Journal of Applied Bacteriology 1993, **75**, 373–380

Effects of the *in vitro* fermentation of oligofructose and inulin by bacteria growing in the human large intestine

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4461/02/93: accepted 4 May 1993

X. WANG AND G.R. GIBSON. 1993. The *in vitro* fermentability of oligofructose and inulin was compared with a range of reference carbohydrates by measuring bacterial end-product formation in batch culture. Short chain fatty acid and gas formation indicated that these substrates, which occur naturally in the diet and reach the colon in a largely intact form, were utilized by mixed populations of gut bacteria. Bacterial growth data showed that oligofructose and inulin exerted a preferential stimulatory effect on numbers of the health-promoting genus *Bifidobacterium*, whilst maintaining populations of potential pathogens (*Escherichia coli*, *Clostridium*) at relatively low levels. Pure culture studies confirmed the enhanced ability of bifidobacteria to utilize these substrates in comparison with glucose. Batch culture experiments demonstrated that the growth of *Bifidobacterium infantis* had an inhibitory effect towards *E. coli* and *Clostridium perfringens*. Potentially, an increase in the concentration of these substrates in the diet may therefore improve the composition of the large intestinal microflora and have positive effects on the quality of the Western diet.

INTRODUCTION

The human large intestine harbours a nutritionally and physiologically diverse community of bacteria of which species belonging to the genera *Bacteroides*, *Bifidobacterium* and *Eubacterium* are numerically predominant (Moore and Holdeman 1974; Finegold *et al.* 1983). Other quantitatively less important groups include *Lactobacillus*, *Clostridium*, enterobacteria and Gram-positive cocci (Finegold *et al.* 1974). Substrates for the growth of colonic bacteria may be either dietary or endogenous in origin and are derived from proteinaceous or carbohydrate-based material (Macfarlane and Cummings 1991). Substrate utilization in the large gut involves a series of related bacterial interactions with the formation of various end-products. The type of fermentation product formed is thought to be related to the amount and form of substrate available, the metabolic pathways of the bacteria involved and the extent of breakdown (Macfarlane 1991).

Bacteria in the colon may be separated into two broad-based categories. For example, genera such as *Bifidobacterium* and *Lactobacillus* are often considered salutary micro-organisms with respect to human health. These bacteria are thought to create conditions unfavourable for the growth of potentially pathogenic species (Tamura 1983). Conversely, many enterobacteria and clostridia can be undesirable micro-organisms. This event may occur either by invasive action or the production of toxic metabolites (Balows *et al.* 1991). Stimulation of growth of the former group and suppression of the latter is of obvious benefit to the host, and there is currently some interest in the addition to the diet of health-promoting substrates. Recent studies, largely by Japanese workers, have indicated that some oligofructoses may preferentially stimulate selected populations of colonic bacteria in mixed faecal culture (Okada *et al.* 1984; Hidaka *et al.* 1986; Mitsuoka *et al.* 1987).

Inulin is a naturally-occurring storage oligomer of fructose found in many plants, such as onion, garlic, artichoke and chicory (Yazawa *et al.* 1978). The sugar consists of a polymer of D-fructose linked by β (2-1) bonds with an α (1-2) linked D-glucose at the terminal end of the molecule (Yazawa and Tamura 1982). The degree of polymerization (DP) of inulin may range from 2 to 60 with oligofructose defined as the fraction of inulin with a DP below 20. These substances have been shown to be resistant to the degradative properties of the gastrointestinal tract and reach the colon in a largely intact form (Rumessen *et al.* 1990).

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Therein, they may serve as growth substrates for the gut microflora.

Little is known about the fermentation of oligofructose or inulin, particularly by faecal micro-organisms from Western populations. In this study we therefore compared the utilization of these substrates and other carbohydrates by colonic bacteria *in vitro*. The experiments were carried out in batch culture fermenters inoculated with mixed bacteria as well as defined pure cultures. Potential consequences for the host were addressed, in particular effects on the growth of selected genera of gut bacteria.

MATERIALS AND METHODS

Growth substrates

Oligofructose and inulin were extracted from chicory and supplied by Raffinerie Tirlemontoise (Tienen, Belgium). Inulin was 94% pure with fructose (1%), glucose (0·1%) and sucrose (5%) as impurities. Oligofructose was 98% pure with fructose (0·9%), glucose (<0·1%) and sucrose (0·9%) as contaminating substances. All other carbohydrates and chemicals used in the experiments were obtained from Sigma unless otherwise stated.

Bacteria

Bifidobacterium infantis (NCFB 2205), *B. catenulatum* (NCFB 2246), *B. longum* (NCFB 2259), *B. pseudolongum* (NCFB 2244), *B. breve* (NCFB 2257), *B. angulatum* (NCFB 2238), *B. adolescentis* (NCFB 2230), *B. bifidum* (NCFB 2203) and *Clostridium perfringens* (NCFB 8427) were obtained from the National Collection of Food Bacteria (Reading, UK).

Fermentation experiments

Batch culture fermenters (70 ml working volume) were inoculated with 5% (w/v) faecal slurries and 0·7% (w/v) carbohydrate added. The fermenters were incubated at 37°C for 48 h and samples for gas (1 ml) and liquid (2 ml) analyses were removed after 0, 3, 6, 9, 12, 24 and 48 h. Slurries were prepared by homogenizing fresh faeces in anaerobic sodium phosphate buffer (0·1 mol l^{-1}; pH 7·0). The experiments were carried out with triplicate specimens from each of six healthy volunteers (age 24–45) who had no preceding history of gastrointestinal disorder and had not been prescribed antibiotics for at least 3 months prior to the study. Before incubation the fermenters containing the slurries were gassed out with high purity argon for 10 min, leaving a slight positive pressure of gas. Substrates used in the experiments were oligofructose, inulin, glucose, arabinose, galactose, fructose, lactose, sucrose, lactulose, cellobiose, sorbitol, lactitol, Lintner's starch (BDH), polydextrose (Pfizer), pectin, maltitol and arabinogalactan. Slurries containing no additions served as controls.

Fermentation product analysis

Hydrogen, methane and carbon dioxide concentrations in gas headspaces overlying the slurries were determined by the gas chromatographic procedures of Allison and Macfarlane (1988). Acetate, propionate, butyrate, lactate and succinate were extracted and measured as described by Holdeman *et al.* (1977). Triplicate samples were used for each determination.

Growth of mixed faecal bacteria

Faecal slurries were prepared from three volunteers as previously described and added to 280 ml volume batch culture fermenters. Substrates (oligofructose, inulin, glucose, sucrose, polydextrose, starch) were added separately to give a final concentration of 0·7% (w/v). The fermenters were continually stirred, sparged with oxygen-free nitrogen and maintained at a pH of 7·0 and temperature of 37°C. Liquid samples (1 ml) were removed from each fermenter after 0, 6, 9, 12 and 24 h and serially diluted in an anaerobic cabinet (10 : 10 : 80; H_2 : CO_2 : N_2 atmosphere) with half-strength Wilkins Chalgren Anaerobic Broth (Oxoid). Triplicate plates were then inoculated with 0·1 ml samples and incubated aerobically or anaerobically as appropriate, at 37°C. Bacteria were enumerated on Wilkins Chalgren Agar (Oxoid; total anaerobes), Nutrient Agar (Oxoid; total aerobes), Bacteroides mineral salts medium (Macfarlane *et al.* 1989; *B. fragilis* group), MRS Agar (Oxoid; lactobacilli), Reinforced Clostridial Agar plus supplements (Munoa and Pares 1988; bifidobacteria), Sulphite-Polymyxin Milk Agar (Mevissen-Verhage *et al.* 1987; clostridia), MacConkey's No. 2 (Oxoid; coliforms) and Azide Blood Agar Base (Oxoid; Gram-positive cocci). Single colonies were removed from selective media plates and grown in peptone yeast glucose (PYG) broth (Holdeman *et al.* 1977). Subsequently, the bacteria were characterized to genus level on the basis of colonial appearance, Gram reaction, spore production, cell morphology and fermentation end-product formation (Holdeman *et al.*

1977). Differences in bacterial numbers after 0 and 12 h incubation with the various substrates were subjected to statistical analysis with a paired *t*-test.

Growth of selected bacteria utilizing oligofructose

Active cultures (0·5 ml) of *B. infantis*, *B. catenulatum*, *B. longum*, *B. pseudolongum*, *B. breve*, *B. angulatum*, *B. adolescentis*, *B. bifidum*, *Escherichia coli* and *Cl. perfringens* were added to PYG broth, in triplicate, with either oligofructose or glucose (0·5% w/v) as the sole source of carbon and energy. Specific growth rates of the bacteria were measured in 25 ml serum bottles by monitoring $O.D._{650}$ changes as described by Pirt (1985). The pH of the fermenters was maintained at 7·0 throughout the experiment.

Competition experiments

Batch culture fermenters (280 ml) were inoculated with actively growing cultures of *B. infantis*, *E. coli* and *Cl. perfringens*. The growth medium was PYG broth with either oligofructose or glucose as the carbon source (0·5% w/v). Samples were removed periodically up to an incubation time of 60 h to determine viable counts. The selective media described above were used and single colonies removed for further identification. The initial pH of the fermenters was 7·0 and was not controlled during the experiment which was performed in triplicate.

Effect of pH on bacterial growth

The ability of *B. infantis*, *E. coli* and *Cl. perfringens* to withstand the effects of low pH was compared by inoculation of glucose-containing growth media as described earlier. Triplicate media were adjusted to a range of pH values (3·5–7·0) and specific growth rates determined.

RESULTS

Carbohydrate fermentation

Gas and short chain fatty acid production was measured in faecal slurries with various carbohydrates added as bacterial growth substrates. All substrates tested were utilized to some extent as evidenced by a formation of fermentation end-products during the incubation period. Production patterns of CO_2, CH_4, acetate, propionate, butyrate, lactate and succinate were similar for each of the carbohydrates during the incubations but the relative rates and amounts formed varied depending on the individual substrates tested.

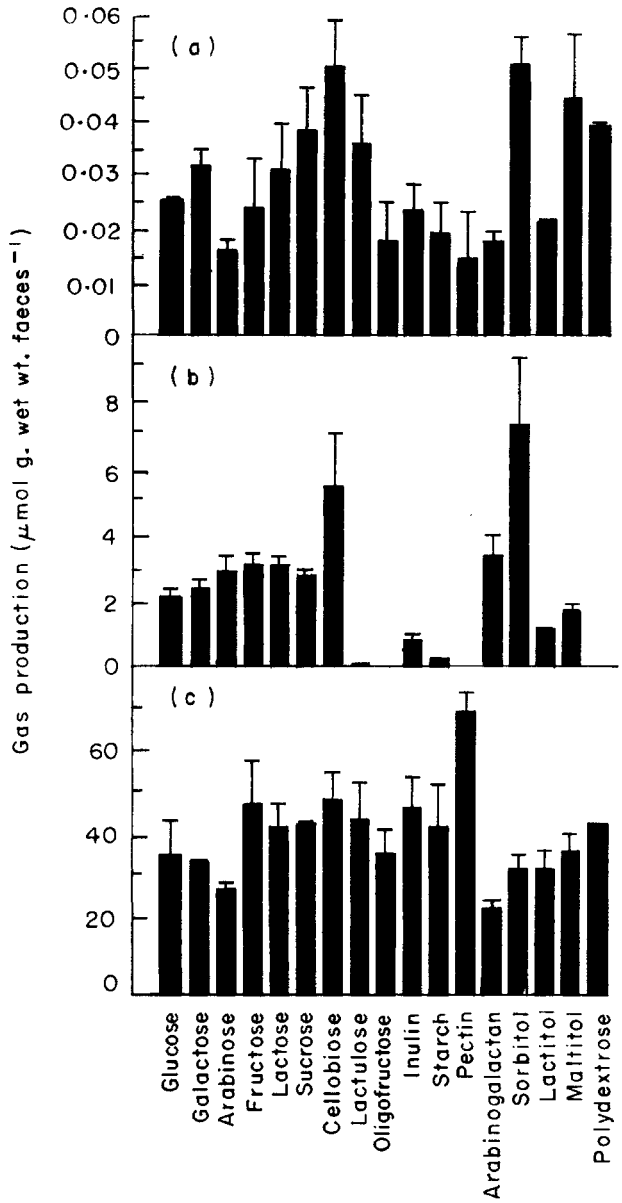

Fig. 1 Gas production after 24 h incubation of faecal slurries (5% w/v) containing various carbohydrates as growth substrates (0·7% w/v). (a) H_2; (b) CH_4; (c) CO_2. Results are mean values of triplicate incubations from six separate volunteers. Error bars show S.E.M. values

Gas production results showed that cellobiose, polydextrose and sucrose produced high concentrations of H_2, as did the sugar alcohols sorbitol and maltitol (Fig. 1a). However, H_2 contributed only a minor proportion of the fermentation end-products in these experiments. Methane production was highest from sorbitol and cellobiose, whilst the highest CO_2 accumulation occurred when pectin was used as growth substrate (Fig. 1b,c).

The highest volatile fatty acid (VFA) production was from lactulose, lactose, maltitol and galactose, with low amounts from cellobiose and arabinogalactan fermentations (Fig. 2). The results in Table 1 show molar ratios of VFA at the end of the incubation period and demonstrate that starch was a good substrate for butyrate production, whilst lactose, lactulose, lactitol, sucrose, galactose and oligofructose produced relatively higher proportions of acetate. Lactate and succinate are important intermediates in carbohydrate fermentation (Macfarlane 1991). All the substrates tested caused an accumulation of succinate, to varying extents, whilst lactate was highest when oligofructose, lactulose or lactose were added to the fermentation system (Fig. 3). A complete disappearance of oligofructose or inulin was observed after 9 h incubation (results not shown).

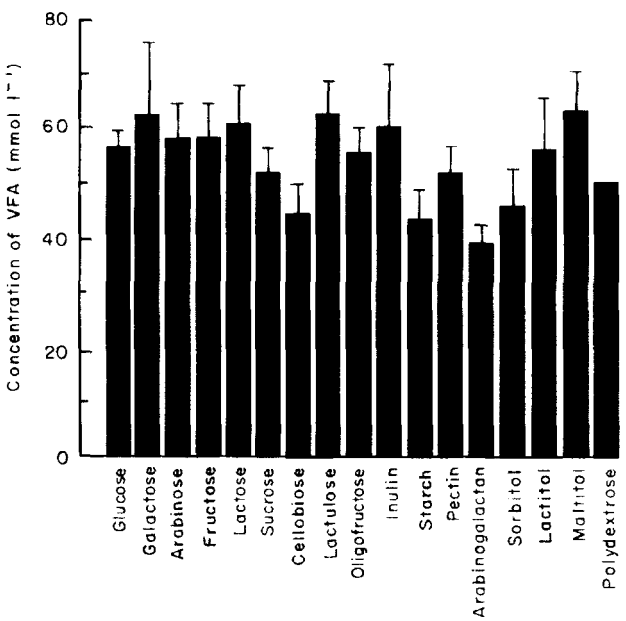

Fig. 2 Total volatile fatty acid (VFA) production after 24 h incubation of faecal slurries (5% w/v) containing various carbohydrates as growth substrates (0·7% w/v). Results are mean values of triplicate incubations from six separate volunteers. Error bars show S.E.M. values

Table 1 Molar ratios of acetate, propionate and butyrate produced from carbohydrate fermentation by slurries of mixed human faecal bacteria*

Substrate	Acetate	Propionate	Butyrate
Inulin	72 ± 6	19 ± 5	8 ± 2
Oligofructose	78 ± 4	14 ± 2	8 ± 2
Glucose	70 ± 6	15 ± 4	5 ± 4
Fructose	67 ± 5	20 ± 4	13 ± 3
Sucrose	82 ± 2	10 ± 2	8 ± 1
Galactose	76 ± 6	15 ± 3	9 ± 3
Arabinose	59 ± 3	31 ± 3	9 ± 2
Lactose	80 ± 7	13 ± 4	7 ± 3
Lactulose	81 ± 5	12 ± 4	7 ± 2
Cellobiose	69 ± 3	19 ± 1	12 ± 3
Sorbitol	61 ± 6	23 ± 5	16 ± 6
Lactitol	85 ± 4	9 ± 2	6 ± 2
Starch	58 ± 6	17 ± 3	25 ± 3
Polydextrose	61 ± 3	25 ± 3	14 ± 1
Pectin	75 ± 2	16 ± 1	10 ± 1
Arabinogalactan	68 ± 1	24 ± 2	8 ± 1
Maltitol	57 ± 6	26 ± 6	17 ± 1

* Slurries were prepared with a 5% (w/v) concentration of faeces with 0·7% (w/v) carbohydrate added (results show mean values ± S.E.M. of triplicate determinations from six volunteers).

Enumeration of selected bacterial genera utilizing carbohydrates

Viable populations of bacteria increased during the initial 12 h of carbohydrate fermentation (Table 2). This effect was most marked for bifidobacteria when oligofructose and inulin were used as growth substrates, although not to a significant extent ($P = 0·05$). In contrast, the other carbo-

Table 2 Growth of selected populations of colonic bacteria in batch culture fermenters with various carbohydrates added*

	Log_{10} bacterial numbers (g wet weight faeces)⁻¹											
	Pectin		Fructose		Polydextrose		Starch		Oligofructose		Inulin	
	0 h	12 h	0 h	12 h	0 h	12 h	0 h	12 h	0 h	12 h	0 h	12 h
Total aerobes	7·4 ± 0·7	8·5 ± 0·9	7·5 ± 0·9	8·8 ± 0·9	7·4 ± 0·9	8·4 ± 0·9	7·3 ± 0·7	8·0 ± 0·7	7·7 ± 0·2	8·4 ± 0·5	8·3 ± 0·5	8·3 ± 0·5
Total anaerobes	10·4 ± 0·5	11·8 ± 0·9	10·5 ± 1·3	11·7 ± 1·5	10·4 ± 0·6	11·5 ± 0·6	10·7 ± 0·6	12·0 ± 0·9	10·8 ± 0·3	11·8 ± 0·7	10·4 ± 0·5	12·1 ± 0·7
Bacteroides	9·5 ± 0·4	10·2 ± 0·7	9·7 ± 0·6	10·4 ± 0·8	9·4 ± 0·8	10·8 ± 0·9	9·5 ± 0·4	10·4 ± 1·0	9·8 ± 0·5	10·1 ± 0·9	10·0 ± 0·7	10·3 ± 0·4
Bifidobacteria	9·7 ± 0·3	10·5 ± 0·8	10·1 ± 0·7	10·6 ± 0·9	9·7 ± 0·7	10·5 ± 0·8	10·0 ± 0·6	10·4 ± 0·6	10·1 ± 0·7	11·5 ± 0·6	9·8 ± 0·4	11·1 ± 0·5
Clostridia	8·5 ± 1·4	8·6 ± 0·7	8·6 ± 0·9	9·1 ± 0·7	8·5 ± 1·0	10·5 ± 1·3	8·5 ± 1·0	8·5 ± 0·8	8·1 ± 0·7	8·2 ± 0·8	8·4 ± 0·8	8·6 ± 0·7
Coliforms	6·1 ± 0·9	6·3 ± 1·7	6·3 ± 0·4	7·9 ± 1·0	6·1 ± 1·0	8·3 ± 1·7	6·1 ± 0·8	8·0 ± 0·3	6·8 ± 0·8	7·1 ± 1·0	4·9 ± 0·8	5·5 ± 0·8
Lactobacilli	8·6 ± 0·8	9·0 ± 0·7	8·7 ± 0·9	9·1 ± 0·8	8·5 ± 1·0	9·0 ± 1·5	8·5 ± 1·0	9·5 ± 1·0	8·7 ± 0·9	9·1 ± 0·8	6·5 ± 0·7	6·5 ± 0·6
Gram + ve cocci	7·2 ± 1·3	7·9 ± 0·9	7·2 ± 1·0	7·1 ± 1·0	7·2 ± 1·0	7·4 ± 1·4	7·2 ± 1·0	7·2 ± 1·0	6·6 ± 0·9	7·5 ± 0·9	6·7 ± 0·4	7·3 ± 0·8

* Substrates were added to give a final concentration of 0·7% (w/v). The fermenters were inoculated with mixed faecal bacteria (5% w/v). Results are mean values ± S.E.M. of triplicate determinations for each of three volunteers.

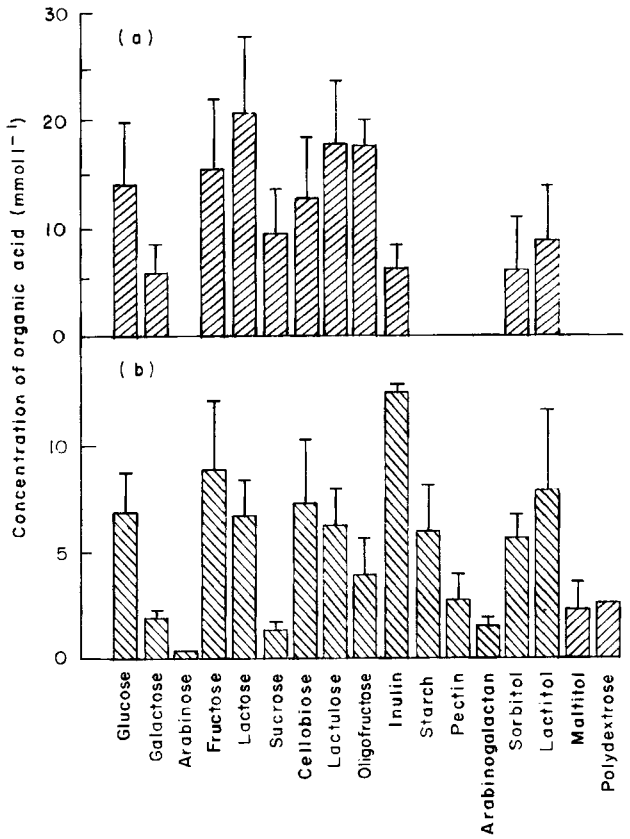

Fig. 3 Lactate and succinate production after 24 h incubation of faecal slurries (5% w/v) containing various carbohydrates as growth substrates (0·7% w/v). (a) Lactate; (b) succinate. Results are mean values of triplicate incubations from six separate volunteers. Error bars show S.E.M. values

hydrates tested exerted a more general effect upon bacterial genera, in that an increase occurred for the majority of groups enumerated.

Comparative bacterial growth with oligofructose as the substrate

To confirm the enhanced ability of bifidobacteria to utilize oligofructose, PYG broth was separately inoculated with eight species and growth compared with that of *E. coli* and *Cl. perfringens*. Oligofructose or glucose provided the sole source of carbon and energy in these fermenters. Table 3 shows that all the strains of bifidobacteria grew well in oligofructose-containing media. In comparison, growth on glucose (apart from *B. longum*) was somewhat lower. The highest specific growth rates were recorded with *B. pseudolongum*, *B. infantis* and *B. catenulatum*, whilst the lowest was with *B. adolescentis*. In contrast, *E. coli* and *Cl. perfringens* both grew better when glucose was used as a source of carbon and energy, in comparison with oligofructose.

Table 3 Growth rates of selected pure cultures of colonic bacteria utilizing oligofructose or glucose as carbon and energy sources*

	Specific growth rate (h^{-1})	
Bacterium	Oligofructose	Glucose
Bifidobacterium infantis	0·297 ± 0·028	0·173 ± 0·043
adolescentis	0·160 ± 0·001	0·124 ± 0·042
catenulatum	0·270 ± 0·039	0·201 ± 0·064
bifidum	0·183 ± 0·024	0·180 ± 0·010
pseudolongum	0·303 ± 0·035	0·243 ± 0·029
breve	0·204 ± 0·050	0·132 ± 0·042
angulatum	0·261 ± 0·020	0·218 ± 0·012
longum	0·208 ± 0·022	0·215 ± 0·084
Escherichia coli	0·212 ± 0·010	0·242 ± 0·011
Clostridium perfringens	0·216 ± 0·021	0·259 ± 0·022

* Bacteria were grown in batch culture with 0·5% (w/v) substrate added. Results are means of triplicate incubations ± S.E.M.

Competition experiments

Batch culture fermenters were inoculated with pure cultures of *B. infantis*, *E. coli* and *Cl. perfringens*. Prior to inoculation the bacteria were grown in overnight cultures to give approximately equal cell densities. To establish the relative abilities of these bacteria to compete for nutrients the systems were operated for 60 h with either oligofructose or glucose as the carbon source. In the initial stages of oligofructose fermentation (0–14 h), bacterial counts of all three species were high (approximately 10^{7}–10^{11} ml^{-1} growth medium, Fig. 4a). The culture pH fell in this time from 7·0 to approximately 5·5 and this was followed by a decline in viable counts of *E. coli* and *Cl. perfringens* to reach zero after 36 h (*E. coli*) or 52 h (*Cl. perfringens*) incubation. Populations of *B. infantis* remained relatively unaffected during this time.

In the glucose-containing fermenters, populations of each of the three bacteria were again high until 14 h incubation (Fig. 4b). Subsequently, numbers of *E. coli* and *Cl. perfringens* decreased, albeit at a lower rate than in the oligofructose fermentations. Fermentation end-product analysis showed that acetate and lactate were produced in relatively high amounts particularly after 14 h incubation (Fig. 5a,b).

Effect of pH of bacterial growth

Table 4 shows that the specific growth rates of *B. infantis*, *E. coli* and *Cl. perfringens* were approximately equal at neutral pH values. When the pH was lower, however, growth of the bifidobacterium remained relatively unaffected. The ability of *B. infantis* to withstand the effects of acidic conditions was demonstrated at pH 5·0 and 4·5,

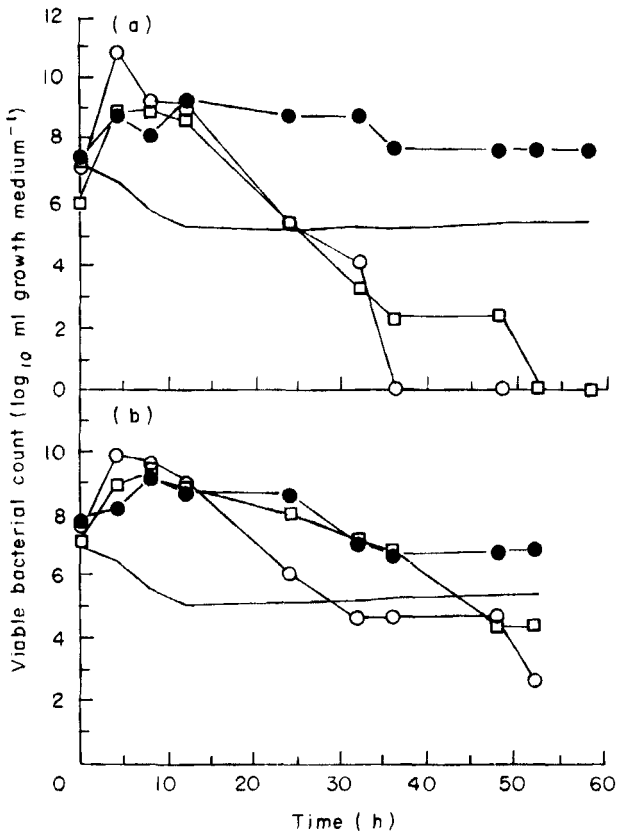

Fig. 4 Growth of: ●, *Bifidobacterium infantis*, ○, *Escherichia coli* and □, *Clostridium perfringens* in batch culture with oligofructose or glucose as growth substrate. (a) Oligofructose; (b) glucose. Results are mean values of triplicate determinations. —, pH

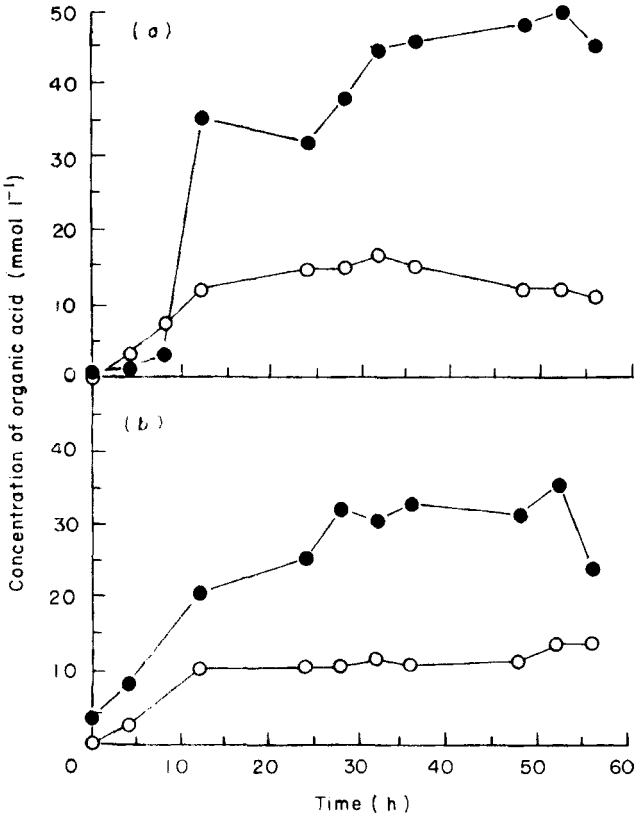

Fig. 5 ●, Acetate and ○, lactate production from a mixed batch culture of *Bifidobacterium infantis*, *Escherichia coli* and *Clostridium perfringens* with oligofructose or glucose as growth substrate. (a) Oligofructose; (b) glucose. Results are mean values of triplicate determinations

Table 4 Effect of culture pH on the growth of *Bifidobacterium infantis*, *Escherichia coli* and *Clostridium perfringens**

	Specific growth rate (h^{-1})		
pH	*B. infantis*	*E. coli*	*Cl. perfringens*
7·0	0·170 ± 0·007	0·199 ± 0·007	0·169 ± 0·008
6·5	0·211 ± 0·008	0·185 ± 0·009	0·163 ± 0·008
6·0	0·206 ± 0·008	0·164 ± 0·009	0·143 ± 0·019
5·7	0·175 ± 0·013	0·111 ± 0·017	0·149 ± 0·011
5·5	0·167 ± 0·006	0·105 ± 0·004	0·126 ± 0·013
5·2	0·127 ± 0·006	0·053 ± 0·001	0·125 ± 0·023
5·0	0·082 ± 0·006	NG	NG
4·5	0·016 ± 0·001	NG	NG
4·0	NG	NG	NG
3·5	NG	NG	NG

* Bacteria were grown in batch culture with 0·5% (w/v) substrate (glucose) added. Results are means of triplicate determinations ± S.E.M.
NG, No growth.

which completely inhibited the growth of *E. coli* and *Cl. perfringens*.

DISCUSSION

Fermentation of carbohydrates by colonic bacteria mainly produces short chain fatty acids and gases (Macfarlane and Cummings 1991). Short chain fatty acids are partly absorbed by the colonic epithelium (Cummings and Macfarlane 1991) and gases may be expelled in breath or flatus (Levitt and Ingelfinger 1968) or, in the case of hydrogen, further utilized by terminal oxidizers such as sulphate-reducing (Gibson *et al.* 1988) and methanogenic bacteria (Miller *et al.* 1982). The principal dietary carbohydrates available for fermentation are starch, non-starch polysaccharides (NSP, dietary fibre), sugar alcohols, other unabsorbed sugars, synthetic carbohydrates, food additives and oligosaccharides (Cummings and Macfarlane 1991). The positive effects on health of resistance starch and NSP fermentation have been recognized for some years. For example, starch fermentation *in vitro* produces a relatively

high concentration of butyrate which may act as a respiratory fuel for colonocytes and a regulator of cell growth (Leder and Leder 1975; Kim *et al.* 1982). An increase in the amount of dietary fibre reaching the large gut leads to improved bowel habit and increased stool output (Cummings 1986). In contrast, however, the possible beneficial effects of the fermentation of other dietary carbohydrates has not received much attention. In Japan, certain oligosaccharides are commonly added to the diet for health purposes. In the West this course has not been followed.

A number of studies have indicated that oligofructose and inulin are not degraded in the upper gastrointestinal tract and therefore reach the colon in an intact form (Nilsson *et al.*, 1988; Barwald *et al.* 1989; Rumessen *et al.* 1990). These substances cannot be detected in faecal samples, however, even after inulin feeding (Heupke and Blanckenburg 1934), indicating that they are fermented by large intestinal bacteria. The *in vitro* results shown in Figs 1–3 confirm this. Both oligofructose and inulin were utilized by mixed cultures of faecal bacteria from each of the six volunteers tested. From the nutritional viewpoint, this fermentation may offer a number of advantages. For instance, stool bulking, higher production and absorption of short chain fatty acids and a decrease in intestinal transit time should all occur. Of importance, the composition and activities of bacteria involved in their metabolism will be of significance to the host.

The enhanced capabilities of bifidobacteria to grow on oligofructose in comparison with other carbohydrates are shown in Table 2. The other substrates tested exerted a much less specific effect in that an increase in numbers of many of the bacterial genera tested occurred. With oligofructose a high proportion of the total anaerobic bacterial count was due to growth of bifidobacteria. These results seem to confirm observations made by Japanese workers on the bifidogenic effects of fructo-oligosaccharides and inulin (Yazawa *et al.* 1978; Yazawa and Tamura 1982; Okada *et al.* 1984; Hidaka *et al.* 1986; Mitsuoka *et al.* 1987).

Populations of bifidobacteria can represent up to 95% of the total gut flora in breast-fed infants, in comparison with about 25% in the adult. It is thought that such a proliferation of these micro-organisms contributes to the purported health advantages of breast-fed compared with bottle-fed infants. Although bifidobacteria have been implicated in a number of opportunistic anaerobic infections (Miller-Catchpole 1989), these bacteria are generally regarded as beneficial for host health. There are a number of potential reasons for this: (i) they are recognized as being immunomodulators, for example, *B. infantis* antigens have been used to promote immunological attack against malignant cells (Miller-Catchpole 1989); (ii) bifidobacteria can produce a number of vitamins (B_1, B_6, B_{12}, folic acid), digestive enzymes and lysozyme (Tamura 1983), (iii) they have also been used to restore the normal intestinal flora during antibiotic therapy (Miller-Catchpole 1989); (iv) more importantly, the major end-products of the bifidus pathway are acetic and lactic acids (Bezkorovainy 1989). A lowering of gastrointestinal pH may cause binding of potentially toxic NH_3 with hydrogen to produce NH_4^+ which is non-diffusible and therefore has the effect of lowering blood ammonia levels. Also, these strong acids may have a detrimental effect on the growth of other colonic bacteria, including pathogenic species. Figure 4 shows that numbers of *E. coli* and *Cl. perfringens* can be lowered by incubation in defined mixed culture with *B. infantis*, particularly when oligofructose was used as a source of carbon and energy. During this time acetate and lactate were excreted into the culture medium (Fig. 5) which may have the effect of suppressing *E. coli* and *Cl. perfringens* growth.

Results in this study suggest that the addition of oligofructose or inulin to the diet may cause an improvement in the composition of the gut microflora. This may arise because of a stimulation of bifidobacterial numbers, in comparison with other bacterial genera. Polydextrose is a synthetic polymer, consisting of glucose, sorbitol and citric acid, which is also used as a food additive. Our results show that although polydextrose is fermented, its effect on bacterial numbers was somewhat more general and probably therefore less advantageous than oligofructose (Table 2). Inulin and oligofructose have the additional property of being naturally present in plants and vegetables.

Oligofructose and inulin are not hydrolysed during passage to the colon, thus their caloric value is likely to be reduced. Other studies have shown that the feeding of oligofructose to rats causes a significant decrease in serum triglycerides, blood cholesterol and total lipids (Hata *et al.* 1982; Tadeka and Niizato 1982; Tokunaga *et al.* 1986). From the initial *in vitro* results shown in this paper it would also seem that an increase in the concentration of fructose-based oligosaccharides in the diet may alter the gut microflora in such a manner that numbers of bifidobacteria may be selectively stimulated. This may potentially have further positive implications for host welfare.

ACKNOWLEDGEMENTS

The authors are very grateful to Raffinerie Tirlemontoise, Tienen, Belgium for supporting this study.

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