

In vitro fermentation of commercial α -gluco-oligosaccharide by faecal microbiota from lean and obese human subjects

Shahrul R. Sarbini¹, Sofia Kolida², Glenn R. Gibson² and Robert A. Rastall^{2*}

¹Department of Crop Science, Faculty of Agriculture and Food Science, Universiti Putra Malaysia, Jalan Nyabau, 97008 Bintulu, Sarawak, Malaysia

²Department of Food and Nutritional Sciences, University of Reading, Whiteknights, PO Box 226, Reading RG6 6AP, UK

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Abstract

The fermentation selectivity of a commercial source of α -gluco-oligosaccharides (BioEcolians; Solabia) was investigated *in vitro*. Fermentation by faecal bacteria from four lean and four obese healthy adults was determined in anaerobic, pH-controlled faecal batch cultures. Inulin was used as a positive prebiotic control. Samples were obtained at 0, 10, 24 and 36 h for bacterial enumeration by fluorescent *in situ* hybridisation and SCFA analyses. Gas production during fermentation was investigated in non-pH-controlled batch cultures. α -Gluco-oligosaccharides significantly increased the *Bifidobacterium* sp. population compared with the control. Other bacterial groups enumerated were unaffected with the exception of an increase in the *Bacteroides*–*Prevotella* group and a decrease in *Faecalibacterium prausnitzii* on both α -gluco-oligosaccharides and inulin compared with baseline. An increase in acetate and propionate was seen on both substrates. The fermentation of α -gluco-oligosaccharides produced less total gas at a more gradual rate of production than inulin. Generally, substrates fermented with the obese microbiota produced similar results to the lean fermentation regarding bacteriology and metabolic activity. No significant difference at baseline (0 h) was detected between the lean and obese individuals in any of the faecal bacterial groups studied.

Key words: Gluco-oligosaccharides; Prebiotics; Obese subjects

It is now well established that the composition of the colonic microbiota can be modified by the introduction of prebiotics to improve or maintain host health⁽¹⁾. The efficacy of a prebiotic can be evaluated by *in vitro* batch-culture fermentation systems which have been compared and validated against human and animal *in vivo* data⁽²⁾. Batch-culture fermentation systems provide a simple, rapid and inexpensive method of evaluating the prebiotic potential of carbohydrates.

To date, the majority of studies on prebiotics have focused on inulin, fructo-oligosaccharides and galacto-oligosaccharides due to their selective fermentation by bifidobacteria and history of safe commercial use. Nevertheless, there are potential prebiotic oligosaccharides still under investigation, such as α -gluco-oligosaccharides. These gluco-oligosaccharides are selectively metabolised by *Bifidobacterium*, *Lactobacillus* and *Bacteroides* but are poorly metabolised by potentially pathogenic bacteria such as enterobacteria and *Clostridium*⁽³⁾. Even though they exhibit promising characteristics, the evidence is not sufficient to classify them as prebiotics presently⁽⁴⁾. All studies to date have been carried out by

culture-dependent methods, which are not reliable for the analysis of complex bacterial samples. Therefore, further investigation using culture-independent (DNA-based) methods is needed to verify these initial findings.

The human gut microbiota is dominated by two major phyla, the Bacteroidetes and Firmicutes⁽⁵⁾. A study has observed that genetically obese mice had a higher proportion of Firmicutes relative to Bacteroidetes compared with lean mice⁽⁶⁾. It is also thought that the gut microbiota of obese mice may be more efficient at salvaging energy from the diet than the microbiota of lean mice^(7,8). A further *in vivo* human study has suggested that obese individuals had a higher Firmicutes:Bacteroidetes ratio compared with lean ones⁽⁹⁾. However, all the above-mentioned studies only determined changes in Bacteroidetes and Firmicutes, each containing various genera that have diverse metabolic capabilities. Identifying changes at the phylum rather than the genus level could be misleading. Some recent studies have failed to observe differences in Bacteroidetes between lean and obese individuals^(10,11). Therefore, the role of the gut

Abbreviations: DP, degree of polymerisation; OA, organic acid.

* **Corresponding author:** Professor R. A. Rastall, fax +44 118 931 0080, email r.a.rastall@reading.ac.uk

microbiota in obesity remains unclear. Some *in vivo* animal studies have demonstrated that the composition of the diet, not the obese state, resulted in changes of the gut microbiota^(12,13). This raises the possibility of developing prebiotics that influence the microbiota composition and could be consumed as part of a weight management diet.

The aim of the present study is to evaluate the fermentation selectivity of a commercial preparation of α -gluco-oligosaccharides (BioEcolians⁽¹⁴⁾) by the human faecal microbiota and to assess its prebiotic potential towards lean and obese adults.

Materials and methods

Materials

Unless stated otherwise, all reagents and chemicals used were purchased from Sigma Laboratories. α -Gluco-oligosaccharides were provided by Solabia (Pantin). Inulin Frutafit TEX (Sensus) with a degree of polymerisation (DP) >22 was used as a positive control.

Faecal inocula

Faecal samples were obtained from four lean human volunteers (BMI 19–23 kg/m²; age 30–36 years) and four obese human volunteers (BMI 35–40 kg/m²; age 30–36 years) who were free of known metabolic and gastrointestinal diseases (e.g. diabetes, ulcerative colitis, Crohn's disease, irritable bowel syndrome, peptic ulcers and cancer). The samples were collected on site, kept in an anaerobic cabinet (10% H₂, 10% CO₂ and 80% N₂) and used within a maximum of 15 min after collection. The samples were diluted 1:10 (w/w) in anaerobic PBS (0.1 mol/l, pH 7.4) and homogenised in a stomacher (Stomacher 400; Seward) for 2 min at normal speed.

In vitro fermentations

Sterile stirred batch-culture fermentation systems (50 ml working volume) were set up, and aseptically filled with 45 ml sterile, pre-reduced, basal medium (peptone water 2 g/l (Oxoid), yeast extract 2 g/l (Oxoid), NaCl 0.1 g/l, K₂HPO₄ 0.04 g/l, KH₂PO₄ 0.04 g/l, MgSO₄·7H₂O 0.01 g/l, CaCl₂·6H₂O 0.01 g/l,

NaHCO₃ 2 g/l, Tween-80 2 ml (BDH), haemin 0.05 g/l, vitamin K₁ 10 μ l, cysteine.HCl 0.5 g/l, bile salts 0.5 g/l, pH 7.0) and gassed overnight with O₂-free N₂ (15 ml/min). The carbohydrate substrates were added to the respective fermentation vessels just before the addition of the faecal slurry. Concentration of the test substrates was 1% (w/v) in 50 ml culture fluid (0.5 g). The temperature was kept at 37°C and the pH was controlled between 6.7 and 6.9 using an automated pH controller (Fermac 260; Electrolab). Each vessel was inoculated with 5 ml of fresh faecal slurry (1:10, w/w). The batch cultures were run over a period of 36 h and 5 ml samples were obtained from each vessel at 0, 10, 24 and 36 h for fluorescent *in situ* hybridisation⁽¹⁵⁾ and SCFA analysis. Finally, eight replicate batch culture fermentations were set up, each inoculated with one of eight different human faecal inocula (four lean and four obese).

Bacterial enumeration

Synthetic oligonucleotide probes targeting specific regions of the 16S ribosomal RNA molecule, labelled with the fluorescent dye Cy3, were utilised for the enumeration of bacterial groups: Chis150⁽¹⁶⁾, Lab158⁽¹⁷⁾, Erec482⁽¹⁶⁾, Prop853⁽¹⁸⁾, Fpra655⁽¹⁹⁾, Rbro730/Rfla729⁽²⁰⁾, Bac303⁽²¹⁾, Bif164⁽²²⁾ and Ato291⁽²³⁾ (Table 1). Samples (375 μ l) obtained from each vessel at each sampling time were fixed for 4 h (4°C) in 1125 μ l (4%, w/v) paraformaldehyde. Fixed cells were centrifuged at 13 000 g for 5 min and washed twice in 1 ml filtered sterilised PBS. The washed cells were resuspended in 150 μ l filtered PBS and stored in 150 μ l ethanol (99%) at –20°C for at least 1 h before further processing. Samples (10 μ l) were diluted in a suitable volume of PBS in order to obtain 20–100 fluorescent cells in each field of view and 20 μ l of the above solution were added to each well of a six-well polytetrafluoroethylene/poly-L-lysine-coated slide (Tekdon, Inc.). The samples were dried for 15 min in a drying chamber (46°C). They were then dehydrated, using an alcohol series (50, 80 and 96% (v/v) ethanol) for 3 min in each solution. Slides were returned in the drying oven for 2 min to evaporate excess ethanol before adding the hybridisation mixture. The hybridisation mixture (50 μ l consisting of 5 μ l probe and 45 μ l hybridisation buffer) was added to each well and left to hybridise for 4 h in a micro-array hybridisation incubator (Grant-Boekel). After hybridisation,

Table 1. 16S ribosomal RNA oligonucleotide probes used in the present study

Probe name	Specificity	Sequence (5'–3')
Chis150	Most of the <i>Clostridium histolyticum</i> group (<i>Clostridium</i> clusters I and II) ⁽¹⁶⁾	TTATGCGGTATTAATCTYCCTT
Lab158	<i>Lactobacillus</i> – <i>Enterococcus</i> ⁽¹⁷⁾	GGTATTAGCAYCTGTTTCCA
Erec482	Most of the <i>Clostridium coccooides</i> – <i>Eubacterium rectale</i> group (<i>Clostridium</i> clusters XIVa and XIVb) ⁽¹⁶⁾	GCTTCTTAGTCARGTACCG
Prop853	<i>Clostridium</i> cluster IX ⁽¹⁸⁾	ATTGCGTAACT CCGGC
Fpra655	<i>Faecalibacterium prausnitzii</i> and relatives ⁽¹⁹⁾	CGC CTA CCT CTG CAC TAC
Rbro730	<i>Clostridium sporosphaeroides</i> , <i>Ruminococcus bromii</i> , <i>Clostridium leptum</i> ⁽²⁰⁾	TAAAGCCCAGYAGGCCGC
Rfla729	<i>Ruminococcus albus</i> , <i>Ruminococcus flavefaciens</i> ⁽²⁰⁾	AAAGCCCAGTAAGCCGCC
Bac303	Most Bacteroidaceae and Prevotellaceae, some Porphyromonadaceae ⁽²¹⁾	CCAATGTGGGGGACCTT
Bif164	<i>Bifidobacterium</i> spp. ⁽²²⁾	CATCCGGCATTACCACCC
Ato291	<i>Atopobium</i> cluster ⁽²³⁾	GGTCGGTCTCTCAACCC

slides were washed in 50 ml washing buffer for 15 min. They were then dipped in cold water for a few seconds and dried with compressed air. Thereafter, 5 μ l of polyvinyl alcohol mounting medium with 1,4-diazabicyclo(2,2,2)octane were added onto each well and a cover slip was placed on each slide (20 mm, thickness no. 1; VWR). The slides were examined under an epifluorescence microscope (Eclipse 400; Nikon) using the Fluor 100 lens. For each well, fifteen different fields of view were enumerated.

Organic acid analyses

Analysis was performed using an ion-exclusion HPLC system (LaChrom Merck Hitachi) equipped with a pump (L-7100), an RI detector (L-7490) and an autosampler (L-7200). Data were collected using Jones Chromatography Limited for Windows 2.0 software. The column used was an ion-exclusion Rezex ROA-Organic Acid H⁺(8%) (300 \times 7.80 mm; Phenomenex). Guard columns were SecurityGuard™ Carbo-H⁺4 \times 3.0 mm cartridges (Phenomenex). The eluent used was 0.0025 mM-H₂SO₄ in HPLC-grade water.

Samples (1 ml) from each fermentation time point were centrifuged at 13 000 *g* for 10 min. Supernatants were filtered through a 0.22 μ m filter unit (Millipore) and 20 μ l were injected into the HPLC, operating at a flow rate of 0.5 ml/min with a heated column at 84.2°C. The sample run-time was 35 min. Sample quantification was carried out using calibration curves for lactate, acetate, propionate and butyrate at concentrations of 12.5, 25, 50, 75 and 100 mM.

Rate of gas production

Sterile glass Balch tubes (18 \times 150 mm; Bellco) containing 13.5 ml pre-reduced basal medium (peptone water 2 g/l (Oxoid), yeast extract 2 g/l (Oxoid), NaCl 0.1 g/l, K₂HPO₄ 0.04 g/l, KH₂PO₄ 0.04 g/l, MgSO₄.7H₂O 0.01 g/l, CaCl₂.6H₂O 0.01 g/l, NaHCO₃ 2 g/l, Tween-80 2 ml, haemin 0.05 g/l, vitamin K₁ 10 μ l, cysteine.HCl 0.5 g/l, bile salts 0.5 g/l, pH 7.0) were placed in the anaerobic cabinet and kept overnight. Substrates (1:10, w/v) were added to the fermentation tubes just before the addition of the faecal inocula (1:10, w/v). The tubes were then sealed with a gas-impermeable butyl rubber septum (Bellco) and aluminium crimp (Sigma-Aldrich). The tubes were incubated at 37°C with constant agitation. The volume of gas generated by faecal bacteria from each substrate was measured every 3 h up to 36 h fermentation by inserting a sterile needle (BD, 23G X 1 inch) attached to a transducer (Gems Sensors) into the butyl rubber septum of each tube. The pressure build-up in the headspace was measured in pounds per square inch. After each measurement, the headspace of each tube was allowed to equilibrate with the atmosphere. The gas production experiments were performed in four replicates for each substrate. Quantification of gas volume (ml) was carried out using calibration curves of air pressure (pounds per square inch) by injecting known volumes of air into the culture tubes (0.5–7 ml).

Statistical analyses

Statistical analysis was performed using SPSS for Windows (version 16.0; SPSS, Inc.). One-way ANOVA and *post hoc* Tukey's tests were used to determine the significant difference of substrate used on bacterial group population, SCFA production and gas production. Principal component analysis was performed using XLSTAT-Pro software (Addinsoft) in accordance with Pearson's correlation test to identify correlated variables among the target bacterial groups at baseline (0 h) in the lean and obese faecal microbiota populations. Differences were deemed significant when $P < 0.05$.

Results

Bacterial enumeration

The average bacterial concentrations of the test substrates fermented by the lean and obese human faecal slurries are shown in Table 2. There was a significant increase in the Bif164 populations in the lean fermentations following the response to α -gluco-oligosaccharides at all time points compared with 0 h. Furthermore, the Bif164 populations at 36 h fermentation were significantly higher in α -gluco-oligosaccharides compared with inulin ($P < 0.05$). Other members of the actinobacteria group, i.e. Ato291, on α -gluco-oligosaccharides were significantly lesser in comparison with inulin at 36 h. There were significant increases in the Bac303 group on both α -gluco-oligosaccharides and inulin at 24 and 36 h, respectively. A significant decrease in Fpra655 was observed with both test substrates ($P < 0.01$) at all time points. No significant changes were detected in the other bacterial populations. Also, there were no significant changes in the total cell count (enumerated by 4',6-diamidino-2-phenylindole stain) on both substrates of the lean fermentation.

Analyses of the obese faecal microbiota composition at baseline (0 h) did not reveal any significant differences in the specific bacterial groups between the obese and lean human faecal microbiota. The total cell counts were higher in the obese human faecal samples compared with lean faecal samples ($P < 0.05$). However, a considerably higher percentage of the baseline microbiota was accounted for in the lean cultures compared with the obese cultures by the probe set used. The principal component analysis results for all parameters related to gut bacteria and the distribution of the faecal microbiota from all donors at baseline (0 h) are shown in Fig. 1. The first and second principal components were responsible for 63% of the total variance. The projection of the parameters in the plane by these principal components did not separate the lean and obese faecal microbiota and no clustering was observed, indicating no significant differences among the faecal inocula in accordance with Pearson's correlation test ($P > 0.05$).

There was a significant increase of Bif164 in response to α -gluco-oligosaccharides tested at all time points from 0 h, which was similar to the lean fermentation. Also, the Bif164 populations at 36 h with α -gluco-oligosaccharides were significantly higher compared with inulin fermentation ($P < 0.05$). Generally, substrates fermented with the obese microbiota produced similar results to the lean fermentation,

Table 2. Bacterial populations (\log_{10} cells/ml batch culture fluid) in pH-controlled batch cultures at 0, 10, 24 and 36 h inoculated with the lean and obese faecal microbiota (Mean values and standard deviations, n 4)

Probe/stain	Time (h)	Lean faecal fermentation				Obese faecal fermentation			
		α -Gluco-oligosaccharides		Inulin		α -Gluco-oligosaccharides		Inulin	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
Chis150	0	7.55	0.51	7.55	0.51	7.98	0.13	7.98	0.13
	10	7.49	0.33	7.64	0.38	7.89	0.29	7.71	0.18
	24	7.52	0.32	7.90	0.44	7.94	0.45	7.62	0.21
	36	7.60	0.43	7.76	0.51	7.48	0.21	7.52	0.27
Lab158	0	7.77	0.31	7.77	0.31	8.16	0.28	8.16	0.28
	10	7.74	0.38	8.06	0.46	8.72*	0.24	8.76*	0.33
	24	7.50	0.09	8.10	0.63	8.69 ^b	0.26	8.77 ^{b*}	0.36
	36	7.74 ^a	0.11	8.44	0.29	8.53 ^b	0.20	8.54	0.45
Erec482	0	8.53	0.17	8.53	0.17	8.60	0.18	8.60	0.18
	10	8.39	0.33	8.34	0.33	8.53	0.46	8.84	0.39
	24	8.16	0.39	8.36	0.30	8.62	0.47	8.55	0.39
	36	8.25	0.30	8.49	0.28	8.47	0.29	8.60	0.40
Prop853	0	8.41	0.29	8.41	0.29	8.28	0.28	8.28	0.60
	10	8.26	0.30	8.45	0.17	8.68 ^b	0.17	8.44	0.26
	24	8.46	0.48	8.52	0.16	8.33	0.38	8.26	0.27
	36	8.32	0.52	8.42	0.22	8.24	0.46	8.04	0.59
Fpra655	0	8.58	0.34	8.58	0.34	8.57	0.10	8.57	0.34
	10	7.81**	0.15	7.83**	0.16	8.05*	0.46	8.33	0.16
	24	7.88**	0.24	7.93**	0.19	8.06*	0.32	8.05*	0.19
	36	7.70**	0.07	7.80**	0.09	7.73**	0.18	7.72**	0.09
Rbro730/Rfla729	0	8.36	0.44	8.36	0.44	8.67	0.54	8.67	0.44
	10	8.33	0.71	8.17	0.45	8.21	0.27	8.26*	0.45
	24	8.09	0.41	8.33	0.56	8.18	0.56	8.32*	0.56
	36	8.07	0.27	8.22	0.45	7.84*	0.31	8.21*	0.45
Bac303	0	8.48	0.38	8.48	0.38	8.61	0.33	8.61	0.33
	10	9.10	0.27	9.07	0.21	9.41*	0.11	9.17**	0.36
	24	9.05	0.45	9.27*	0.28	9.36*	0.20	9.05**	0.27
	36	9.34*	0.27	8.98	0.24	8.86	0.43	8.72	0.30
Bif164	0	8.03	0.14	8.03	0.14	7.84	0.19	7.84	0.19
	10	8.80**	0.18	8.42	0.43	8.90**	0.11	8.57**	0.31
	24	8.98**	0.17	8.59	0.25	8.75**	0.39	8.50**	0.25
	36	8.99 ^{a***}	0.27	8.10	0.47	8.91 ^{a***}	0.21	8.29	0.24
Ato291	0	8.15	0.45	8.15	0.45	7.95	0.28	7.95	0.28
	10	8.35	0.20	8.47	0.66	8.50*	0.09	8.60	0.36
	24	8.31	0.16	8.74	0.34	8.39	0.19	8.40	0.38
	36	8.40 ^a	0.22	8.85	0.20	8.29	0.25	8.39	0.20
DAPI	0	9.46	0.12	9.46	0.12	9.74 ^b	0.11	9.74 ^b	0.11
	10	9.67	0.13	9.70	0.11	9.81	0.07	9.86	0.25
	24	9.80	0.19	9.74	0.16	9.85	0.18	9.87	0.19
	36	9.87	0.21	9.69	0.22	9.84	0.26	9.85	0.23

Chis150, *Clostridium histolyticum*; Lab158, *Lactobacillus/Enterococcus*; Erec482, *Eubacterium rectale/Clostridium coccoides*; Prop853, *Clostridium* cluster IX; Fpra655, *Faecalibacterium prausnitzii*; Rbro730/Rfla729, *Ruminococcus bromii/Ruminococcus flavefaciens*; Bac303, *Bacteroides/Prevotella*; Bif164, *Bifidobacterium* spp.; Ato291, *Atopobium* cluster; DAPI, 4',6-diamidino-2-phenylindole.

Mean value was significantly different from that at 0h: * $P < 0.05$, ** $P < 0.01$.

^{a,b} Mean values with unlike superscript letters were significantly higher/lower in comparison with inulin and the lean human faecal fermentation values of the same sampling hour ($P < 0.05$).

i.e. increasing Bac303 populations and decreasing Fpra655 populations. However, during the obese slurry fermentation, the populations of Lab158 increased with both substrates, which was not the case in the lean fermentation ($P < 0.05$).

The percentages of the major bacterial phyla in the fermentation using the lean and obese persons' inocula are shown in Table 3, where the total bacterial number of Chis150, Lab158, Erec482, Prop853, Fpra655 and Rbro730/Rfla729 represents Firmicutes; Bac303 represents Bacteroidetes; Bif164 and Ato291 represent Actinobacteria. The starting bacterial populations in both lean and obese faecal slurries

(0h) demonstrate that Firmicutes was the predominant group followed by Bacteroidetes and then Actinobacteria. No significant difference was detected in all major bacterial phyla ($P > 0.05$) between the lean and obese faecal fermentations.

Organic acid analyses

Table 4 shows organic acid (OA) concentrations in the lean and obese faecal cultures. Generally, fermentation of the lean faecal microbiota of both substrates produced all OA

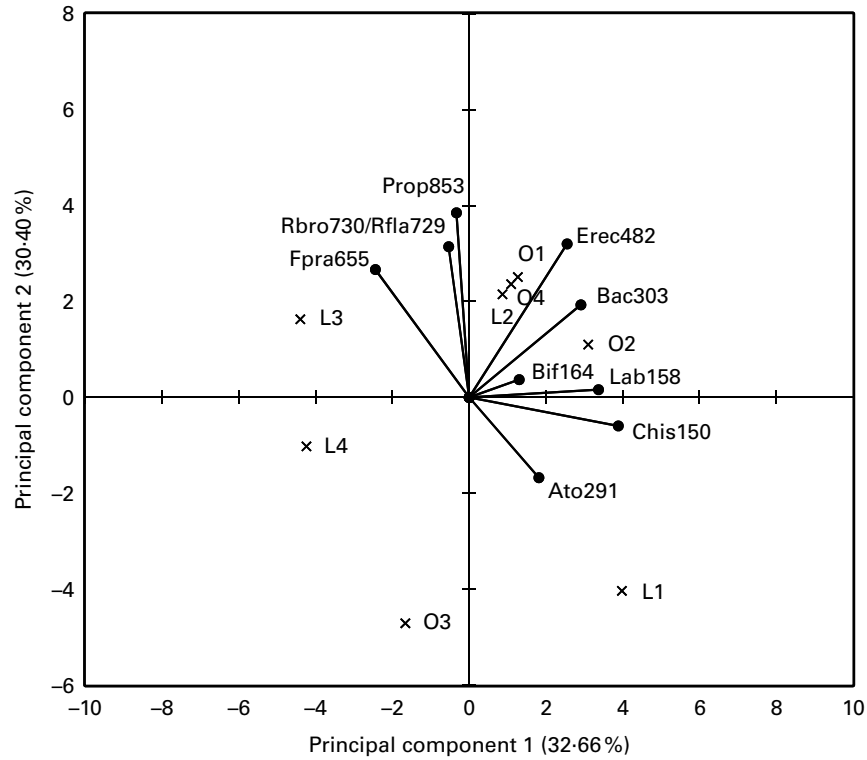


Fig. 1. Principal component analysis score plot of the faecal microbiota groups at baseline (0 h) for the lean (L, *n* 4) and obese (O, *n* 4) donors enumerated using fluorescent *in situ* hybridisation (Chis150, *Clostridium histolyticum*; Lab158, *Lactobacillus/Enterococcus*; Erec482, *Eubacterium rectale/Clostridium coccoides*; Prop853, *Clostridium cluster IX*; Fpra655, *Faecalibacterium prausnitzii*; Rbro730/Rfla729, *Ruminococcus bromii/Ruminococcus flavefaciens*; Bac303, *Bacteroides/Prevotella*; Bif164, *Bifidobacterium* spp.; Ato291, *Atopobium* cluster). The plot shows no clustering, indicating no significant differences between the L and O donors according to Pearson's correlation test ($P > 0.05$).

except butyrate. Lactate significantly increased early in the α -gluco-oligosaccharide fermentation (10 h) but later diminished. Total OA concentrations significantly increased on both test substrates after 10 h of fermentation ($P < 0.01$). In addition, total OA concentrations with α -gluco-oligosaccharides at 36 h were significantly higher compared with inulin. Acetate was the most prevalent SCFA on both test substrates accounting for $> 50\%$ of the total OA produced followed by propionate and butyrate. Significant increases in acetate were found with both test substrates. The highest acetate concentration was observed following α -gluco-oligosaccharide

fermentation at 36 h, which was significantly higher than inulin. Significant increases in propionate were found with both substrates, with α -gluco-oligosaccharides giving higher propionate levels. There is no significant difference in the acetate:propionate ratio observed in the fermentation of α -gluco-oligosaccharide compared with inulin.

Substrates fermented with the obese microbiota exhibited similar OA patterns to the lean fermentation, i.e. a significantly higher total OA concentration with α -gluco-oligosaccharides compared with inulin at 36 h. However, for α -gluco-oligosaccharides, the acetate:propionate ratio in the obese faecal

Table 3. Percentage of the major bacterial phyla in pH-controlled batch cultures of the lean and obese human faecal fermentations at 0, 10, 24 and 36 h*

Substrate	Time (h)	Phylum proportion (%)							
		Firmicutes		Bacteroidetes		Actinobacteria		Firmicutes/ Bacteroidetes	
		Lean	Obese	Lean	Obese	Lean	Obese	Lean	Obese
α -Gluco-oligosaccharides	0	68.4	74.8	17.7	18.2	14.0	7.0	3.9	4.1
	10	32.9	31.6	41.6	47.8	25.5	20.6	0.8	0.7
	24	25.6	32.2	42.6	50.0	31.8	17.8	0.6	0.6
	36	17.3	35.5	53.0	26.8	29.7	37.7	0.3	1.3
Inulin	0	69.8	73.6	16.2	18.3	14.1	8.1	6.4	5.3
	10	36.6	46.7	38.5	34.9	24.9	18.4	0.9	2.5
	24	33.9	49.8	43.2	32.3	22.9	17.9	1.0	1.9
	36	41.3	55.6	30.1	25.2	28.6	19.2	1.6	4.7

* No significant difference was detected in all major bacterial phyla ($P > 0.05$) between the lean and obese faecal fermentations.

fermentation was significantly lower than that in the lean faecal fermentation.

Gas production

The total gas production after 36 h in the lean and obese fermentations is shown in Fig. 2. No significant differences were observed in the total gas produced between the lean and obese fermentations. Fig. 3 shows the gas production patterns in the lean faecal fermentation. Fermentation of α -gluco-oligosaccharides produced gas which peaked at 3 h. Meanwhile, fermentation of inulin produced the highest amount of gas at 6 h. α -Gluco-oligosaccharides produced a slower rate of gas combined with lower total gas production compared with inulin ($P < 0.05$). This indicates that α -gluco-oligosaccharides were fermented more slowly to produce a more gradual build-up of gas compared with inulin. Fig. 4 shows the gas production patterns in the obese faecal fermentation. Fermentation of α -gluco-oligosaccharides produced gas which peaked at the same time with inulin at 3 h. However, thereafter, the gas production rate of α -gluco-oligosaccharide was slower than that of inulin. The fermentation of α -gluco-oligosaccharides with the obese faecal inocula was significantly faster than that with the lean faecal inocula ($P < 0.05$). However, no significant difference on the gas production rate of inulin was observed between the lean and obese faecal fermentations ($P > 0.05$).

Discussion

This is the first study to evaluate commercial α -gluco-oligosaccharides (BioEcolians) in a mixed human faecal culture system using modern DNA-based microbiological methods. α -Gluco-oligosaccharides gave rise to significant increases in *Bifidobacterium* populations when compared with inulin. This may be related to the difference in the molecular weight of the substrates tested. α -Gluco-oligosaccharides used in the present study have a DP of 5–6⁽²⁴⁾. Our current understanding of prebiotic substances is that low-molecular-weight oligosaccharides are more selectively fermented by bifidobacteria and lactobacilli than high-molecular-weight carbohydrates⁽⁴⁾. A study has demonstrated that lower-DP oligodextrans produced by controlled enzymatic hydrolysis resulted in higher fermentation selectivity for bifidobacteria, compared with the parent dextran molecule and other oligodextran fractions with a higher average DP⁽²⁵⁾, with a DP of 3 giving higher prebiotic activity than a DP of 2⁽²⁶⁾. This may be due to the fact that the low molecular mass means more non-reducing ends per unit mass, which are susceptible to attack by various exo-acting α - and β -glucosidases produced by colonic bacteria such as *Bifidobacterium* spp.⁽²⁷⁾. Little is known about the biochemical characteristics of α -glucosidase enzymes from bifidobacteria, although it is a common activity observed among this genus⁽²⁸⁾.

We previously demonstrated that an increase in α -1,2 branching of dextrans did not lead to higher selectivity for

Table 4. SCFA and lactic acid concentrations (mM) in pH-controlled batch cultures at 0, 10, 24 and 36 h inoculated with the lean and obese faecal microbiota

(Mean values and standard deviations, n 4)

Organic acid	Time (h)	Lean faecal fermentation				Obese faecal fermentation			
		α -Gluco-oligosaccharides		Inulin		α -Gluco-oligosaccharides		Inulin	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
Lactate	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	10	5.39*	4.33	5.16	5.12	3.00	3.93	3.10**	2.55
	24	0.00	0.00	1.24	2.47	0.00	0.00	0.00	0.00
	36	0.08	0.17	0.40	0.80	0.00	0.00	0.00	0.00
Acetate	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	10	26.20*	12.15	25.27**	8.97	30.96**	18.45	28.31**	9.64
	24	43.88**	10.52	34.59**	3.96	39.20**	16.79	28.69**	6.15
	36	48.90***	9.21	35.43**	3.83	41.04**	5.37	19.69**	10.84
Propionate	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	10	9.28	8.81	10.97	5.26	12.36*	5.78	10.08**	4.51
	24	21.83	10.20	18.76*	7.72	22.56**	8.92	11.70**	2.82
	36	24.19*	8.50	19.74*	7.04	23.50**	3.05	10.90**	1.27
Butyrate	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	10	1.52	2.18	5.33	6.96	6.01	5.64	13.77	13.18
	24	2.73	3.25	8.30	5.12	7.63	5.66	11.90	8.69
	36	3.90 ^a	2.71	8.49	4.29	8.23	4.16	11.92	5.02
Total organic acids	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	10	37.00**	21.01	41.57**	16.10	49.32*	29.75	52.16**	26.74
	24	68.44**	16.94	61.65**	3.92	69.40**	31.10	52.29**	15.23
	36	77.00***	7.64	63.66**	2.03	72.76 ^a **	11.86	42.5**	13.96
Acetate:propionate	10	6.52	6.55	2.65*	1.11	2.42 ^b **	0.33	3.01**	0.97
	24	2.32	0.94	2.16	1.01	1.72**	0.08	2.47**	0.17
	36	2.24	0.84	2.13	1.21	1.75**	0.00	1.90**	1.09

Mean value was significantly different from that at 0 h: * $P < 0.05$, ** $P < 0.01$.

^{a,b} Mean values with unlike superscript letters were significantly higher/lower in comparison with inulin and the lean human faecal fermentation values of the same sampling hour ($P < 0.05$).

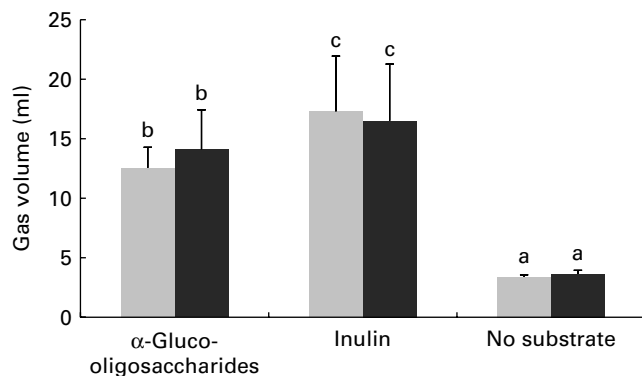


Fig. 2. Total gas production (ml) from 36 h non-pH-controlled batch culture fermentation inoculated with lean (■) and obese (■) faecal microbiota (n 4). Values are means, with standard deviations represented by vertical bars. ^{a,b,c}Mean values with unlike letters were significantly different among the treatments ($P < 0.05$), with 'a' being the least amount of total gas and 'c' being the highest amount of total gas.

bifidobacteria. However, branching may decrease the gastrointestinal digestibility of the 1 kDa dextrans as assessed by the higher total dietary fibre content of branched 1 kDa dextrans than linear 1 kDa dextrans⁽²⁹⁾. A study has found that α -1,2- and α -1,6-linked disaccharides were selective for *Bifidobacterium* spp. using *in vitro* faecal cultures⁽³⁰⁾. These observations are consistent with α -gluco-oligosaccharides used in the present study, which consist of a linear α -1,6 glucan backbone with α -1,2 and α -1,3 branching⁽¹⁴⁾.

An *in vivo* animal study has demonstrated that gluco-oligosaccharides fed to gnotobiotic rats (inoculated with human faecal microbiota) did not influence major bacterial populations (including bifidobacteria) as opposed to rats fed fructo-oligosaccharides⁽³¹⁾. However, this study used culture-based techniques to enumerate bacterial populations disregarding the non-cultivable species. Nevertheless, gluco-oligosaccharides increased β -galactosidase and α -glucosidase activities and decreased β -glucuronidase, which could be considered beneficial for the host. β -Glucuronidase is involved in the generation of toxic and carcinogenic metabolites⁽³²⁾, whereas β -galactosidase and α -glucosidase activities can

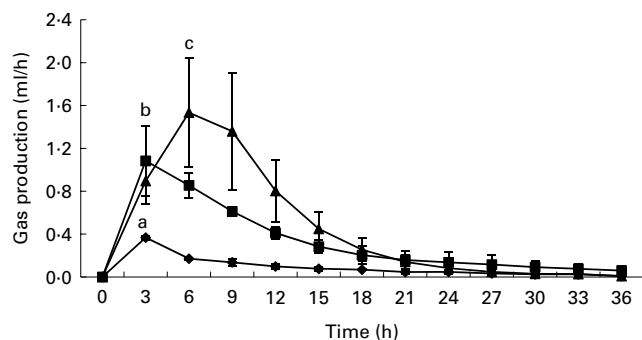


Fig. 3. Gas production pattern expressed in ml/h from non-pH-controlled batch culture (n 4) inoculated with lean faecal microbiota. Values are means, with standard deviations represented by vertical bars. ^{a,b,c}Mean values with unlike letters were significantly different among the treatments ($P < 0.05$), with 'a' being the slowest gas rate and 'c' being the fastest gas rate. ▲, α -Gluco-oligosaccharides; ■, inulin; ◆, no substrate.

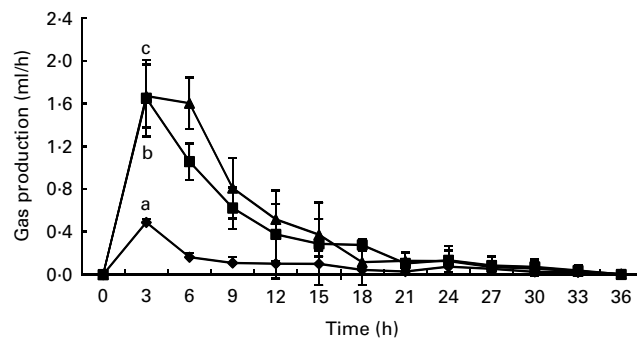


Fig. 4. Gas production pattern expressed in ml/h from non-pH-controlled batch culture (n 4) inoculated with obese faecal microbiota. Values are means, with standard deviations represented by vertical bars. ^{a,b,c}Mean values with unlike letters were significantly different among the treatments ($P < 0.05$), with 'a' being the slowest gas rate and 'c' being the fastest gas rate. ▲, α -Gluco-oligosaccharides; ■, inulin; ◆, no substrate.

improve carbohydrate fermentation to SCFA⁽³³⁾. A study has shown that α -glucosidase from *Bifidobacterium breve* UCC2003 belongs to a subgroup of the glycosyl hydrolase family 13, the α -1,6-glucosidases (*EC* 3.2.1.10), which exhibits hydrolytic activity towards α -1,6-linked carbohydrates such as panose, isomaltose and isomaltotriose⁽³⁴⁾. It has also been demonstrated, in non-pH-controlled fermentations, that α -1,2- and α -1,6-linked disaccharides were selective for *Bifidobacterium* spp.⁽³⁵⁾. The decrease in *Faecalibacterium prausnitzii* was seen in all fermentations using faecal inocula from lean and obese donors. Another study has demonstrated that a clinical improvement of Crohn's disease was correlated with a significant decrease in *F. prausnitzii*⁽³⁶⁾.

A preliminary human study has suggested differences between lean and obese human gut microbiota based on sequencing 16S ribosomal RNA genes from stool samples⁽⁹⁾. However, through the present findings, we did not see any significant difference in the major colonic bacterial groups (except for Lab158) between lean and obese, either on the baseline or subsequent fermentation. In addition, the fermentation of α -gluco-oligosaccharides significantly increased Bif164 and Bac303, and decreased Fpra655 in the obese faecal fermentation. This was similar to the lean faecal fermentation, which may suggest that the changes appear to be mainly in response to the substrates. Obesity is thought to be associated with low-grade systemic inflammation^(37–41). High numbers of bifidobacteria may decrease endotoxaemia and pro-inflammatory cytokines, further improving glucose tolerance and glucose-induced insulin secretion⁽⁴²⁾.

Some studies have observed altered proportions of Firmicutes and Bacteroidetes in obese compared with lean mice^(6,7,43). It has also been reported that Firmicutes concentrations were higher in the faeces of obese than lean human subjects^(7,44). However, we observed here that the number of Bacteroidetes, Firmicutes and Actinobacteria in baseline faecal samples from obese donors was similar to those from lean donors. Nevertheless, it has to be noted that the present study only involved four donors that could possibly limit the statistical significance of this particular observation.

Fermentation of α -gluco-oligosaccharides induced the production of lactate early in the fermentation, which later declined. This is likely to be due to lactate conversion into SCFA by cross-feeding of other bacteria. The significantly higher concentration of acetate observed on α -gluco-oligosaccharides compared with inulin at 36 h is probably due to a higher population of *Bifidobacterium*⁽⁴⁵⁾. There was also an increase in propionate concentration on both substrates in accordance with a significant increase in the *Bacteroides*–*Prevotella* group, as these are known to be propionate producers. It has been postulated that propionate may have anti-obesity properties through the reduction of fatty acid levels in plasma⁽⁴⁶⁾. High plasma fatty acids are known to cause inflammation, leading to insulin resistance⁽⁴⁷⁾. These beneficial effects are usually linked with a reduction in body weight and have been demonstrated to increase satiety⁽⁴⁸⁾. Acetate may act as a precursor for cholesterol synthesis, whereas propionate may inhibit this process. Therefore, a low acetate:propionate ratio may be of interest in regulating serum cholesterol levels⁽⁴⁹⁾. In the present *in vitro* study, the fermentation of α -gluco-oligosaccharides produced a significantly lower acetate:propionate ratio in the obese faecal fermentation than in the lean faecal fermentation. Therefore, it is suggested to some extent that this type of α -gluco-oligosaccharides may demonstrate an anti-obesity effect through its propionate production especially when used in obese people. However, this has to be demonstrated *in vivo*.

Known producers of propionate include *Bacteroides* spp. and *Clostridium*. An example is *Bacteroides fragilis* that is prevalent in the human gut microbial community, which produces substantial amounts of propionate from succinate and fumarate⁽⁵⁰⁾. Species within the *Clostridium histolyticum* group can also produce propionate, for example *Clostridium homopropionicum*^(51,52). Clostridial cluster IX contains known propionate producers⁽⁵²⁾ and species within it (e.g. *Succiniclasticum ruminis* and *Succinispira mobilis*) have been reported to convert succinate into propionate^(53,54). Through the present study, we only observed significant increases in *Bacteroides*–*Prevotella* populations but not in the *Clostridium* group, i.e. *C. histolyticum* and *Clostridium* cluster IX. Therefore, we suggest that propionate may have been produced by the *Bacteroides*–*Prevotella* group. In addition, a previous study has shown that *Bacteroides thetaio-taomicron* is highly efficient in utilising α -1,2 and α -1,6 glycosidic linkages that occur in α -gluco-oligosaccharides⁽⁵⁵⁾.

There is no significant increase in butyrate in the fermentation of both substrates. This may be due to the decrease in the population of major butyrate producers such as *F. prausnitzii*, *Clostridium coccoides*–*Eubacterium rectale* and *Ruminococcus* groups. An *in vitro* study has demonstrated that fermentation of α -gluco-oligosaccharides resulted in a lower concentration of butyrate compared with fructo-oligosaccharides⁽⁵⁶⁾, which is in agreement with the present findings. In addition, an *in vitro* study has shown that α -gluco-oligosaccharide fermentation resulted in significantly lower butyrate concentration compared with short-, medium- and long-chain fructo-oligosaccharides⁽⁵⁷⁾.

Gas production in the large intestine is part of the normal digestive function caused by fermentation of carbohydrate by the gut microbiota. These gases are inevitable fermentation products but are also the main reported disincentive to consumption of prebiotics. Undesirable symptoms relating to gas production in the gut have been widely reported in human prebiotic feeding studies⁽⁵⁸⁾. Gastrointestinal discomfort usually occurs with high carbohydrate intakes (more than 20 g/d)⁽⁵⁹⁾. Although it is known that prebiotic target bacteria, i.e. bifidobacteria, do not produce gas, they do produce lactate which can be utilised by gas-producing bacteria such as *Clostridium* spp., sulphate-reducing bacteria and *Bacteroides*^(60,61). Clostridial clusters IV and XIVa, which are also butyrate producers, have been shown to produce gases, mainly CO₂ and H₂^(62,63). Fermentation of α -gluco-oligosaccharides resulted in lower quantities of gas, and this was produced more gradually than the fermentation of inulin with both lean and obese faecal slurries. This is in accordance with a previous study which demonstrated that fermentation of gluco-oligosaccharides produced significantly lower amounts of total gas than fructo-oligosaccharides⁽³¹⁾.

The *in vitro* study described here suggested that α -gluco-oligosaccharides (BioEcolians; Solabia) have bifidogenic activity. This, in combination with the fact that glycosidic linkages present results in a very high resistance to hydrolysis by digestive enzymes in both humans and animals⁽⁶⁴⁾, makes this type of α -gluco-oligosaccharides an interesting candidate prebiotic and is worthy of further *in vivo* evaluation. Also, we suggested that the substrate (or diet) but probably not the individual's obese condition can influence the microbiota composition. However, further *in vivo* work is needed to ultimately confirm this.

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