

## ***In vitro* fermentability of dextran, oligodextran and maltodextrin by human gut bacteria**

Estibaliz Olano-Martin, Konstantinos C. Mountzouris, Glenn R. Gibson and Robert A. Rastall\*

Department of Food Science and Technology, The University of Reading, PO Box 226, Whiteknights, Reading RG6 6AP, UK

(Received 14 January 1999 – Revised 9 September 1999 – Accepted 27 September 1999)

Anaerobic batch culture fermenters were used for a preliminary screening of the *in vitro* utilization by human gut microflora of dextran and novel oligodextrans (I, II and III) produced in the University of Reading (UK). Glucose and fructooligosaccharides (FOS) were used as reference carbohydrates. As expected, FOS acted as a good prebiotic in that it selectively increased numbers of bifidobacteria in the early stages of the fermentation. Dextran and oligodextrans each resulted in an enrichment of bifidobacteria in the batch cultures, with high levels of persistence up to 48 h. They also produced elevated levels of butyrate ranging from 5 to 14.85 mmol/l. To more effectively simulate conditions that prevail in different regions of the large intestine, a three-stage continuous culture cascade system was used to study further the fermentation of dextran, a low-molecular-mass oligodextran (IV) and maltodextrin. Oligodextran IV was shown to be the best substrate for bifidobacteria and lactobacilli with steady-state populations of bifidobacteria and lactobacilli being higher in all three vessels of the gut model than the respective populations resulting from dextran and maltodextrin. A maximum difference of 1.9 log was observed in vessel 1 for both bifidobacteria and lactobacilli in the case of dextran fermentation, while 1.4 log and 0.8 log in vessel 3 were the maximum differences for bifidobacteria and lactobacilli when maltodextrin was used as the carbohydrate source. Moreover, dextran and oligodextran appeared to stimulate butyrate production, with a maximum production up to 25.39 mmol/l in vessel 3 when fermenting dextran, followed by 21.70 mmol/l in the case of oligodextran IV and only 12.64 mmol/l in the case of maltodextrin.

### **Oligosaccharides: Gut fermentation: Prebiotics: Butyrate**

The microflora present in the human large intestine is thought to comprise at least 400 culturable bacterial species, although the likely microbial diversity is much greater than this (Gibson & Roberfroid, 1995). As a result of the metabolic activities of different bacteria, this complex ecological niche plays a major role in human nutrition, and possibly health (McCartney & Gibson, 1998).

Many undigested food materials, principally carbohydrates and proteins, can enter the large intestine and are susceptible to metabolism by the microbiota. In general, gut fermentation is characterized by carbohydrate metabolism in the proximal colon, with increased proteolysis in the distal gut (Macfarlane *et al.* 1992). Short-chain fatty acids (SCFA), particularly butyrate, and vitamins are desirable end-products of this process. Physiologically undesirable metabolites include carcinogens, other tumour-promoters and bacterial toxins (Cummings & Macfarlane, 1991; Tomomatsu, 1994). Because of this dichotomy of activities, there is currently much interest in determining appropriate

dietary components that stimulate a benign, or beneficial, gut microflora.

Despite our relative lack of understanding of the ecological interactions between bacterial species in the colon, it is possible to identify certain markers that may be associated with a healthy hindgut. These include populations of species belonging to the genera *Lactobacillus* and *Bifidobacterium* (Mizota, 1996; Yaeshima, 1996) and the production of butyrate (Cummings, 1984; Cummings & Macfarlane, 1991). Butyrate acts as the preferred energy source for colonic epithelial cells (Delzenne & Roberfroid, 1994) and it has been shown to stimulate apoptosis in colon cells *in vitro* (Hague *et al.* 1995). Lactobacilli and bifidobacteria may inhibit gut colonization by pathogens and are thought to exert other protective effects against acute and chronic gut disorders (Fuller, 1989). For this reason, current prebiotics are largely targeted towards these genera. A prebiotic has been defined by Gibson & Roberfroid (1995) as 'a non-digestible food ingredient that beneficially affects

**Abbreviations:** DP, degree of polymerization; FOS, fructooligosaccharides; IMO, isomaltooligosaccharides; LAB, lactic acid bacteria; SCFA, short-chain fatty acids; V, vessel.

\* **Corresponding author:** Dr Robert A. Rastall, fax +44 (0) 118 931 0080, email r.a.rastall@afnovell.reading.ac.uk

the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health'.

Currently a commercially produced isomaltooligosaccharide (IMO) preparation is the market leader in the prebiotic oligosaccharide sector in Japan (Japanscan, 1998), with annual sales of approximately 10 000 tonnes. This commercial preparation is a mixture of IMO such as isomaltose (230 g/kg) and isomaltotriose (170 g/kg) but it also contains high amounts (approximately 300 g/kg) of other non-isomalto oligosaccharides such as panose, maltose, maltotriose, nigerose and kojibiose. Approximately 260 g/kg of this preparation consists of higher oligosaccharides having a degree of polymerization (DP) ranging from 4 to 6. IMO are partly digestible in the small intestine, while the degree of digestion decreases for components having DP  $\geq 3$  (Kaneko *et al.* 1995).

The development of a prebiotic that allows the growth of elevated numbers of lactic acid bacteria (LAB), whilst stimulating butyrate production, would be advantageous. We have recently investigated ways of generating oligodextrans with controlled physico-chemical characteristics using enzyme biotechnology. The manufacturing process involves the enzymic depolymerization of industrial-grade dextran using an endo-dextranase in membrane bioreactors (Mountzouris *et al.* 1999) to effectively control the molecular size of the oligodextran products. It is expected that dextran and the produced oligodextrans would be less digestible than IMO for two main reasons: (a) their predominant  $\alpha$ -1 $\rightarrow$ 6 glucosidic linkages would escape digestion by the luminal enzymes, and (b) their higher molecular mass components (i.e. DP  $\geq 3$ ) would be also less susceptible to membrane digestion by the disaccharidases on the epithelial surface of the small intestine. Thus, the oligodextrans developed as described earlier would be expected to reach the colon more-or-less intact as they were eaten.

The aim of the present work was to generate some information on the *in vitro* fermentability of oligodextrans by human gut microflora. *In vitro* fermentations using batch and continuous culture methods have been regarded as acceptable models for the study of large intestinal function

(Salminen *et al.* 1998). Initially, batch culture fermentations were considered as the only possible option for experimentation due to the limited amounts of the novel oligodextrans produced on a laboratory scale according to the method of Mountzouris *et al.* (1999). The study of the *in vitro* fermentability of one oligodextran type in a more efficient validated three-stage continuous culture system (Macfarlane *et al.* 1998) was enabled, following the development of a continuous membrane bioreactor system (KC Mountzouris, unpublished results) facilitating the production of oligodextrans in larger amounts. Dextran and a food-grade maltodextrin were also tested using the three-stage continuous culture system (Macfarlane *et al.* 1998), the former because it is the polysaccharide used in the production of the oligodextrans and the latter because starch is associated with high *in vitro* production of butyrate (Wang & Gibson, 1993).

## Materials and methods

### General reagents

Unless otherwise specified, all chemicals were purchased from Sigma (Poole, Dorset, UK). Microbiological culture media and other additions were obtained from Oxoid (Basingstoke, Hants., UK). Fructooligosaccharides (FOS; Oligofructose P95) were obtained from Orafiti (Tienen, Belgium). A food-grade maltodextrin preparation, 'Goldex 20' with a dextrose equivalent value in the range of 17–20, was kindly supplied by ABR Foods Ltd (Corby, Northants., UK).

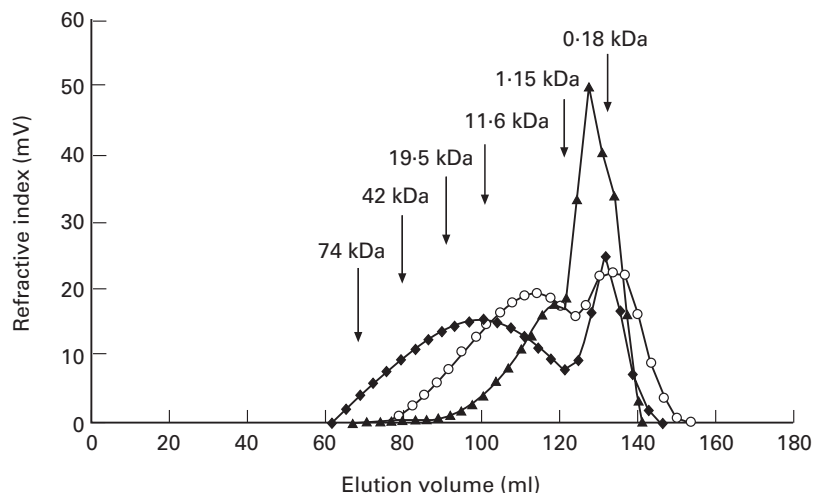
The oligodextrans are currently manufactured according to a process developed by Mountzouris *et al.* (1999). Three different sizes of oligodextrans were used in the batch culture experiment: oligodextrans I, II, and III. Another oligodextran (IV) produced in a continuous process (KC Mountzouris, unpublished results) was used for the gut model. The composition of the oligodextrans with respect to their DP is given in Table 1 and the overall molecular mass distributions of oligodextrans I, II and III are shown in Fig. 1.

**Table 1.** Composition (g/kg) of oligodextrans used in the batch and gut model experiments with respect to their degree of glucose polymerization (DP)

	Oligodextran I* (g/kg)	Oligodextran II* (g/kg)	Oligodextran III* (g/kg)	Oligodextran IV† (g/kg)
DP 1	62.2	75.2	151.3	9.8
DP 2	185.3	50.7	0.0	154.6
DP 3	269.6	98.2	58.2	264.6
DP 4	41.1	27.9	11.6	46.1
DP 5	11.4	12.4	6.6	14.8
DP 6	14.3	12.9	8.3	22.1
DP 7	20.0	13.5	8.8	31.8
DP 8	22.2	14.7	8.3	36.6
DP 9	18.4	14.5	7.6	32.8
DP 10	12.9	15.0	7.6	28.4
DP 11+	342.4	665.0	731.7	358.5

\* Oligodextrans used in the batch experiments. The molecular mass distributions of oligodextran I, II and III are shown in Fig. 1.

† Oligodextran used in the three-stage continuous culture experiment.



**Fig. 1.** Molecular mass distributions of oligodextran I (▲), oligodextran II (○) and oligodextran III (◆) used in the batch culture fermentations. Arrows indicate the elution volumes of the standards used to calibrate the chromatographic column (Sephacryl S-200 HR). Data were adapted from Mountzouris *et al.* (1999).

#### Batch culture fermentations

Sterile batch culture fermenters (50 ml capacity) were filled with a pre-reduced culture medium and inoculated with slurried faecal bacteria to give a final concentration of 100 g/l. Carbohydrates (glucose, oligodextran I, oligodextran II, oligodextran III, dextran, FOS) were added separately before inoculation, to give a final concentration of 10 g/l. The closed fermenters were maintained under a headspace of O<sub>2</sub>-free N<sub>2</sub>. Faecal slurries were prepared by homogenizing freshly voided faeces in anoxic 0.1 M-sodium phosphate buffer, pH 7.0. Details of the process are given by Wang & Gibson (1993).

The basal culture medium consisted of (g/l): peptone water 2.0, yeast extract 2.0, NaCl 0.1, K<sub>2</sub>HPO<sub>4</sub> 0.04, KH<sub>2</sub>PO<sub>4</sub> 0.04, MgSO<sub>4</sub>·7H<sub>2</sub>O 0.01, CaCl<sub>2</sub>·2H<sub>2</sub>O 0.01, NaHCO<sub>3</sub> 2.0, cysteine HCl 0.5, bile salts 0.5. The following liquid additions were made (ml/l): Tween 80 2, phyloquinone 10; haemin solution 1.

Fermenters were incubated at 37° and triplicate samples (1 ml) removed after 0, 6, 24 and 48 h for the enumeration of bacteria on appropriate agars and analysis of fermentation end-products. The initial pH of the culture medium was 7.0 and was not controlled during the incubation period. All additions, inoculations and incubation, sampling and plating were carried out inside an anaerobic cabinet containing H<sub>2</sub>-CO<sub>2</sub>-N<sub>2</sub> (10:10:80, by vol.).

#### Three-stage continuous culture system

To simulate the different physical and nutritional characteristics of the proximal, transverse and distal colons, a three-stage continuous culture (gut model) system was inoculated with a faecal bacterial inoculum. The system comprised a cascade of three fermenters, connected in series, such that culture medium was pumped into vessel (V) 1, which sequentially fed V2 then V3 and ultimately a waste unit. V1 and V2 had operating volumes of 250 ml, whilst the

volume in V3 was 300 ml. The respective pH values in each of the fermenters were 5.5, 6.2 and 7.1. Each vessel was magnetically stirred and maintained under anaerobic conditions by continuous sparging with O<sub>2</sub>-free N<sub>2</sub>. Temperature (37°) and pH were controlled automatically. As such V1 simulated the acidic conditions, fast bacterial growth and high substrate availability that prevail in the proximal (right) colon. In contrast, V3 was held at a neutral pH, bacteria grew more slowly and were substrate limited, conditions similar to those in the distal (left) colon. Overall retention time in the system was 60 h. The system has been previously validated as giving a close approximation to fermentation events *in situ* (Macfarlane *et al.* 1998). Culture media and faecal slurries were prepared as previously described.

One experiment was carried out for each C source: dextran, oligodextran IV and maltodextrin. Dilution rates in the system were 0.016/h for dextran and maltodextrin and 0.02/h for oligodextran IV. The carbohydrate source was added to the fermentation medium before autoclaving to give a final concentration of 10 g/l. The vessels were filled with 200 ml fermentation medium and 50 ml inoculum.

The system was allowed to equilibrate for 24 h, after which time the medium pump was started. Samples (1 ml) were removed from the inoculum after at least seven turn-overs from each vessel for the enumeration of selected bacterial genera and fermentation end-product analyses. Sampling from each vessel was performed in triplicate.

#### Enumeration of bacteria

Samples (1 ml) of batch or continuous culture were serially diluted to 10<sup>-7</sup> in an anaerobic cabinet with pre-reduced half strength peptone water, enriched with 0.5 g cysteine HCl/l. Portions (20 µl) of each dilution were then inoculated onto a range of agars designed to be selective for predominant colonic bacteria. The following growth media were used:

nutrient agar (total aerobes), Wilkins Chalgren agar (total anaerobes), MacConkey agar no. 3 (coliforms), Rogosa agar (lactobacilli), reinforced clostridia agar (clostridia), Beeren's agar (bifidobacteria), Brucella agar (bacteroides). Various additions were made to the agars in order to improve selectivity (Wang & Gibson, 1993). Agar plates were placed into an anaerobic cabinet at least 16 h before inoculation to allow them to pre-reduce. After incubation, single colonies were counted and characterized to genus level on the basis of colonial appearance, Gram reaction, microscopic characteristics and limited biochemical profiles as described by Wang & Gibson (1993). For further confirmation of culture identity, some randomly selected isolates were further characterized by sequencing specific regions of 16S-rRNA (McCartney & Gibson, 1998).

#### Analysis of fermentation end-products

Samples (1 ml) from the cultures were centrifuged at 13 000 *g* for 15 min to remove bacteria and other particulate material.

Analysis of SCFA was carried out by HPLC. Acetate, propionate and butyrate were determined on a BIO-RAD Aminex HPX-87H column (300 × 7.8 mm). Degassed 0.005 M-H<sub>2</sub>SO<sub>4</sub> was used as eluent at a flow rate of 0.6 ml/min and an operating temperature of 50°. Organic acids were detected by u.v. absorbance at a wavelength of 215 nm. The injection volume was 20 µl sample.

Residual carbohydrate in the samples was determined by the phenol-sulfuric assay for total sugar (Dubois *et al.* 1956).

## Results

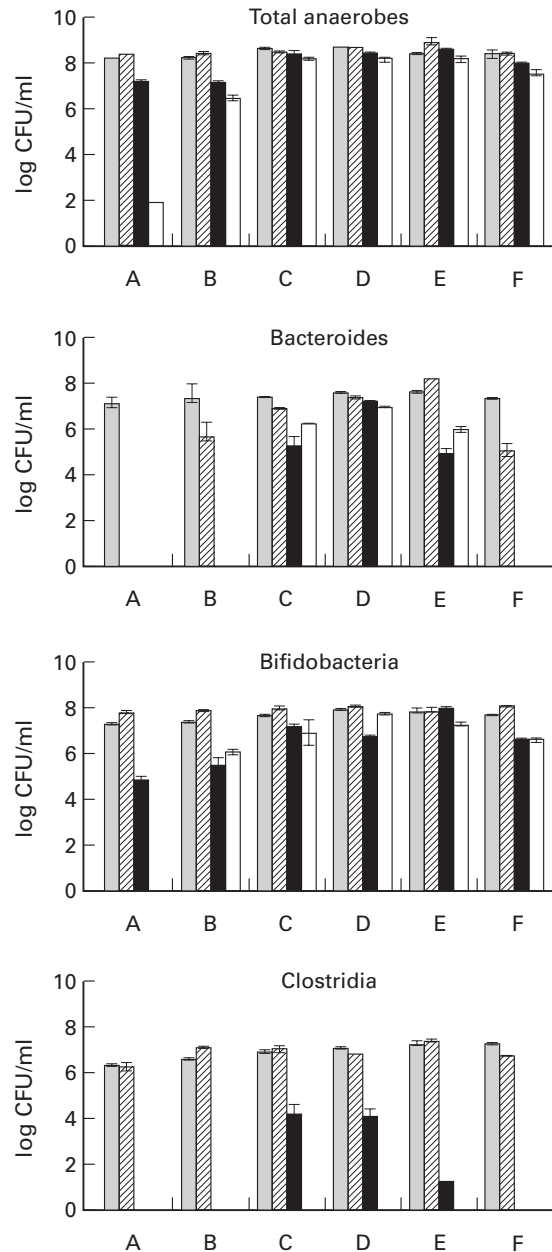
### Influence of molecular mass on the fermentation of oligodextrans in batch culture

Counts of selected bacterial genera are shown in Fig. 2. During the first 6 h of fermentation, counts of total anaerobes were seen to increase slightly giving a maximum increase of 0.5 log in the case of dextran. Generally, high levels of total anaerobes were maintained in the batch systems growing on oligodextrans, dextran and FOS for up to 48 h, the effect being more pronounced in the case of oligodextrans II and III and dextran. The oligodextrans II, III and dextran were shown to allow clostridia counts (Fig. 2) to persist for up to 24 h, but this did not happen in the case of FOS and oligodextran I.

Oligodextran I and FOS (Fig. 2) displayed quite similar behaviour with respect to their selectivity towards *Bacteroides* and bifidobacteria. A decrease in *Bacteroides* counts by 1.6 and 2.3 log occurred during the first 6 h for oligodextran I and FOS respectively. Bifidobacteria increased by a maximum of 0.6 log within the first 6 h in the case of oligodextran I, and by 0.4 log in the case of FOS. High levels of bifidobacteria appeared to persist for up to 48 h mostly in the case of oligodextrans II, III and dextran.

Generation of SCFA (Fig. 3) was used to determine the fermentability of the different carbohydrate substrates in the batch culture fermenters. All the carbohydrates tested were utilized to some degree (Fig. 4) but SCFA production patterns were variable (Fig. 3).

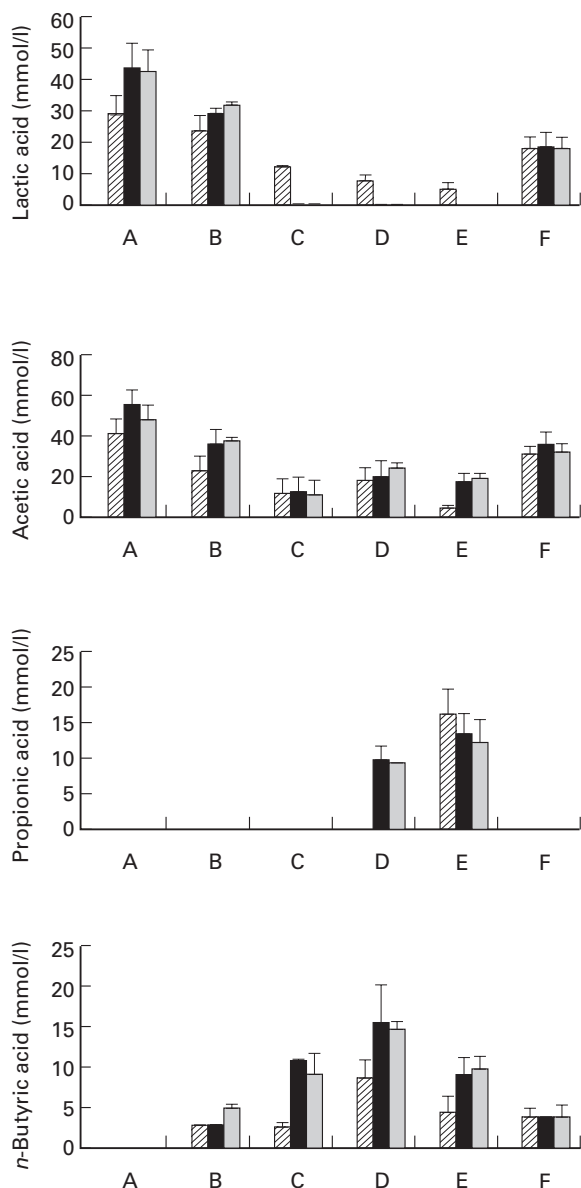
The utilization of the oligodextrans showed dependence



**Fig. 2.** Counts (log colony-forming units (CFU)/ml) of selected gut bacterial genera at 0 (□), 6 (▨), 24 (■) and 48 (◻) h in batch culture experiments using glucose (A), oligodextran I (B), oligodextran II (C), oligodextran III (D), dextran (E) and fructooligosaccharides (F) as carbohydrate sources. Values are means of triplicate determinations on the same culture, with standard deviations represented by vertical bars.

on their molecular mass: glucose > oligodextran I > oligodextran II > oligodextran III > dextran. FOS had its own utilization pattern (up to 50% within 48 h) which ranged between those of oligodextrans I and III (Fig. 4).

The SCFA data (Fig. 3) showed that carbohydrates with predominantly  $\alpha$ -1→6 glucosidic linkages (dextran and oligodextrans) were good substrates for butyrate formation. Both the low-molecular-mass oligodextran I and FOS had similar SCFA patterns (Fig. 3). Their fermentation did not produce large quantities of butyrate; 3.9 mmol/l for FOS and



**Fig. 3.** Production of lactic acid, acetic acid, propionic acid and butyric acid by human gut microflora at 0 (□), 6 (▨), 24 (■) and 48 (▩) h in batch culture experiments using glucose (A), oligodextran I (B), oligodextran II (C), oligodextran III (D), dextran (E) and fructo-oligosaccharides (F) as carbohydrate sources. Values are means of duplicate determinations on the same culture, with standard deviations represented by vertical bars.

5 mmol/l for oligodextran I. As the average molecular mass of oligodextrans increased, elevated amounts of butyrate were detected, giving a maximum of 14.9 mmol/l for oligodextran III. Propionate was only formed during the metabolism of dextran and high-molecular-mass oligodextran III (Fig. 3).

#### *Comparative growth of colonic bacteria using a three-stage continuous culture system*

An assessment of bacterial numbers in a validated gut model system (Macfarlane *et al.* 1998) was carried out, with

dextran, oligodextran IV and maltodextrin as carbohydrate sources. Oligodextran IV was chosen because this preparation can currently be manufactured continuously on a larger (100 g) scale and has a composition similar to that of oligodextran I (Table 1), shown to have a comparable fermentation pattern with FOS in batch cultures. Several groups of intestinal bacteria were selected as markers of the general colonic population to determine how the carbohydrate source may influence the microbial composition.

Steady-state counts of bacterial responses to the carbohydrate sources are presented in Table 2. Anaerobic bacteria were more numerous than aerobes in all three vessels for all the carbohydrate sources tested. *Bacteroides* species predominated in all three vessels during dextran fermentation but only in V2 and V3 during the oligodextran IV fermentation. Maltodextrin fermentation did not increase *Bacteroides* species, with steady-state populations being lower than the corresponding ones during oligodextran IV fermentation by 1.9 log and 3.2 log in V1 and V3 respectively. Steady-state clostridia populations in the oligodextran IV fermentation were lower by a minimum of 0.4 log in V2 compared with dextran fermentation. Clostridia counts in maltodextrin fermentation were higher by 1.1 log in V1 and lower by 1.4 log in V3 compared with oligodextran fermentation.

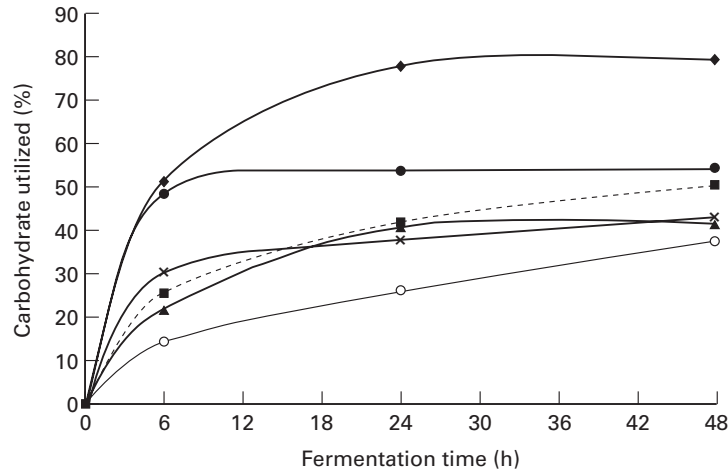
Oligodextran IV was the best growth substrate for bifidobacteria and lactobacilli from the carbohydrates tested (Table 2) with the highest counts found in V1. Steady-state populations of bifidobacteria and lactobacilli when oligodextran IV was used as the carbohydrate source were higher in all three vessels than the respective populations in the case of dextran and maltodextrin fermentations. Dextran resulted in 1.9 log smaller bifidobacteria and lactobacilli populations in V1 compared with oligodextran IV, whereas maximum differences of 1.4 log and 0.8 log were seen in V3 for bifidobacteria and lactobacilli respectively (Table 2) in the case of maltodextrin.

Total SCFA production in all vessels increased when dextran or oligodextran IV was added into the system, with the highest production recorded in V2 and V3 (Table 3). Lactate was detected only when maltodextrin was the C source. The production of lactic acid indicates saccharolytic activity, but lactic acid is an electron sink and is normally further oxidized to other SCFA by bacteria growing in the medium. The dextran and oligodextran IV fermentations showed high production of butyric acid, reaching levels of 25.39 mmol/l and 21.70 mmol/l in V3 respectively (Table 3).

The slower fermentability of dextran led to a higher persistence of total sugars through V1, allowing 87.5% of the total carbohydrate source to reach V2 (Fig. 5). The amount of oligodextran IV reaching vessel 2 was 34%, followed by 22% in the case of maltodextrin.

## Discussion

The aim of the present work was to generate new information on the *in vitro* utilization of novel oligodextrans produced via membrane reactors (Mountzouris *et al.* 1999), by human gut microflora, for their possible application as functional food ingredients and in particular as prebiotics.



**Fig. 4.** Carbohydrate utilization (%) during batch culture fermentations at 0, 6, 24 and 48 h using different substrates. (◆), Glucose; (●), oligodextran I; (x), oligodextran II; (▲), oligodextran III; (○), dextran; (-■-), fructooligosaccharides. Values are means of triplicate determinations on the same culture.

LAB are desirable microbial groups in the gut. Through the dietary route, both probiotics (Fuller, 1989) and prebiotics (Gibson & Roberfroid, 1995) serve to increase populations of LAB in the large intestine. It would appear that fortification of selected components of the gut flora is nutritionally beneficial. An adjunct to this is the generation

of certain fermentation metabolites, of which butyrate has much current significance (Cummings, 1984; Hague *et al.* 1995; Salminen *et al.* 1998; Wang & Friedman, 1998).

The low-molecular-mass oligodextran I (Table 1 and Fig. 1) showed similar bifidogenic behaviour (Fig. 2) to that of FOS in the batch culture study. FOS displayed its

**Table 2.** Steady-state bacterial counts ( $\log_{10}$  colony-forming units/ml) of selected colonic bacteria in each of the three vessels of a validated three-stage continuous culture system (Macfarlane *et al.* 1998), using dextran, oligodextran IV and maltodextrin as carbohydrate sources

(Mean values and standard deviations for three samples on the same culture)

Bacteria	Vessel 1		Vessel 2		Vessel 3	
	Mean	SD	Mean	SD	Mean	SD
<b>Dextran</b>						
Coliforms	6.89	0.15	6.56	0.03	5.50	0.02
Total aerobes	7.07	0.08	6.94	0.04	6.54	0.05
Total anaerobes	9.40	0.04	9.93	0.07	9.17	0.04
Bifidobacteria	7.37	0.17	7.13	0.10	7.37	0.17
Lactobacilli	7.56	0.12	7.42	0.13	6.98	0.03
Bacteroides	8.87	0.13	9.94	0.03	9.61	0.02
Clostridia	8.44	0.10	9.57	0.02	9.39	0.10
<b>Oligodextran IV</b>						
Coliforms	5.68	0.01	6.41	0.06	6.84	0.14
Total aerobes	7.47	0.03	8.05	0.01	8.23	0.06
Total anaerobes	9.46	0.03	9.61	0.05	10.13	0.02
Bifidobacteria	9.31	0.12	8.67	0.07	8.44	0.06
Lactobacilli	8.85	0.01	8.38	0.12	8.29	0.06
Bacteroides	8.22	0.04	9.35	0.06	9.10	0.08
Clostridia	7.13	0.07	9.23	0.08	8.84	0.06
<b>Maltodextrin</b>						
Coliforms	6.17	0.04	6.99	0.07	6.84	0.09
Total aerobes	6.47	0.08	7.15	0.12	6.94	0.12
Total anaerobes	8.44	0.05	8.68	0.05	8.78	0.02
Bifidobacteria	8.39	0.10	7.65	0.11	6.99	0.13
Lactobacilli	8.38	0.07	8.11	0.10	7.46	0.11
Bacteroides	6.26	0.10	6.02	0.04	5.88	0.06
Clostridia	8.27	0.10	7.40	0.02	7.39	0.02

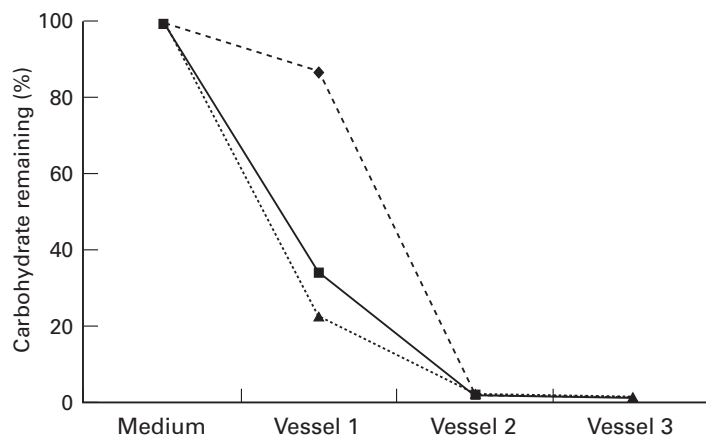
**Table 3.** Steady-state production of short-chain fatty acids (SCFA) in each of the three vessels of a validated three-stage continuous culture system (Macfarlane *et al.* 1998), using dextran, oligodextran IV and maltodextrin as carbohydrate sources  
(Mean values and standard deviations for two determinations on the same culture)

SCFA (mmol/l)	Vessel 1		Vessel 2		Vessel 3	
	Mean	SD	Mean	SD	Mean	SD
<b>Dextran</b>						
Lactic acid	0.00	–	0.00	–	0.00	–
Acetic acid	12.51	0.29	36.63	1.47	39.04	4.8
Propionic acid	4.10	0.08	21.59	0.11	22.28	0.88
Butyric acid	15.69	0.50	23.34	1.20	25.39	4.10
<b>Oligodextran IV</b>						
Lactic acid	0.00	–	0.00	–	0.00	–
Acetic acid	24.14	2.72	35.85	4.95	42.72	1.50
Propionic acid	0.00	–	16.34	0.69	18.63	0.32
Butyric acid	18.00	5.03	19.31	0.92	21.70	0.78
<b>Maltodextrin</b>						
Lactic acid	99.60	7.65	94.47	0.97	12.94	2.26
Acetic acid	7.88	1.78	13.71	0.52	31.58	2.55
Propionic acid	0.00	–	0.00	–	46.69	1.64
Butyric acid	1.37	1.94	3.71	0.50	12.64	0.71

well-known bifidogenic character (Wang & Gibson, 1993; Gibson & Wang, 1994) by stimulating the proliferation of bifidobacteria during the first 6 h of batch culture (Fig. 2), whilst decreasing the counts of other less desirable bacteria (i.e. bacteroides, clostridia). The bifidogenic properties of the low-molecular-mass oligodextran IV (Table 1) were also seen during its fermentation in the more representative three-stage continuous culture system. Bifidobacteria and lactobacilli grew at high numbers (8.3–9.3 log) in all the vessels and their steady-state populations were higher by far (i.e. at least 0.5 log for the lactobacilli and 0.9 log for the bifidobacteria) than the respective ones in the case of dextran and maltodextrin fermentations (Table 2). Overall, the LAB populations seen in the oligodextran IV fermentation reported here were higher (i.e. at least 2.1 log for lactobacilli and 0.5 log for bifidobacteria) than the respective steady-state LAB populations of FOS fermentation in a

study conducted by Gibson & Wang (1994), using a similar three-stage continuous culture system.

In the batch cultures, the low-molecular-mass oligodextran I resulted in higher amounts of bifidobacteria during the early stages of the fermentation compared with dextran and oligodextrans II and III (Fig. 1). In the continuous culture system, dextran steady-state LAB populations were also at least 1 log lower in all the vessels than the respective ones from the low-molecular-mass oligodextran IV (Table 2). The bifidogenic differences between the high-molecular-mass oligodextrans II, III and dextran and the low-molecular-mass oligodextrans (I and IV) could possibly be attributed to the differences in their DP. The effect of DP of the  $\beta$ -2→1 fructans on the inulinase activities has been considered as the reason why FOS has been shown to be the preferred growth substrate by many strains of bifidobacteria compared with glucose, inulin and branched FOS (Gibson & Wang,



**Fig. 5.** Percentage of carbohydrate remaining in each vessel of the three-stage continuous culture system at steady-state, using different substrates. (-◆-), Dextran; (-■-), oligodextran IV; (-▲-), maltodextrin. Values are means of triplicate determinations on the same culture.

1994). In the case of dextranases the extraction of exo- and endo-dextranases from *Bifidobacterium bifidum* has been reported (Sidebotham, 1974). However, the effect of oligodextran DP on the dextranase activities of various colonic genera is unknown and will have to be determined in future studies.

The carbohydrate utilization pattern of the oligodextrans and dextran in the batch cultures was shown to be dependent on their overall molecular mass, with oligodextran I being utilized to a greater extent than dextran (Fig. 4). Similar behaviour was also observed in the three-stage continuous system where dextran persisted through V2 to a greater extent than oligodextran IV (Fig. 5). *In vivo*, this might suggest that higher molecular-mass oligodextrans are more likely to persist through the colon.

In the batch cultures it was seen that clostridia counts were reduced after 6 h but also persisted up to 24 h (Fig. 2) in the cases of oligodextrans II, III and dextran. Use of a validated gut model showed that bacterial counts during dextran and oligodextran IV fermentation gave elevated numbers of clostridia as the pH increased. Clostridia are known not to grow in acidic conditions (Gibson & Roberfroid, 1995), with *Clostridium perfringens* not growing at culture pH values below 5 (Wang & Gibson, 1993). *Clostridium* species were promoted mainly in V2 in dextran and oligodextran IV fermentation, where the pH was higher (i.e. pH 6.2) than in V1 and carbohydrate was still overflowing in high amounts from V1 (Fig. 5). Maltodextrin fermentation was characterized by high numbers of clostridia in V1 which were subsequently reduced by 0.8 log in V2 and V3 (Table 2).

It is clear that gut bacteria cannot be reliably identified through phenotypic procedures (Collins & Gibson, 1999). Hence, the *in vitro* study reported here used a molecular characterization of some predominant fermentative organisms.

Sequencing of the 16S-rRNA genes of random isolates from a growth medium designed to be selective for *Clostridium* species showed that the predominant species recovered was *Clostridium perfringens*. This observation was in good agreement with the results reported by Kohmoto *et al.* (1991) in which increases in the counts of clostridia and especially *C. perfringens* in the faeces of healthy adults were observed following oral administration of commercial IMO. Previously the growth of *C. perfringens* on commercial IMO had been shown by Kohmoto *et al.* (1988).

Butyrate is not a major end-fermentation product of bacteroides, or of the common target probiotic bacteria (i.e. bifidobacteria and lactobacilli), whereas it is for eubacteria and clostridia. Interestingly, butyrate generation in V2 and V3, which correspond to the transverse and distal colons respectively, was enhanced with both the dextran and oligodextran IV (Table 3), indicating a saccharolytic metabolism. As such, clostridia may well have been contributing towards butyrate formation.

More importantly dextran and oligodextran resulted in the production of almost twice the amount of butyrate compared with maltodextrin (Table 3). Butyrate currently attracts more attention than other SCFA as a desirable metabolite of gut bacterial function, through its interaction

with colonocytes (Cummings, 1984). Butyrate is the primary respiratory fuel in the distal colonic mucosa. The epithelial cells of the intestinal surface obtain from its metabolism 60–70% of the energy needed (Scheppach, 1994). Anti-neoplastic properties have also been attributed to butyrate: in cancer-cell studies it prolongs doubling time and reduces the growth rate of human large-bowel cancer cells (Cummings & Macfarlane, 1991; Gamet *et al.* 1992; Scheppach *et al.* 1995). Butyrate has been shown *in vitro* not only to induce apoptosis in a number of colonic tumour cell lines but also to be the most effective inducer of apoptosis compared with propionate and acetate (Hague *et al.* 1995). SCFA mixtures that mimic the digestion products of wheat bran, oat bran and pectin have been shown to induce growth arrest in a human colon carcinoma cell line by induction of the p21 and p27 cyclin-directed kinase inhibitors (Wang & Friedman, 1998). The relative efficiency of these SCFA mixtures in inducing growth arrest was directly proportional to the amount of butyrate present in the mixture (Wang & Friedman, 1998). The data obtained from the gut model system during the oligodextran IV fermentation (Table 3) showed a production of 42.7 mmol/l acetate, 18.7 mmol/l propionate and 21.70 mmol/l butyrate in V3 of the gut model which represents the left colon, the predominant site for onset of colon cancer. In developed countries the colon is the second commonest site of cancer, and this disease is a major source of sickness and mortality. The possible biological properties of butyrate directed towards bowel cancer are of relevance, and it would therefore seem to be desirable to promote its production in the colon.

A dietary component that allows both increased prevalence of LAB, as well as increased butyrate levels would appear to hold much promise as a functional food ingredient. In the present study, it has been observed that the novel low-molecular-mass oligodextran IV promoted the growth of bifidobacteria and lactobacilli while producing butyrate.

The *in vitro* studies described in this paper suggest that oligodextrans have potential as modulatory agents for the gut microflora. The next phase of this research is to scale up production in order to facilitate *in vivo* studies.

### Acknowledgement

The authors would like to thank the European Commission FAIR-CT 96-5022 for funding part of this project.

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