight OAr and PGOS. This concurs to some extent with previous studies using *in vitro* batch fermentation of high bifidogenic OAr with DP of 1 or 2 (1).

Pectins have been investigated for their potential as prebiotic carbohydrates (24, 25, 29, 30). Based on the current study, it would seem that pectins rich in galactan, arabinan (such as potato and sugar beet pectin, respectively), and methylated oligogalacturonides are worthy of further investigation in more-sophisticated models of the human gut and ultimately, human volunteer trials.

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