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ORIGINAL ARTICLE

In vitro fermentation of sugar beet arabinan and arabino-oligosaccharides by the human gut microflora

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Abstract

Aims: To determine the fermentation profiles by human gut bacteria of arabino-oligosaccharides of varying degree of polymerization.
Materials and Methods: Sugar beet arabinan was hydrolyzed with a commercial pectinase and eight fractions, of varying molecular weight, were isolated by gel-filtration chromatography. Hydrolysis fractions, arabinose, arabinan and fructo-oligosaccharides were fermented anaerobically by gut bacteria. Total bacteria, bifidobacteria, bacteroides, lactobacilli and the *Clostridium perfringens/histolyticum* sub. grp. were enumerated using fluorescent *in situ* hybridization.
Results: Bifidobacteria were stimulated to different extents depending on molecular weight, i.e. maximum increase in bifidobacteria after 48 h was seen on the lower molecular weight fractions. Lactobacilli fluctuated depending on the initial inoculum levels. Bacteroides numbers varied according to fraction; arabinan, arabinose and higher oligosaccharides (degree of polymerization, dp > 8) resulted in significant increases at 24 h. Only carbohydrate mixtures with dp of 1–2 resulted in significant increases at 48 h (log 8·77 ± 0·23). Clostridia decreased on all substrates.
Conclusions: Arabino-oligosaccharides can be considered as potential prebiotics.
Significance and Impact of the Study: Arabinan is widely available as it is a component of sugar beet pulp, a co-product from the sugar beet industry. Generation of prebiotic functionality from arabinan would represent significant added value to a renewable resource.

Introduction

There has been much interest recently in the use of non-digestible oligosaccharides (NDO) as functional food components targeted at gut health (Roberfroid and Slavin 2000). Some NDO are selectively fermented in the human colon, and can be described as prebiotics (Gibson and Roberfroid 1995). The currently accepted criterion for prebiotic activity is an increase in populations of *Bifidobacterium* sp. and *Lactobacillus* sp. with a decrease in less desirable bacterial groups such as species of *Clostridium* and *Bacteroides* (Gibson and Roberfroid 1995). There is a wide range of prebiotic oligosaccharides on the market in Japan with lesser use in Europe (Crittenden and Playne 1996). The properties of these prebiotics have not been fully characterized, although some comparative studies do exist (Rycroft *et al.* 2001a; Palframan *et al.* 2002). In view of the growing interest in prebiotic oligosaccharides, it is of interest to investigate the potential for generation of novel forms from readily available, renewable carbohydrate sources.

One such carbohydrate resource is sugar beet pulp. Large quantities are produced annually in major sugar beet growing areas of Europe, Japan and the USA and this material is very rich in arabinan. Beet pulp is traditionally used as an animal feed, usually combined with molasses and dried to

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give a high energy feed for ruminants. Several attempts have been made to add value to this material and current commercial products include a dietary fibre called Fibrex and beet pectin from Herbstreith and Fox.

Arabinan is a pectic polysaccharide consisting of highly branched α1,5-linked chains of arabinofuranoside residues (Oosterveld *et al.* 1996, 2000). Arabinan has a molecular weight ranging from range 5700 to 10 000 kDa. Arabinan from sugar beet pulp represents 15–21%, w w^{-1} of the dry pulp after sucrose extraction. Crude arabinan extracted from sugar beet pulp contains arabinose, galactose and rhamnose in approximate ratios of 80 : 15 : 5 and has a galacturonic acid content of 6–10% (McCleary *et al.* 1989). Arabinan from sugar beet pulp is used as a traditional route to the isolation of L-arabinose in classical carbohydrate text books (Jones and Tanaka 1965).

Enzyme modification of arabinans has been employed by Cooper and co-workers to produce an essentially linear (debranched) arabinan using rigorously purified α-L-arabinofuranosidase (devoid of endo-arabinase) from a commercially available pectinase (Cooper *et al.* 1992).

The aim of this work was to investigate the fermentation properties of arabinan and enzymatically derived oligosaccharides by the human gut flora. The sugars were fractionated and the fractions tested using batch culture fermentations inoculated with a human faecal inoculum. Microbial populations were enumerated using fluorescent *in situ* hybridization (FISH).

Materials and methods

Materials

Arabinan, MW ≤ 10 kDa, was obtained from British Sugar (Peterborough, UK). It was extracted by treatment of sugar beet pulp with calcium hydroxide at 95°C followed by addition of carbon dioxide to reduce the concentration of calcium by precipitating out calcium carbonate. The resultant filtered crude extract was purified using a combination of ultra/diafiltration and spray drying (McCleary *et al.* 1989). Viscozyme® L (Novozymes, Bagsvaerd, Denmark) is a commercial enzyme used as a cell-wall lyase to reduce viscosity of pectin-containing solutions.

Enzymatic hydrolysis

Arabinan (5%, w/v) was reconstituted in 0·2 M sodium succinate buffer, pH 4·5, then centrifuged for 10 min at 5200 × ***g*** to remove any particulate material. The clear supernatant was then filtered through a 0·7 μm filter (Millipore, UK) and stored at 4°C until required.

Viscozyme (3 ml) was purified by diafiltration using Vectraspin™ centrifuge tubes fitted with a 20 kDA MWCO membrane (Whatman, UK). Tubes were filled and centrifuged for 10 min at 5000 × ***g***. Sodium succinate buffer 0·2 M, pH 4·5, equivalent to the permeate volume, was added and the centrifugation repeated three times in total.

A mixture (50 ml) of arabinan solution (5%, w/v) and viscozyme was prepared by mixing five volumes of arabinan solution to one volume of diafiltered viscozyme.

This mixture was incubated at 35°C for 22 h. Every 2 h, a 1 ml sample was taken and boiled for 3 min, followed by centrifugation for 5 min at 10 000 × ***g***. The supernatant was then assayed for reducing sugar content using the Nelson–Somogyi assay (Somogyi 1952) with arabinose as the standard in order to determine the degree of hydrolysis.

Gel filtration chromatography on bio-gel P4

A sample (5 ml) of supernatant from the enzyme hydrolysis reaction at 12–16 h was applied to a column of Bio-Gel P4 (90 × 2·5 cm) and eluted at a flow rate of 0·75 ml min^{-1} in deionized water. Fractions (5 ml) were collected. Triplicate samples (200 μl) from each tube were assayed for total sugar using the phenol–sulphuric acid assay (Dubois *et al.* 1956) with arabinose as the standard. Each fraction was collected and freeze dried.

Analysis of fractions by high performance anion exchange chromatography (HPAEC)

The qualitative composition of each fraction following lyophilization was analysed by high performance anion exchange chromatography with pulsed amperometric detection (HPAE-PAD) using an 8220i Dionex ion chromatograph. A solution (1 mg ml^{-1}) of each fraction was prepared in HPLC grade water and filtered through a 0·2 μm filter. The column was a Carbopac PA100 column eluted at room temperature and the injection volume for each sample was 20 μl. The column was eluted at 1 ml min^{-1} in 200 mM NaOH for 5 min, followed by a gradient over 40 min from 0 to 45% of 550 mM sodium acetate in 200 mM NaOH, followed by a gradient to 100% sodium acetate eluent over 50 min and a further 10 min with 100% sodium acetate eluent. A pulsed amperometric detector was used with the following settings: 420 ms at 0·05 V, 180 ms at 0·75 V and 420 ms at −0·15 V. Peaks were analysed using Turbochrom software (Perkin Elmer, Fremont, CA, USA). Standards of arabinobiose to arabino-octaose (Megazyme, Ireland) were used as calibration standards to indicate approximate degrees of polymerization (dp) elution times. Authentic standards to allow quantification of each oligosaccharide in the hydrolysates were not available.

Preparation of faecal inoculum for batch cultures

Donors (three healthy adults, who had not received antibiotics or pre/probiotics for the previous 6 months and had no history of gastrointestinal disorder) provided faecal samples. These were diluted with sterile 0·17 M phosphate buffered saline (PBS, 8·0 gl^{-1} NaCl, 0·2 gl^{-1} KCl, 1·15 $gl^{-1}Na_2HPO_4$ and 0·2 $gl^{-1}KH_2PO_4$ pH 7·3, Oxoid, UK) to 10%, w/v. Diluted samples were then homogenized in a stomacher (Seward, Worthing, UK) for 2 min.

Sample preparation

In addition to the experimental materials, fructo-oligosaccharides (FOS) were used as a positive control. It was not deemed necessary to include a control with no added substrate as it is well established that faecal bacteria do not significantly increase in the absence of added carbohydrate (Wang and Gibson 1993).

Arabinan, arabinose, freeze-dried arabinan fractions and FOS (0·15 g) were each weighed into triplicate 1·5 ml capacity microcentrifuge tubes. Tubes were kept overnight in an anaerobic cabinet (Don Whitley Scientific ltd, Shipley, UK) under an atmosphere of $H_2 : CO_2 : N_2$ (10 : 10 : 80, by volume).

A carbohydrate-free basal medium was prepared to consist of (g l^{-1}):

Peptone water 2·0, yeast extract 2·0, NaCl 0·1, K_2HPO_4 0·04, KH_2PO_4 0·04, $MgSO_4$ 0·01, $CaCl_2{\cdot}6H_2O$ 0·01, $NaHCO_3$ 2·0, cysteine HCl 0·5, bile salts 0·5 and Tween 80 2·0 ml l^{-1}, haemin solution (50 mg ml^{-1}) 1 ml l^{-1}, vitamin K 10 $\mu l\ l^{-1}$ and resazurine 1 mg ml l^{-1}. The medium (900 ml) was autoclaved (121°C for 15 min) and kept in an anaerobic cabinet (10% H_2; 10% CO_2; 80% N_2) whilst hot to assure anaerobicity. The cooled medium was inoculated with fresh faecal suspension (100 ml l^{-1}) and the mixture thoroughly shaken. Then, 1·5 ml were dispensed into each tube containing carbohydrates and shaken thoroughly to ensure dissolution. A sample (5 ml) was taken from the inoculum to determine microbial counts and short chain fatty acid levels at time 0. All tubes were maintained at 37°C and a sample (0·375 ml) taken after 24 and 48 h incubation to determine microbial counts.

Analysis of microbial populations by fluorescence *in situ h*ybridization

In a 1·5 ml microcentrifuge tube, 0·375 ml of the sample was mixed with 1·125 ml of 4% (w/v) filtered paraformaldehyde solution and fixed overnight at 4°C. The tubes (1·5 ml) were centrifuged at 13 000 × ***g*** for 5 min. The supernatant was then removed and the pellets resuspended in 1 ml of filtered 0·17 M PBS, pH 7·3. The cells were then re-centrifuged at 13 000 × ***g*** for 5 min. This wash was repeated a further two times. The supernatant was removed, 150 μl of PBS and 150 μl of 100% ethanol added and the pellets resuspended thoroughly before being stored at −20°C. In a 1·5 ml microcentrifuge tube, 0·2 ml of filtered double-strength hybridization buffer (40 mmol l^{-1} Tris–HCl pH 7·2, 1·8 mol 1^{-1} NaCl containing 20 ml l^{-1} of 10%, w/v sodium dodecyl sulphate), 64 μl of HPLC water and 16 μl of the fixed cells was added. In a 0·5 ml microcentrifuge tube, 10 μl of probe solution (50 ng μl^{-1}), was mixed with 90 μl of the above hybridization mixture.

The probe sequences and references are shown in Table 1. The mixture was vortexed and incubated in an oven at 50°C for Bif 164 and His 150 and at 45°C for Lab 158 and Bac 303. All samples were allowed to hybridise overnight. In a 7 ml centrifuge tube, 5·0 ml of pre-warmed hybridization buffer (20 mmol l^{-1} Tris–HCl pH 7·2, 0·9 mol 1^{-1} NaCl) was added followed by 20 μl of 4,6-diamidino-2-phenylindole (500 ng ml^{-1}). Hybridization mixture (2–150 μl, Table 1) was added and vortexed. The mixture was returned to the oven at its corresponding temperature for 30 min. The stored wash mixture was filtered through a 0·2 μm filter (Millipore, UK) under vacuum. The filter was then placed on a slide, a drop of 'Slow Fade^{TM} (Molecular Probes, Leiden, the Netherlands) added and covered with a coverslip. Slides were counted under UV light at 461 nm to determine the total number of DAPI-stained bacteria. A green light at 565 nm was used for counting of Cy-3 labelled bacterial

Table 1 Sequences of the probes used in the FISH enumeration and the incubation temperatures used

Probe Name	Probe sequence	Incubation temperature (°C)	Bacterial group	Reference
Bif164	5-CATCCGGCATTACCACCC-3	50	*Bifidobacterium* spp.	Langendijk *et al.* (1995)
Bac 303	5-CCAATGTGGGGGACCTT-3	45	*Bacteroides* spp.	Manz *et al.* (1996)
His 150	5-TTATGCGGTATTAATAT(C/T)CCTTT-3	50	*Clostridium histolyticum* subgroup	Franks *et al.* (1998)
Lab 158	5-GGTATTAGCAYCTTCCA-3	45	*Lactobacillus/Enterococcus* subgroup	Harmsen *et al.* (1999)

probes. A minimum of 15 fields of view were counted for each enumeration.

Analysis of short chain fatty acids (SCFA) by HPLC

Samples (0·5 ml) from each faecal batch culture were centrifuged at 13 000 × ***g*** for 10 min to remove bacterial cells and any particulate material. Acetate, propionate, butyrate and lactate concentrations were determined by HPLC on an Aminex HPX-87H column (300 × 7·8 mm, Bio-Rad, Watford, Herts, UK). Degassed 5 mM H_2SO_4 was used as an eluent at a flow rate of 0·6 ml min^{-1} and an operating temperature of 50°C. Organic acids were detected by UV at a wavelength of 220 nm, and calibrated against standards of corresponding organic acids at concentrations between 10 and 100 mmol ml^{-1}. The injection volume was 20 μl.

Results

Enzymatic hydrolysis

Arabinose (reducing sugar) liberated from arabinan reached a maximum level after 6 h of incubation with viscozyme (54 mg ml^{-1} protein) at 35°C (Fig. 1).

Gel filtration chromatography on Bio-Gel P-4

Arabinan hydrolysate was applied to a column of Bio-Gel P-4 (Fig. 2). The major fractions were partially hydrolyzed arabinan and arabinose. Fractions (1–8) of different dp were present but in smaller amounts.

HPAE-PAD analysis

Oligosaccharides contained in each fraction resulting from arabinan hydrolysis are shown in Fig. 3. Oligosaccharides with a dp higher than eight were not determined.

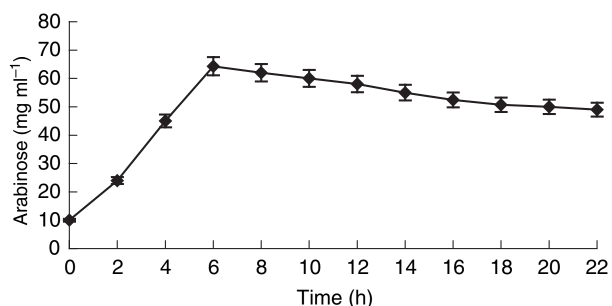

Figure 1 Hydrolysis of arabinan (5% w/v) by Viscozyme [5 : 1 arabinan:viscozyme, 54 mg protein ml^{-1}] at 35°C expressed as liberated reducing sugar (arabinose equivalents). Averages of triplicate readings were plotted.

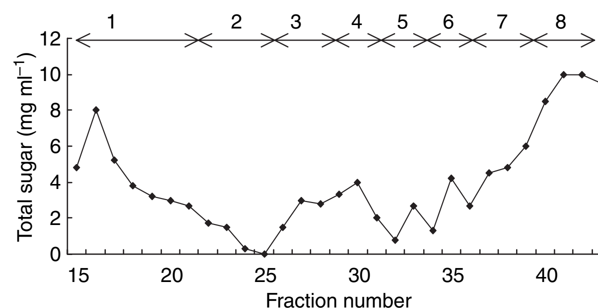

Figure 2 Chromatogram of arabinan hydrolysate on Bio-Gel P-4. A sample (5 ml) was applied to the column and eluted at a flow rate of 0·75 ml min^{-1}. Fractions (5 ml) were collected and total carbohydrate determined as arabinose equivalents by the phenol sulphuric acid assay. Fractions (1–8) were pooled to obtain different AOS structures.

Effect of dp of arabino-oligosaccharides on bacterial population changes

The effect of arabino-oligosaccharides on microbial populations in the faecal inoculum is shown in Table 2. In general, there were increases in total counts for all substrates, however these were not significant ($P > 0·05$). At 48 h of incubation there were significant differences in total count in cultures grown on arabinose, fractions 7 and 8.

There was a possible relationship between bifidobacteria numbers and dp. As dp decreased bifidobacteria numbers increased. Fractions 5, 7, 8 (Fig. 3) and arabinose showed significant increases at 24 h over inoculum of log 8·56, 8·97 8·95 and 8·77 respectively. Cultures on arabinan and FOS, in addition to fractions 5, 7, 8 and arabinose, have significantly ($P < 0·05$) increased bifidobacterial numbers by 48 h. All fractions, arabinose, arabinan and FOS showed insignificant changes in numbers of lactobacilli at 24 and 48 h.

Although arabinan resulted in increases in the numbers of bifidobacteria, arabinan, arabinose, fractions 1 and 3 also resulted in significant increases ($P < 0·05$) in bacteroides numbers after 24 h (log 0·49, 0·41, 0·60 and 0·39 respectively). Initially, fractions of higher dps (F1–4) resulted in increased clostridial (*perfringens/histolyticum* sub. grp.) numbers at 24 h while arabinan, arabinose, FOS and fractions 5–8 gave decreased numbers. At 48 h, clostridial numbers were significantly decreased ($P < 0·05$) from inoculum levels for fractions 1–7 and FOS, but not arabinan, arabinose and fraction 8.

The lower molecular weight oligosaccharides were thus more selective for bifidobacteria than arabinan or higher molecular weight fractions.

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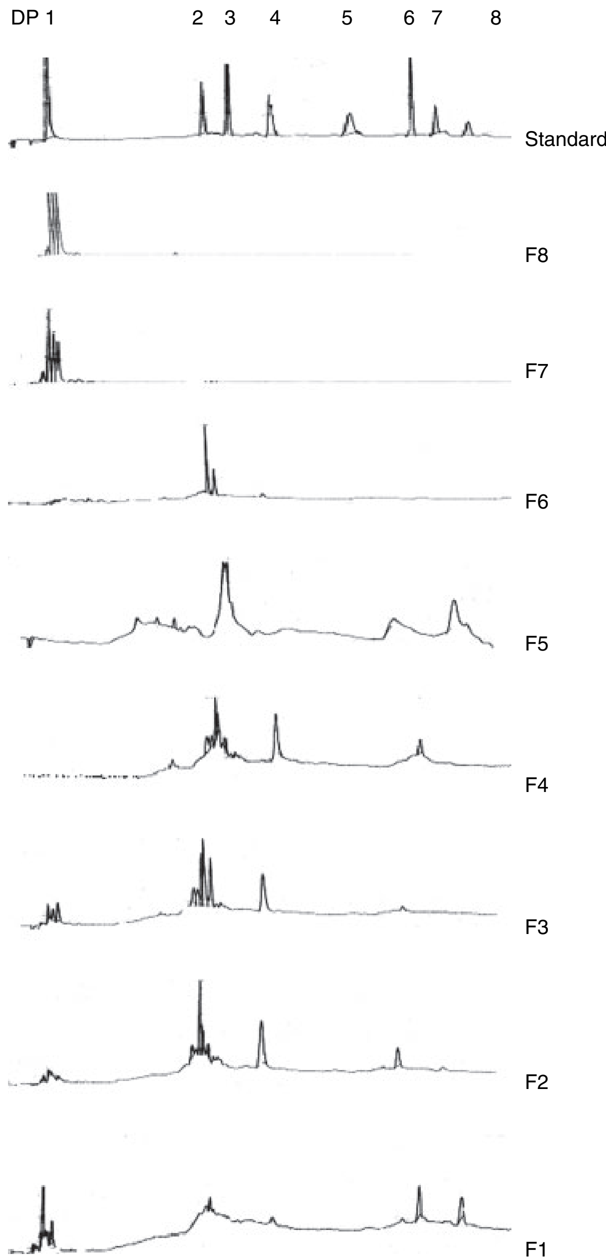

Figure 3 Chromatograms of different fractions obtained from arabinan hydrolysis by viscozyme and fractionation by Bio-Gel P4. All oligosaccharides with dp ≤ 8 were detected by HPAE-PAD.

Production of short chain fatty acids (SCFA)

Amounts of SCFA (propionate, acetate and butyrate) produced by fermentation of arabinan, AOS, arabinose and FOS are shown in Table 3. Acetate was predominant for all substrates except for fractions 4 and 5. In all substrates tested, the propionate molar ratio remained less than 50% of the total, while the butyrate level did not exceed 20% molar ratio on all substrates except fraction 6.

Table 2 Bacterial count ($\log_{10}$ ml^{-1}) of three donors ± SD, each sample was obtained from a static culture, then cells were fixed in 4% paraformaldehyde, hybridized *in situ* with a fluorescent oligonucleotide probe for 18 h in an oven and counted under UV light at 461 nm

	Inoculum	Arabinan	Arabinose	FOS	Fraction 1	Fraction 2	Fraction 3	Fraction 4	Fraction 5	Fraction 6	Fraction 7	Fraction 8
24 h												
Total	8·83 ± 0·22	9·01 ± 0·17	9·09 ± 0·24	9·04 ± 0·15	9·09 ± 0·17	9·1 ± 0·15	9·18 ± 0·03*	9·25 ± 0·19	9·12 ± 0·10	9·06 ± 0·18	9·13 ± 0·11	9·12 ± 0·18
Bifidobacteria	8·15 ± 0·17	8·43 ± 0·08	8·82 ± 0·22*	8·77 ± 0·9	8·27 ± 0·14	8·23 ± 0·19	8·39 ± 0·02	8·47 ± 0·22	8·56 ± 0·09*	8·55 ± 0·31	8·97 ± 0·14*	8·95 ± 0·26*
Bacteroides	8·35 ± 0·09	8·84 ± 0·17*	8·76 ± 0·13*	8·58 ± 0·38	8·95 ± 0·06*	8·56 ± 0·26	8·74 ± 0·05*	8·68 ± 0·09	8·56 ± 0·13	8·64 ± 0·12	8·56 ± 0·31	8·61 ± 0·09
Lactobacilli	6·56 ± 0·96	6·37 ± 0·64	7·13 ± 0·11*	7·71 ± 0·09*	6·46 ± 0·80	6·47 ± 0·81	6·45 ± 0·78	6·37 ± 0·65	6·36 ± 0·64	6·36 ± 0·64	6·71 ± 0·62	6·75 ± 0·65
Clostridia	7·36 ± 0·13	6·84 ± 0·73	6·81 ± 0·71	6·47 ± 0·82	7·84 ± 0·73	7·38 ± 0·60	7·48 ± 0·29	7·38 ± 0·31	6·97 ± 0·86	6·47 ± 0·81	6·53 ± 0·92	6·93 ± 0·87
48 h												
Total	8·83 ± 0·22	9·28 ± 0·18	9·4 ± 0·12*	9·38 ± 0·24	8·96 ± 0·19	8·89 ± 0·35	9·07 ± 0·04	8·97 ± 0·17	9·12 ± 0·16	9·13 ± 0·12	9·27* ± 0·07	9·42* ± 0·14
Bifidobacteria	8·15 ± 0·17	8·83 ± 0·26*	9·03 ± 0·13*	8·97 ± 0·40*	8·31 ± 0·04	8·31 ± 0·43	8·52 ± 0·22	8·63 ± 0·13	8·67 ± 0·08*	8·81 ± 0·26	9·1 ± 0·17*	9·1 ± 0·02*
Bacteroides	8·35 ± 0·09	9·02 ± 0·19*	9·2 ± 0·15*	8·66 ± 0·44*	8·38 ± 0·21	8·38 ± 0·20	8·15 ± 0·39	8·43 ± 0·13	8·43 ± 0·29	8·5 ± 0·24	8·6 ± 0·07	8·77 ± 0·23*
Lactobacilli	6·56 ± 0·96	6·32 ± 0·28	6·71 ± 0·64	6·92 ± 1·0	6·81 ± 0·52	6·81 ± 0·76	6·81 ± 0·73	6·63 ± 0·61	6·53 ± 0·48	6·72 ± 0·63	6·82 ± 0·85	6·86 ± 0·94
Clostridia	7·36 ± 0·13	6·53 ± 0·93	6·62 ± 0·60	6·27 ± 0·47*	6·24 ± 0·69*	6·24 ± 0·42*	6·3 ± 0·52*†	6·23 ± 0·40*†	6·28 ± 0·50*†	6·29 ± 0·51*	6·21 ± 0·37*	6·56 ± 0·97

FOS, fructo-oligosaccharides.
*Significantly different from inoculum.
†Significantly different from 24 h ($P < 0·05$).

Table 3 Short chain fatty acids liberated during fermentation

	T0	ARA	ARS	FOS	F1	F2	F3	F4	F5	F6	F7	F8
LA	3·56 ± 0·85	2·93 ± 0·67	5·76 ± 0·84	5·26 ± 1·36	1·90 ± 0·01	2·65 ± 0·26	3·00 ± 1·40	1·86 ± 0·05	1·88 ± 0·11	2·05 ± 0·35	3·74 ± 1·43	6·38 ± 0·12
AA	1·62 ± 1·26	5·44 ± 0·87	4·71 ± 0·91	3·47 ± 0·95	4·09 ± 0·60	6·25 ± 1·68	4·42 ± 0·61	3·81 ± 1·13	3·59 ± 0·83	3·80 ± 1·17	5·67 ± 1·77	3·60 ± 0·91
PA	1·80 ± 0·81	2·50 ± 0·49	2·63 ± 2·71	1·69 ± 1·01	2·12 ± 0·99	4·40 ± 0·63	3·07 ± 0·85	2·61 ± 1·13	2·61 ± 1·00	3·47 ± 1·68	2·58 ± 0·90	3·39 ± 1·27
BA	0·15 ± 0·00	0·74 ± 0·47	0·24 ± 0·16	1·21 ± 0·10	2·94 ± 3·74	1·66 ± 0·79	1·06 ± 0·57	1·05 ± 0·62	0·82 ± 0·23	0·78 ± 0·45	0·72 ± 0·55	0·49 ± 0·58

LA, Lactic acid; AA, Acetic acid; PA, Propionic acid; BA, Butyric acid; F1–F8, Fractions 1–8 respectively; ARA, arabinan; ARS, arabinose; FOS, fructo-oligosaccharides.
All numbers are means of three samples ± SD, expressed as mmol ml^{-1}.

Discussion

This study has investigated the fermentation of a number of arabino-oligosaccharide mixtures with different compositions, by human faecal bacteria. The dp of the product affected bacterial numbers after 24 and 48 h of culture. The effect of dp upon growth of a number of probiotic bacteria has been investigated previously but only in pure culture studies (Van Laere *et al.* 2000).

The metabolism of individual FOS by strains of lactobacilli has been studied (Kaplan and Hutkins 2000). *Lactobacillus plantarum* and *Lactobacillus rhamnosus* only metabolized trisaccharides and tetrasaccharides and these strains could not metabolize the pentasaccharide, suggesting that they may possess transport systems specific for tri- and tetrasaccharides. Moreover, in a study of the metabolism of fractionated galacto-oligosaccharides (GOS), *Bifidobacterium lactis* was found to prefer trisaccharide and tetrasaccharide fractions and displayed a preference for these over glucose (Gopal *et al.* 2001). The present study supports the presence of such transport mechanisms although it should be noted that such transport phenomena are concentration dependant and it was not possible to determine changes in arabino-oligosaccharide concentration in this study.

Generally, the total bacterial count increased in carbohydrate fermentation studies over inoculum. These results agree with those of Olano-Martin *et al.* (2000) who found an increase of log 0·5 in total anaerobes after 6 h of incubation when dextran was used as the substrate. On the other hand, Rycroft *et al.* (2001b) found no significant difference between maltodextrin (dp 17–20) and gentio-oligosaccharides (dp 2–6) in total counts, although the former showed superiority at 5 and 10 h of incubation. *Bifidobacterium spp*. have shown variations in their ability to hydrolyze arabinan and AOS in media containing such carbohydrates (Van Laere *et al.* 2000). In the mixed culture study here, *Bifidobacterium* spp. were shown to prefer low molecular weight fractions rather than high molecular weight fractions.

As a numerically predominant group in the human gut flora, bacteroides can metabolize a wide range of fibres, as they possess a wide range of glycanases and glycosidases. Bacteroides have been reported to hydrolyze different arabinose-containing compounds, such as arabinogalactans and arabinoxylans (Salyers *et al.* 1981; Cooper *et al.* 1985). Therefore, it was expected that there would be an increase in their numbers with arabinan and the higher molecular weight fractions.

The SCFA profile reflects microbial activities (Rycroft *et al.* 2001a). For example, a highly fermentable substrate such as pectin is expected to produce more SCFA than cellulose, a less well fermentable substance (Olano-

Martin *et al.* 2000; Topping and Clifton 2001). However, prediction of a bacterial population according to SCFA is not feasible, since the colonic ecosystem is very complicated and is affected by both internal and external factors.

The present results confirmed those of Bruck *et al.* (2003) that acetate is the predominant acid produced due to faecal bacterial fermentation and of Fardet *et al.* (1996) who reported an *in vitro* fermentation of beet fibre. The amount of acetate produced in this trial decreased with decreasing molecular weight, then increased for fractions composed mainly of monosaccharides (7, 8 and arabinose). Propionate increased with decreasing molecular weight in general and in fractions 4 and 5 in particular, which also showed decreases in acetate level.

This study has shown variations in fermentation properties of AOS fractions. Microbial populations and metabolites varied as a function of molecular weight. Molecular weight correlated with bifidobacterial numbers, which increased with reduced molecular weight. In general, SCFA molar ratios on arabinan and AOS fermentation were of the order previously reported for other arabinose-containing substrates (acetate:propionate:butyrate, 60–70 : 30–40 : 10) Fardet *et al.* (1996).

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