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# ***In vitro* Fermentation of Mixed Linkage Gluco-oligosaccharides Produced by *Gluconobacter oxydans* NCIMB 4943 by the Human Colonic Microflora**

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## **Abstract**

The aim of this study was to develop selectively fermented (prebiotic) carbohydrate molecules which would also result in the generation of butyric acid. Gluco-oligosaccharides produced by *Gluconobacter oxydans* NCIMB 4943 from various types of maltodextrins were evaluated for their fermentation by mixed cultures of human colonic microflora. The selectivity of growth of desirable bacteria (bifidobacteria, lactobacilli) was studied in stirred pH-controlled (6.8) batch cultures. Bacterial populations were enumerated using fluorescent *in situ* hybridization (FISH). Gluco-oligosaccharides resulted in significantly ( $P < 0.05$ ) increased numbers of bifidobacteria and lactobacilli within 24 hours. Bacteroides, clostridial and eubacterial populations were slightly decreased at 48 h. There was very little difference in selectivity between the maltodextrin substrates and the products, although maltodextrin displayed a slightly less selective fermentation than the gluco-oligosaccharide products, also stimulating the growth of bacteroides, clostridia and eubacteria. Gluco-oligosaccharides, produced from G19 maltodextrin, resulted in the best prebiotic effect with the highest prebiotic index (PI) of 5.90 at 48 hours. Acetate, propionate and butyrate were all produced from gluco-oligosaccharides, derived from G19 maltodextrin, at 48 hours but no lactate or formate were detected.

## **Introduction**

Prebiotics are dietary carbohydrates which are fermented in the colon by indigenous populations of beneficial bacteria such as probiotics (Gibson and Roberfroid, 1995). The colonic microflora produces short-chain fatty acids including acetate, propionate and butyrate as end products of carbohydrate metabolism (Macfarlane *et al.*, 1998; Olano-Martin *et al.*, 2000; Rycroft *et al.*, 2001b). Of these, butyrate has attracted a lot of interest as it is used as a source of metabolic energy by colonocytes and is known to induce apoptosis in colonic cancer cell lines (Gillet *et al.*, 1998). For these reasons, a high level of butyrate in the colon may be protective against colon cancer. Traditional probiotic bacteria, like lactobacilli or bifidobacteria, do not however produce butyrate as a metabolic end product.

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Rycroft *et al.* (2001b) reported that glucose-based oligosaccharides were effective prebiotics, at least *in vitro*, although fructooligosaccharides (FOS, inulin) generated the most butyrate and propionate. A preliminary screening of the *in vitro* utilization of oligodextrins derived by enzymatic hydrolysis of commercial dextran, by the human gut microflora indicated that oligodextrins could be regarded as potentially worthwhile food ingredients for both their prebiotic and butyrate generating abilities (Olano-Martin *et al.*, 2000). Several studies have showed that resistant starch results in high butyrate production (Cummings and Macfarlane, 1991; Silvi *et al.*, 1999; Topping *et al.*, 2003).

This study aims to evaluate the fermentability, *in vitro*, of novel gluco-oligosaccharides synthesized by the action of dextran dextrinase from *Gluconobacter oxydans*. This enzyme has been shown to produce dextrans from maltodextrins (Mountzouris *et al.*, 1999), but the selectivity of fermentation of these materials has not been investigated.

## **Materials and methods**

### *Carbohydrate substrates*

Gluco-oligosaccharides were prepared through the action of *Gluconobacter oxydans* NCIMB 4943 using commercial maltodextrins as catalyst (S. Wichienchot, unpublished; Mountzouris *et al.*, 1999). G12, G19 and G37 gluco-oligosaccharides were produced from Glucidex 12 (G12), Glucidex 19 (G19) and Goldex 37 (G37) maltodextrins, respectively. Glucidex 12 and Glucidex19 maltodextrins were supplied by Roquette Ltd, France and Goldex 37 maltodextrin was supplied by ABR Foods Ltd., UK. Inulin (Raffiline ST, Orafti, Tienen, Belgium) was used as a reference prebiotic.

### *Basal medium for stirred pH-controlled batch culture*

Unless otherwise stated, all chemicals and reagents were obtained from Sigma-Aldrich Co. Ltd. (Poole, UK) and bacteriological growth media supplements were obtained from Oxoid Ltd. (Basingstoke, UK).

Basal medium contained (g/l): peptone water, 2; yeast extract, 2; NaCl, 0.1 (Fisher, Loughborough, UK); NaHCO<sub>3</sub>, 2; cysteine-HCl, 0.5; KH<sub>2</sub>PO<sub>4</sub>, 0.04 (BDH, Poole, UK); K<sub>2</sub>HPO<sub>4</sub>, 0.04 (BDH); bile salts, 0.5; CaCl<sub>2</sub>·6H<sub>2</sub>O, 0.01 (BDH); hemin, 0.005; MgSO<sub>4</sub>·7H<sub>2</sub>O, 0.01; Tween 80, 2 ml (BDH); vitamin K<sub>1</sub>, 10 µl; resazurin, 4 ml. A 135 ml of basal medium was aliquoted into Duran bottles and sterilized at 121°C for 20 min (Rycroft *et al.*, 2001b).

### *Prebiotic index (PI) equation*

This generates a quantitative comparison of prebiotic effects through determining changes in flora profiles

during fermentation experiments. The equation assumed that an increase in the populations of bifidobacteria and/or lactobacilli is a positive effect and an increase in bacteroides and clostridia are negative (Palframan *et al.*, 2003). The prebiotic index was therefore calculated as follows:

$$PI = (\text{Bif}/\text{Total}) - (\text{Bac}/\text{Total}) + (\text{Lac}/\text{Total}) - (\text{Clos}/\text{Total})$$

Where: Bif = bifidobacterial numbers at sampling time/numbers at inoculation; Bac = bacteroides numbers at sampling time/numbers at inoculation; Lac = lactobacilli numbers at sampling time/numbers at inoculation; Clos = clostridial numbers at sampling time/numbers at inoculation; and Total = total bacterial numbers at sampling time /numbers at inoculation.

#### *Stirred pH-controlled batch culture fermentation*

Sterile batch culture fermenters (300 ml) were filled with sterile basal medium and pre-reduced overnight by purging with oxygen-free nitrogen gas. Freshly voided human faeces from a healthy donor (who had not taken antibiotics for 3 months beforehand), was made into slurries (100 g/l, w/w) using phosphate buffered saline (PBS), containing (g/l): NaCl 8, KCl 0.2, Na<sub>2</sub>HPO<sub>4</sub>·2H<sub>2</sub>O 1.44, KH<sub>2</sub>PO<sub>4</sub> 0.24, pH 7) and this was mixed in a stomacher for 2 min. Carbohydrate substrate (1.5 g) was dissolved with prereduced, sterile basal medium to give a final concentration of 10 g l<sup>-1</sup> and returned to the sterile fermenter aseptically. Faecal slurries (15 ml) were added into each fermenter and the closed fermenter was maintained under a headspace of oxygen-free nitrogen gas. Fermentation was conducted at 37°C, magnetically stirred and at a controlled pH (6.8±0.1) by addition of 0.5 N NaOH or HCl. Each test carbohydrate was tested in duplicate fermenters. Samples (6 ml) were taken at 0, 24 and 48 hours for the enumeration of bacteria using FISH technique and analyzed for short-chain fatty acid (SCFA) by HPLC (Rycroft *et al.*, 2001b).

#### *Short-chain fatty acid (SCFA) analysis*

Samples (1.5 ml) were centrifuged (17,000xg for 15 min) and the supernatant (20 µl) injected onto an HPLC system (Model 1050, Hewlett Packard) attached to a UV detector (Knauer, Type 298.00; LC Services, Wootton, Bedfordshire, UK) at 210 and 214 nm. The column was an ion-exclusion Aminex HPX-87H 150×7.8 mm ID (Bio-Rad, Watford, Herts) maintained at 50°C with a column heater (Model 1250426; Bio-Rad). The eluent, 0.005 M sulphuric acid in HPLC-grade water, was pumped through the column at a flow rate of 0.6 ml/min. Data from the UV detector were integrated using the ValueChrom™ software package (Bio-Rad) and the concentration of lactate, formate, acetate, propionate and butyrate in the sample determined using external calibration curves (Rycroft *et al.*, 2001b).

#### *Fluorescent in situ hybridization (FISH)*

Genus-specific 16S rRNA-targeted oligonucleotide probes labelled with the fluorescent dye Cy 3 (supplied by Eurogentec Ltd., UK) were used for enumeration of *Bifidobacterium* (Bif 164, Langedijk *et al.*, 1995), *Bacteroides* (Bac 303, Manz *et al.*, 1998), *Lactobacillus/*

*Enterococcus* spp. (Lab 158, Harmsen *et al.*, 2000), *Clostridium perfringens/histolyticum* subgroup (His 150, Franks *et al.*, 1998) and *Clostridium coccooides/Eubacterium rectale* group (Erec 482, Franks *et al.*, 1998). To obtain total bacterial counts, the nucleic acid stain 4', 6-diamidino-2-phenylindole (DAPI) was added to each sample (Porter and Feig, 1980). Cells were fixed on a slide and those stained with DAPI or hybridized with probe and enumerated under a microscope as described below.

Sample (375 µl) taken from the culture medium was added into 1.125 ml filtered 4% (w/v) paraformaldehyde solution (2 g paraformaldehyde in 30 ml of HPLC-grade water and 16.6 ml of 3× PBS (30 tablets/l), adjusted to a total volume of 50 ml with HPLC-grade water, pH adjusted to 7.2 then filtered through 0.2 µm filter), mixed and stored at 4°C overnight to fix the cells. The fixed cells were then washed twice in filtered 1× PBS (pH 7) and resuspended in 150 µl filtered 1× PBS. Ethanol (150 µl) was added and the sample mixed and stored at -20°C for at least 1 h or until needed, but no longer than 3 months. The fixed cells (16 µl) were added to 264 µl pre-warmed hybridization buffer (1.5 ml of a 10% (w/v) sodium dodecyl sulphate (SDS) solution added to 100 ml solution containing (g/l): Tris-HCl, 4.78; NaCl, 79.69, adjusted to pH 7.2 and heated to boiling point then filtered through 0.2 µm membranes). An appropriate volume of this mixture and probe (50 ng/µl) were mixed and placed in the hybridization oven at the required temperature overnight (Bif 164, Erec 482 and His 150 at 50°C; Bac 303 and Lab 158 at 45°C; DAPI at any temperature).

The hybridization samples (5-120 µl) were washed in 7 ml prewarmed, filtered, wash buffer (g/l: Tris-HCl, 3.15; NaCl, 52.60, adjusted pH to 7.2 then filtered through 0.2 µm membrane) contained 20 µl DAPI solution (500 ng/µl) for 30 min at appropriate hybridization temperatures. Samples were vacuum filtered onto a 0.2 µm isopore membrane filters (Millipore Corporation, Herts, UK) and these were mounted in 'SlowFade' (Molecular Probes, Eugene, The Netherlands) on clean slides. Microscope (Nikon Eclipse, E400, Japan) fitted with appropriate filters for the DAPI stain (excited at 550 nm and emits at 461 nm) and the Cy3 dye (excited at 550 nm and emits at 564 nm). A minimum of 15 fields, each containing 10-100 cells, was counted for each slide.

#### *Statistical analysis*

Differences between bacterial counts at 0, 24 and 48 hours of batch culture fermentations were tested for significance using paired *t*-tests, assuming equal variance and considering both sides of the distribution (two-tailed distribution). Differences were considered 95% significant if *P* < 0.05.

## Results

#### *Microbial population changes*

Microbial population changes (mean log<sub>10</sub> cfu/g faeces ± SD) from the fermentation of G12, G19 gluco-oligosaccharides and G12, G19 maltodextrins are summarized in Table 1 and from the fermentation of G37 gluco-oligosaccharides, G37 maltodextrin and inulin are summarized in Table 2.

Table 1. Changes in bacterial populations enumerated using fluorescent in situ hybridization in situ hybridization in stirred pH-controlled batch culture fermentation with G12, G19 gluco-oligosaccharides and G12, G19 maltodextrins.

Target Bacterial Group	G12 gluco-oligosaccharides			G12 maltodextrin			G19 gluco-oligosaccharides			G19 maltodextrin		
	0 h	24 h	48 h	0 h	24 h	48 h	0 h	24 h	48 h	0 h	24 h	48 h
Total bacteria	9.10±0.67 <sup>a</sup>	9.48±0.82 <sup>b</sup>	9.58±0.76 <sup>c</sup>	9.10±0.63 <sup>a</sup>	9.38±0.83 <sup>b</sup>	9.54±0.92 <sup>c</sup>	9.17±0.67 <sup>a</sup>	9.37±0.62 <sup>b</sup>	9.26±0.71 <sup>c</sup>	9.02±0.74 <sup>a</sup>	9.16±0.59 <sup>ab</sup>	8.97±0.76 <sup>c</sup>
<i>Bifidobacterium</i>	7.74±0.42 <sup>a</sup>	8.47±0.52 <sup>b</sup>	8.32±0.59 <sup>c</sup>	8.13±0.78 <sup>a</sup>	9.07±0.98 <sup>b</sup>	9.01±0.92 <sup>c</sup>	7.79±0.56 <sup>a</sup>	8.51±0.58 <sup>b</sup>	8.49±0.58 <sup>b</sup>	7.77±0.52 <sup>a</sup>	8.36±0.49 <sup>b</sup>	8.40±0.73 <sup>b</sup>
<i>Bacteroides</i>	8.35±0.45 <sup>a</sup>	8.68±0.48 <sup>b</sup>	8.62±0.66 <sup>b</sup>	8.03±0.57 <sup>a</sup>	8.24±0.74 <sup>b</sup>	8.16±0.69 <sup>c</sup>	8.25±0.49 <sup>a</sup>	8.63±0.26 <sup>b</sup>	8.42±0.68 <sup>c</sup>	8.27±0.49 <sup>a</sup>	8.47±0.55 <sup>b</sup>	8.17±0.58 <sup>a</sup>
<i>Lactobacillus/Enterococcus</i>	7.88±0.90 <sup>a</sup>	8.71±0.61 <sup>b</sup>	8.61±0.57 <sup>c</sup>	7.77±0.56 <sup>a</sup>	7.90±0.78 <sup>b</sup>	8.06±0.57 <sup>c</sup>	7.86±0.69 <sup>a</sup>	8.56±0.49 <sup>b</sup>	8.50±0.85 <sup>b</sup>	7.81±0.65 <sup>a</sup>	8.23±0.69 <sup>b</sup>	8.23±0.74 <sup>b</sup>
<i>Clostridium</i> subgrp <i>perfringens/histolyticum</i>	8.00±0.71 <sup>a</sup>	8.64±0.53 <sup>b</sup>	8.10±0.80 <sup>c</sup>	7.61±0.76 <sup>a</sup>	7.67±0.50 <sup>a</sup>	7.79±0.63 <sup>b</sup>	7.95±0.47 <sup>a</sup>	8.36±0.78 <sup>b</sup>	7.82±0.83 <sup>c</sup>	7.94±0.73 <sup>a</sup>	8.12±0.53 <sup>b</sup>	7.87±0.89 <sup>c</sup>
<i>Eubacterium rectale-Clostridium coccoides</i>	8.32±0.56 <sup>a</sup>	8.66±0.60 <sup>b</sup>	8.49±0.72 <sup>c</sup>	8.23±0.50 <sup>a</sup>	8.32±0.62 <sup>ab</sup>	8.52±0.60 <sup>c</sup>	8.23±0.43 <sup>a</sup>	8.58±0.57 <sup>b</sup>	8.18±0.72 <sup>a</sup>	8.27±0.71 <sup>a</sup>	8.36±0.52 <sup>b</sup>	8.25±0.67 <sup>c</sup>

Numbers are mean log<sub>10</sub> cfu/g faeces ± SD of duplicate fermentations.  
 Different letters mean significant differences ( $P < 0.05$ ) for the same bacterial groups.

Table 2. Changes in bacterial populations enumerated using fluorescent in situ hybridization in stirred pH-controlled batch culture fermentation with G37 gluco-oligosaccharides, G37 maltodextrin and inulin.

Target Bacterial Group	G37 gluco-oligosaccharides			G37 maltodextrin			Inulin		
	0 h	24 h	48 h	0 h	24 h	48 h	0 h	24 h	48 h
Total bacteria	8.99±0.59 <sup>a</sup>	9.49±0.90 <sup>b</sup>	9.47±0.73 <sup>b</sup>	9.05±0.82 <sup>a</sup>	9.55±0.85 <sup>b</sup>	9.53±0.01 <sup>b</sup>	9.03±0.67 <sup>a</sup>	9.48±0.79 <sup>b</sup>	9.53±0.62 <sup>b</sup>
<i>Bifidobacterium</i>	8.10±0.61 <sup>a</sup>	9.13±0.90 <sup>b</sup>	8.93±0.86 <sup>c</sup>	8.08±0.70 <sup>a</sup>	8.95±0.87 <sup>b</sup>	8.85±0.78 <sup>c</sup>	8.14±0.69 <sup>a</sup>	9.03±0.76 <sup>b</sup>	9.06±0.88 <sup>b</sup>
<i>Bacteroides</i>	8.25±0.32 <sup>a</sup>	8.73±0.78 <sup>b</sup>	8.69±0.40 <sup>b</sup>	8.05±0.53 <sup>a</sup>	8.11±0.61 <sup>a</sup>	7.99±0.48 <sup>b</sup>	8.36±0.29 <sup>a</sup>	8.72±0.52 <sup>b</sup>	8.58±0.60 <sup>c</sup>
<i>Lactobacillus/Enterococcus</i>	7.51±0.64 <sup>a</sup>	7.86±0.56 <sup>b</sup>	7.83±0.57 <sup>b</sup>	7.61±0.67 <sup>a</sup>	7.91±0.70 <sup>b</sup>	7.92±0.75 <sup>b</sup>	7.51±0.58 <sup>a</sup>	8.14±0.67 <sup>b</sup>	7.95±0.92 <sup>c</sup>
<i>Clostridium</i> subgrp <i>perfringens/histolyticum</i>	7.64±0.61 <sup>a</sup>	8.26±0.75 <sup>b</sup>	8.12±0.71 <sup>c</sup>	7.63±0.56 <sup>a</sup>	7.63±0.60 <sup>a</sup>	7.80±0.70 <sup>b</sup>	7.55±0.58 <sup>a</sup>	8.06±0.54 <sup>b</sup>	8.06±0.56 <sup>b</sup>
<i>Eubacterium rectale-Clostridium coccoides</i>	8.14±0.59 <sup>a</sup>	8.19±0.50 <sup>b</sup>	8.65±0.82 <sup>c</sup>	8.05±0.54 <sup>a</sup>	7.94±0.44 <sup>b</sup>	8.39±0.49 <sup>c</sup>	8.20±0.59 <sup>a</sup>	8.67±0.49 <sup>b</sup>	8.51±0.65 <sup>c</sup>

Numbers are mean log<sub>10</sub> cfu/g faeces ± SD of duplicate fermentations. Different letters mean significant differences (P<0.05) for the same bacterial groups.

All of the carbohydrates tested displayed a selective fermentation with greater increases seen with bifidobacteria than with other groups. There were few differences in selectivity between the maltodextrin substrates and the corresponding oligosaccharide products (Fig. 1).

#### Short-chain fatty acids production

Short-chain fatty acids (mean ± SD) produced by fermentation of G12, G19 gluco-oligosaccharides and G12, G19 maltodextrins were determined by HPLC (Table 3). Generally, acetate, propionate and butyrate were the principal organic acids formed. Formate and lactate were only detected in one incubation only and in smaller concentrations. The preferred butyrogenic oligosaccharide was G12 gluco-oligosaccharide, while most acetate was formed from G12 maltodextrin.

#### Discussion

This is the first study of the *in vitro* fermentability of gluco-oligosaccharides produced by *G. oxydans* NCIMB 4943 by the human colonic microflora. Typically, prebiotic substances are low molecular weight oligosaccharides

which are fermented by selected microflora especially bifidobacteria and/or lactobacilli. However, some higher molecular weight carbohydrates were investigated during this study. Changes of bacterial populations through the fermentation of commercial prebiotics (FOS, inulin, IMO, GOS, lactulose) in stirred pH-controlled batch culture has been previously reported (Palframan *et al.*, 2002). They concluded that FOS (including inulin) demonstrated the greatest bifidogenic effect at pH 6.8 and 1% (w/v) carbohydrate concentration, whereas GOS, IMO and lactulose demonstrated the greatest bifidogenic effect at pH 6 and 2% (w/v) carbohydrate. This showed that various prebiotics demonstrated differing bifidogenic effects under varying conditions *in vitro*. The G19 gluco-oligosaccharides, with a molecular weight of 7.8-65.6 kDa, resulted in a more selective fermentation than did the commercial prebiotic inulin. However, the gluco-oligosaccharides were partially susceptible to amylase hydrolysis and displayed 15% and 25% hydrolysis in the presence of human salivary amylase and intestinal amylase, respectively (data not shown). This would result in lower molecular weight material reaching the colon in

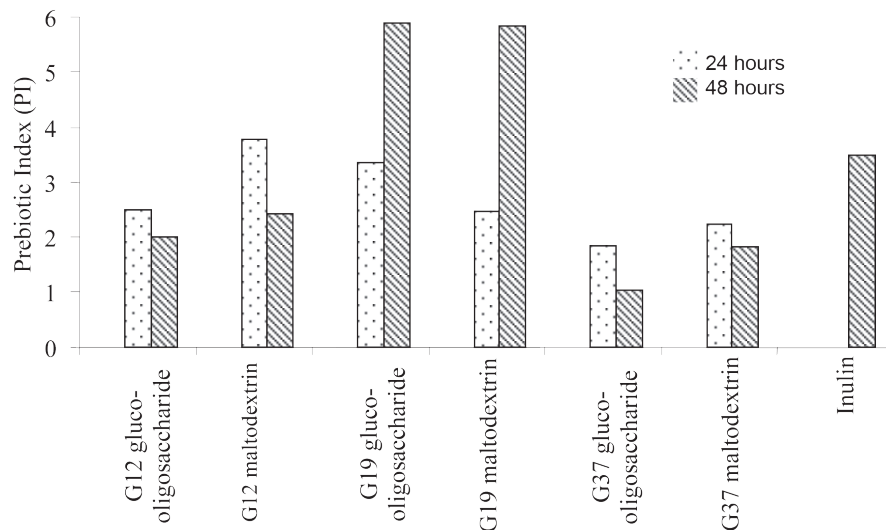


Fig. 1 Prebiotic index (PI) scores from stirred pH-controlled batch culture fermentations of 1% (w/v) G12, G19, G37 gluco-oligosaccharides, G12, G19, G37 maltodextrins and inulin at 24 and 48 hours fermentation.

Table 3. SCFA production by G12, G19 gluco-oligosaccharides and G12, G19 maltodextrins fermentations in stirred pH-controlled batch culture.

Short-chain fatty acid	G12 gluco-oligosaccharides			G12 maltodextrin			G19 gluco-oligosaccharides			G19 maltodextrin		
	0 h	24 h	48 h	0 h	24 h	48 h	0 h	24 h	48 h	0 h	24 h	48 h
Lactate	3.33 ±0.54	ND	ND	ND	ND	ND	1.67 ±0.45	ND	ND	2.64 ±0.70	ND	ND
Formate	ND	ND	ND	ND	ND	ND	ND	0.77 ±0.22	0.37 ±0.12	ND	ND	0.48 ±0.05
Acetate	ND	55.39 ±8.69	62.03 ±11.45	ND	80.96 ±15.83	81.59 ±10.88	7.76 ±1.32	63.48 ±12.40	63.67 ±13.45	ND	58.41 ±12.20	60.98 ±9.90
Propionate	6.30 ±1.38	24.25 ±5.88	28.06 ±2.54	ND	20.88 ±4.56	19.74 ±4.45	3.65 ±0.66	17.34 ±2.15	18.36 ±2.37	5.34 ±1.00	15.61 ±5.12	16.68 ±3.56
Butyrate	ND	10.03 ±1.25	13.44 ±1.46	ND	1.07 ±0.47	2.05 ±0.66	ND	12.96 ±2.00	14.52 ±2.11	ND	6.30 ±1.54	8.45 ±2.25

Values are means ± SD from duplicate fermenters in mM concentrations.  
ND = not detected.

vivo as compared to this *in vitro* study. These data must, therefore, be substantiated in a human volunteer trial.

Rycroft *et al.* (2001b) compared the fermentation properties of fructo-oligosaccharides (FOS), inulin, lactulose, xylo-oligosaccharides (XOS), galacto-oligosaccharides (GOS), soybean oligosaccharides (SOS) and isomalto-oligosaccharides (IMO) in static batch cultures for 24 h. It was found that all the prebiotics tested increased numbers of bifidobacteria while most decreased clostridia. Carbohydrate samples used in the present study had low selective fermentation during the 24 hours, but more selective fermentation was observed after 48 hours. This may be due to carbohydrate samples containing a mixture of low molecular weight (maltodextrin residues) which were firstly non-selectively fermented and a higher molecular weight fraction (oligodextran) which was more selectively fermented by the colonic flora during the later parts of the fermentation. The maltodextrin was not selectively metabolized and as it increased bifidobacteria, lactobacilli, clostridia and bacteroides. Similar results on SCFA production by fermentation of maltodextrin in this study and Rycroft and co-workers (2001a) have been observed. However, this study generated much higher acetate levels. The prebiotic index measurement must be used with care. Whilst it is a useful quantitative measure of selectivity in a comparative study using a common faecal inoculum, the value differs for different individuals and PI values between different published studies cannot be meaningfully compared.

Brouns *et al.* (2002) reported that high concentrations of butyrate were produced through the fermentation of resistant starch (30 mM), whereas wheat bran (15 mM) and pectin (20 mM) generated lower levels of butyrate. The butyrate produced by fermentation of gluco-oligosaccharides in this study showed comparable levels to wheat bran (14.52 mM).

Although maltodextrin gave a prebiotic effect in these *in vitro* fermentations, it can be hydrolyzed by upper gut digestion and small amounts only reach the colon where fermentation occurs. Maltodextrin has also been found to result in the generation of large amounts of gas (8 ml) compared to FOS and GOS (Probert and Gibson, 2002), suggesting a relatively non-selective fermentation. Tuohy *et al.* (2001a) reported consumption 8 g/d of maltodextrin for 14 days followed by 8 g/d inulin-HP for 14 days. Generally, the population changes on consumption of maltodextrin were similar to those seen in this *in vitro* study, however total bacterial numbers were lower. Inulin-HP conferred stimulation of bifidobacteria and clostridia numbers similar to the inulin-ST used in the present study however changes in bacteroides and lactobacilli were different. Human studies are required to understand how these prebiotic properties are manifest *in vivo*.

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Table 4. SCFA production by G37 gluco-oligosaccharides, G37 maltodextrin and inulin fermentations in stirred pH-controlled batch culture.

Short-chain fatty acid	G37 gluco-oligosaccharide			G37 maltodextrin			Inulin		
	0 h	24 h	48 h	0 h	24 h	48 h	0 h	24 h	48 h
Lactate	ND	ND	ND	ND	ND	ND	ND	2.50 ±1.14	ND
Formate	ND	ND	ND	ND	ND	ND	ND	ND	0.66 ±0.15
Acetate	1.96 ±0.89	64.90 ±12.13	79.42 ±15.35	ND	76.65 ±13.35	93.83 ±18.78	6.24 ±1.18	38.62 ±9.89	44.65 ±7.67
Propionate	ND	17.17 ±3.24	21.75 ±5.59	ND	10.09 ±2.50	10.74 ±2.50	5.47 ±1.66	22.78 ±6.55	25.74 ±6.69
Butyrate	ND	7.05 ±1.99	7.40 ±1.85	ND	7.77 ±1.10	5.22 ±1.00	ND	6.81 ±1.26	6.50 ±1.88

Values are means ± SD from duplicate fermenters in mM concentration.  
ND = not detected.

The University of Reading, UK under a split-mode PhD programme.

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