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Carbohydrate Preferences of *Bifidobacterium* Species Isolated from the Human Gut

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Abstract

The growth of nine species of *Bifidobacterium* on media containing glucose, xylose, xylo-oligosaccharides (XOS), xylan or fructo-oligosaccharides (FOS) as the sole carbon source were compared in pure culture. The bifidobacteria differed in fermentation profiles when tested on different carbohydrates. All species grew to their highest final optical density (OD) on a glucose containing medium, with the exception of *B. catenulatum* which demonstrated a preference for xylose over glucose, and XOS over FOS. *B. bifidum* grew to the highest OD on XOS compared to xylose suggesting a specific transport system for the oligosaccharide over the monomer. This is consistent with a lack of β-xylosidase activity present in the culture medium. Lactate, formate and acetate levels were determined and the ratios of these metabolites altered between and within species growing on different carbohydrates. In general, high lactate production correlated with low formate production and low lactate concentrations were obtained at higher levels of formate. Bifidobacteria may alter their metabolic pathways based upon the carbohydrates that are available for their use.

Introduction

Prebiotics such as fructooligosaccharides (Gibson, 1999) and xylooligosaccharides (Vazquez *et al.,* 2000) have been shown to elicit a prebiotic effect both *in vitro* and *in vivo*. Prebiotics are defined as "non-digestible food ingredients that stimulate the growth and/or the activity of one or a limited number of bacteria in the colon, thereby improving the host health" (Gibson and Roberfroid, 1995).

Prebiotics selectively stimulate the growth of commensal probiotic bacteria in the colon due to their fermentation profiles. Species from the genus *Bifidobacterium* are the main target for these prebiotics and numerous clinical trials have demonstrated the bifidogenic nature of prebiotics (Gibson *et al.,* 1995). Bifidobacteria differ from other colonic genera in their fermentation of carbohydrates. They lack the enzymes aldolase and glucose-6-phosphate $NADP^+$ oxidoreductase (De Vriers, 1967) and are therefore unable to carry out the usual glycolysis pathway or the hexose monophosphate shunt pathway. The bifidus pathway depends on the presence of fructose-6-phosphate phosphoketolase (F6PPK) (Fandi *et al.,* 2001). The formation of acetate and lactate as end products of this pathway are significant in terms of the health benefits of probiotics. In some species of bifidobacteria however, pyruvate is converted into formic acid and ethanol, yielding an extra ATP from 1 mole of glucose The presence of the Bifidus pathway allows bifidobacteria to produce more ATP from carbohydrates than conventional hetero and homofermentative pathways. The bifidus pathway yields 2.5 ATP from 1 mole of glucose, as well as theoretically 1.5 moles of acetate and 1 mole lactate. These short chain fatty acids play a part in the purported health promoting properties associated with prebiotics. It is known that acetate produced in the gut is transported to the liver where it is utilised in the formation of ATP. Lactate is a known to possess anti-microbial activity which is active against a number of potentially pathogenic bacteria (Roberfroid *et al.,* 1995).

In order to develop new and improved prebiotic carbohydrates, it is imperative that we fully understand the fermentation pathways and uptake mechanisms that operate in the target organisms *i.e.* bifidobacteria and lactobacilli.

In this study, the fermentation of a number of carbohydrates by nine bifidobacteria species was examined. The carbohydrates tested were xylose, XOS and xylan with FOS and glucose as controls. These carbohydrates were chosen because the fermentation of pentose sugars *i.e.* xylose, are poorly understood in *Bifidobacterium Spp.* As xylooligosaccharides are emerging as prebiotics the understanding of their fermentation by bifidobacteria is of importance. XOS have a structure of $[\beta\text{-Xyl-}(1\text{-}4)\text{-}]_n$, with a chain length between 2 and 9, with xylan having the same structure but a higher molecular weight.

The highest optical density (OD) was used to determine the carbohydrate preferences of each species tested. In order to examine the biochemical activities of the species, high performance liquid chromatography (HPLC) analysis of acetate, lactate and formate concentrations were also carried out. The xylosidase activity of the species able to ferment XOS was also analysed using a β-xylosidase assay, this generated information about the location of xylose and XOS transport mechanisms within each species.

Materials and Methods

Bacterial Growth Media

Bacteria were grown on basal medium containing (g/l): peptone water (Oxoid Ltd. Basingstoke, UK) 2, yeast extract

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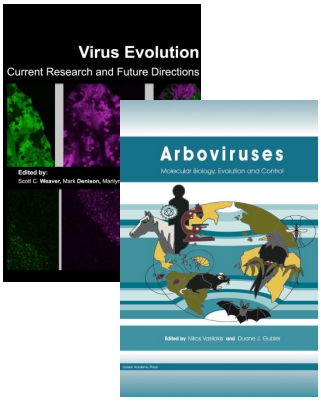

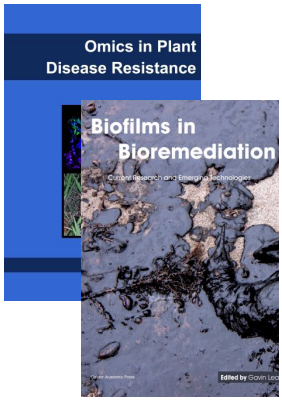

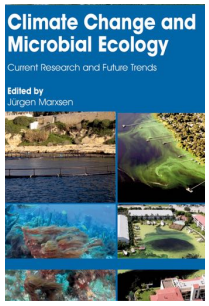

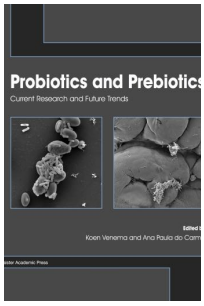

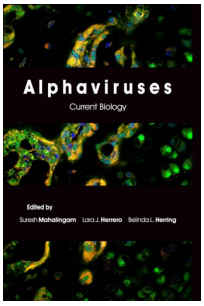

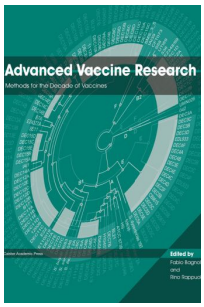

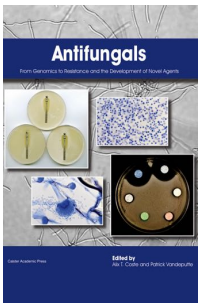

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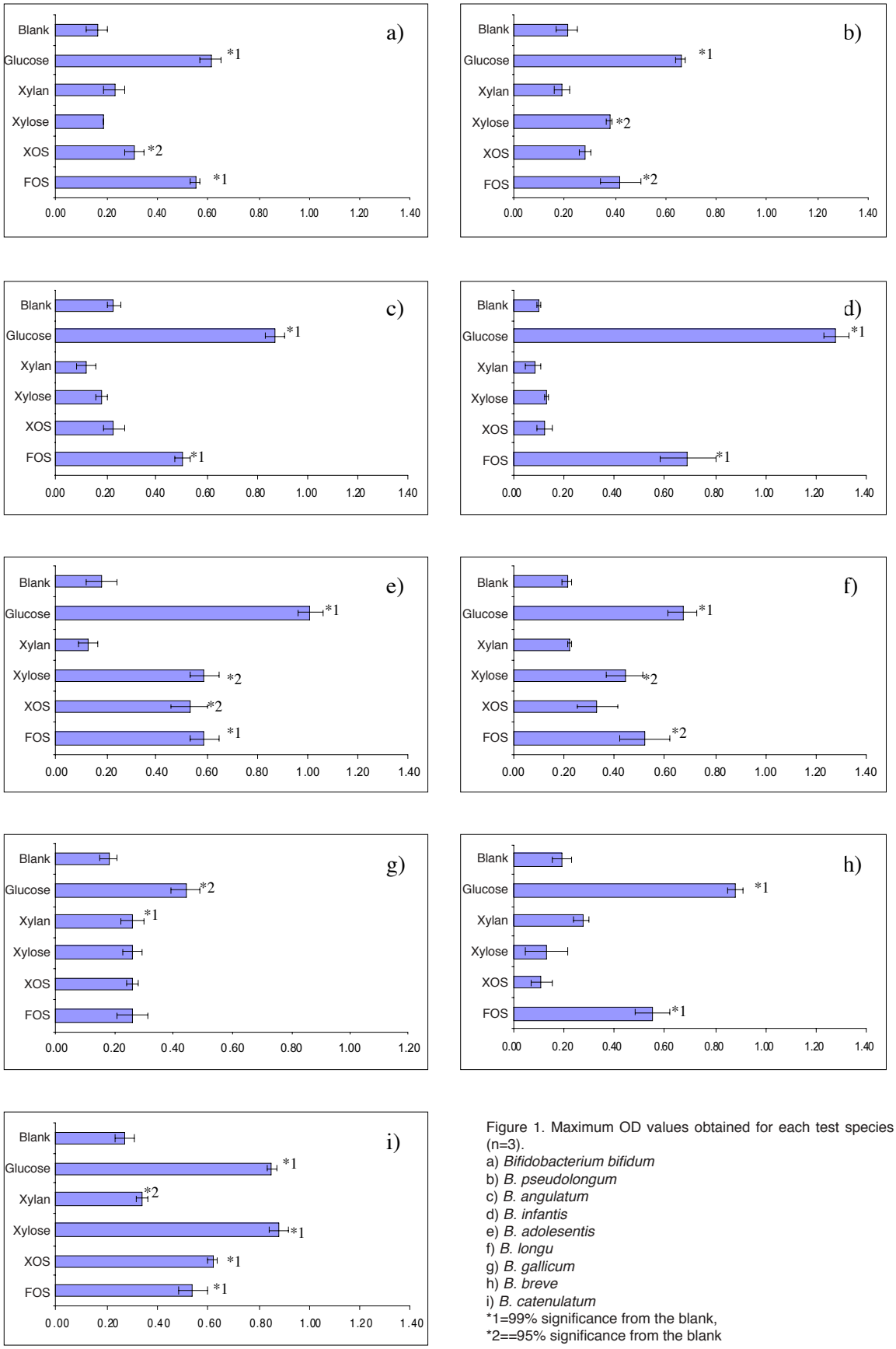

Figure 1. Maximum OD values obtained for each test species (n=3).
a) *Bifidobacterium bifidum*
b) *B. pseudolongum*
c) *B. angulatum*
d) *B. infantis*
e) *B. adolesentis*
f) *B. longu*
g) *B. gallicum*
h) *B. breve*
i) *B. catenulatum*
*1=99% significance from the blank,
*2==95% significance from the blank

(Oxoid) 2; NaCl, 0.1; K_2HPO_4, 0.04; KH_2PO, 0.04; $MgSO_4.7H_2O$, 0.01; $CaCl_2.6H_2O$, 0.01; $NaHCO_3$ 2, haemin (Sigma-Aldrich, Poole, UK) 0.005; L-cysteine HCl (Sigma) 0.5, bile salts (Oxoid) 0.5. This medium was supplemented with Tween 80 (2 ml/L), vitamin K_1 (Sigma) (10μl/L), resazurin (0.001g) and (1% w/v) test carbohydrate (filter sterilised and added after autoclaving). Appropriate additions were made, the medium boiled to dissolve components and then placed into an anaerobic cabinet (10:10:80 carbon dioxide: hydrogen: nitrogen, Don Whitley UK) and allowed to cool. Once cooled it was dispensed in 9ml volumes to Hungate tubes, the tubes were sealed in the cabinet in order to maintain anaerobisity. The Hungate tubes were then autoclaved at 121°C for 20 minutes, once cooled carbohydrates were filtered into added to the media to make an over all concentration of 1% (w/v).

The bacteria used in this study were *Bifidobacterium adolescentis* (DSM 20083), *B. angulatum* (DSM 20098), *B. bifidum* (DSM 20456), *B. breve* (DSM 20213), *B. catenulatum* (DSM 20103), *B. gallicum* (DSM 20093), *B. infantis* (DSM 20088), *B. longum* (DSM 20219) and *B. pseudolongum* (DSM 20099). These strains were used, as they had all been isolated from human faeces. The fermentation profile of these species was determined on the following carbohydrates; fructooligosaccharides (FOS), xylooligosaccharides (XOS), xylose, glucose and xylan as well as a blank with no additional carbohydrate.

The bacterial inocula (all *Bifidobacterium* species were obtained from DSMZ, Braunschweig, Germany) were grown for 48 hours on the above medium with glucose as the added carbohydrate. 1ml of this inoculum was injected aseptically into each tube and incubated at 37°C. Each species was grown on each carbohydrate in triplicate. A blank tube, containing medium with no added carbohydrate was also inoculated for each bifidobacteria species in triplicate, in order to take into account any carry over of glucose.

The OD of the tubes was measured at a wavelength of (650 nm) at 0, 2, 4, 6, 8, 24, 48 and 72 hours post inoculation.

Short Chain Fatty Acid Analysis

Samples (1ml) were taken from each Hungate tube and centrifuged at 10,000g for 5 minutes to remove any bacteria and particulate matter. Short chain fatty acid (SCFA) (lactate, acetate and formate) analysis was carried out using HPLC with an Aminex HPX–87H column (BioRad, Watford, UK) run at 50°C in degassed 0.005M H_2SO_4 at a flow rate of 0.6 ml/hour. The injection volume was 20μl. Organic acids were detected at a wavelength of 210nm using a BioRad UV detector. Organic acid concentrations were analysed with reference to calibration curves using Valuechrom software (BioRad).

β-Xylosidase Assay

80μl of bacterial culture was added to 100μl of 5.5mM p-nitrophenyl β-D-xylo-pyranoside solution (in 0.001M sodium acetate solution, pH 5.0) and incubated at 37°C for 2 hours. 1.1ml of 0.1M calcium carbonate was then added to stop the reaction and develop the colour. Absorbance was then recorded at 420nm.

Results

The maximum optical densities obtained for each species on each carbohydrate are shown in Figure 1. With respect to growth on the carbohydrates tested, the bifidobacteria can be generally put into three groups.

Table 1. Lactic, formic, and acetic acid concentrations (mM/ml) for each test species. Amounts expressed as ratios of acetic acid produced (n=3).

	B. bifidium			*B. angulatum*			*B. breve*		
	Lactic	Formic	Acetic	Lactic	Formic	Acetic	Lactic	Formic	Acetic
Blank	0.11	0.19	1.00	0.00	0.33	1.00	0.28	0.00	1.00
FOS	0.69	0.00	1.00	3.35	0.46	1.00	0.61	0.01	1.00
XOS	0.21	0.22	1.00	0.00	0.27	1.00	0.52	0.52	1.00
Xylose	0.19	0.23	1.00	0.00	0.33	1.00	0.22	0.00	1.00
Xylan	0.03	0.26	1.00	0.00	0.00	1.00	0.00	0.00	1.00

	B. gallicum			*B. infantis*			*B. longum*		
	Lactic	Formic	Acetic	Lactic	Formic	Acetic	Lactic	Formic	Acetic
Blank	0.00	0.22	1.00	0.14	0.18	1.00	0.00	0.00	0.00
FOS	0.00	0.16	1.00	0.74	0.15	1.00	0.25	0.08	1.00
XOS	0.00	0.35	1.00	0.00	0.25	1.00	0.22	0.25	1.00
Xylose	0.00	0.26	1.00	0.11	0.20	1.00	0.98	0.02	1.00
Xylan	0.76	0.00	1.00	0.00	0.07	1.00	0.00	0.09	1.00
Glucose	0.08	0.00	1.00	0.83	0.00	1.00	0.88	0.00	1.00

	B. adolescentis			*B. pseudolongum*			*B. catenulatum*		
	Lactic	Formic	Acetic	Lactic	Formic	Acetic	Lactic	Formic	Acetic
Blank	0.00	0.25	1.00	0.15	0.08	1.00	0.00	0.00	1.00
FOS	0.90	0.00	1.00	0.27	0.14	1.00	0.84	0.00	1.00
XOS	1.01	0.00	1.00	0.62	0.00	1.00	1.17	0.00	1.00
Xylose	0.24	0.11	1.00	0.18	0.21	1.00	1.64	0.00	1.00
Xylan	0.00	0.00	1.00	0.06	0.05	1.00	0.09	0.08	1.00
Glucose	0.80	0.00	1.00	0.73	0.00	1.00	0.82	0.00	1.00

Group 1: *B. angulatum, B. breve, B. pseudolongum, B. longum* and *B. infantis.* These species all displayed a preference for glucose and FOS over the xylose-containing carbohydrates. *B. infantis* did not display any growth on the xylose-containing substrates above that seen with the blank. *B. longum* grew on xylose and XOS, but did not show any growth on xylan compared to the blank.

Group 2: *B. catenulatum*, *B. gallicum* and *B. adolescentis*. These species displayed similar growth on XOS and FOS and all grew well on xylose, with *B. catenulatum* growing equally well on xylose and glucose. *B. adolescentis* grew very well on xylose and XOS but did not show growth in excess of the blank on xylan. *B. gallicum* displayed relatively poor growth on all the carbohydrates tested apart from glucose, however, there was evidence of growth on xylan with this species.

Group 3: *B. bifidum*. This species displayed higher growth on XOS than on xylose, although highest growth rates were seen on glucose and FOS, as was the case with the species in Group 1.

Short chain fatty acid (SCFA) concentrations at the end of fermentation are presented in Table 1. The ratio of lactate: formate: acetate produced, varied according to species and according to growth substrate used. Lactic and acetic acids were the principal SCFA produced on glucose, the exception being *B. bifidum,* which produced formic and acetic acids. Most species produced lactate and acetate from FOS, the exceptions being *B. longum* and *B. pseudolongum*, which both produced lactic, formic and acetic acids. *B. gallicum* produced formate and acetate acids. Variations in SCFA were seen within species growing on xylose and on XOS. *B. bifidum* produced lactic, formic and acetic acids on both substrates and *B. angulatum* and *B. gallicum* both produced formic and acetic acids on both substrates. Other species displayed different SCFA profiles on the XOS and xylose. *B. breve* and *B. longum* produced all three acids on XOS but only lactate on xylose. Conversely, *B. adolescentis* and *B pseudolongum* produced all three acids on xylose but only lactate on XOS. *B infantis* produced all three acids on xylose but only formic and acetic acids on XOS. *B. gallicum* was the only species to produce lactic and acetic acids on xylan. Only *B. bifidum* produced formic acid on this substrate.

β-Xylosidase activities of the microbial cultures are shown in Table 2. Neither *B. bifidum* nor *B. longum* showed any β-xylosidase activity, whereas all other organisms that grew on XOS displayed varying levels of β-xylosidase activity.

Table 2. Xylosidase activity for each species when grown on XOS for 72 hours, compared to maximum OD obtained.

	Maximum Optical Density	U/ml/hour
B.adolescentis	0.53+/-0.07	0.075+/-0.017
B.catenulatum	0.62+/-0.02	0.041+/-0.008
B.longum	0.33+/-0.08	0.002+/-0.002
B.pseudolongum	0.28+/-0.02	0.046+/-0.004
B.bifidum	0.31+/-0.04	0.001+/-0.000
B.gallicum	0.26+/-0.02	0.025+/-0.006
B.gallicum[*1]	0.26+/-0.04	0.028+/-0.004

[*1] *B.gallicum* when grown on xylan

Discussion

At the present time, there is an incomplete understanding of carbohydrate metabolism in bifidobacteria. It is believed that the "bifidus pathway" is the route of carbohydrate fermentation in these micro-organisms (Bezkorovainy, 1989).

Monosaccharides other than glucose can be fed into the bifidus pathway, however, little is known regarding the fermentation of xylose by bifidobacteria. Conceivably, xylose could be handled in the same way as *Lactobacillus pentosus* (Lokman, 1997) and *E. coli*. (Song and Park, 1997) In these organisms, xylose is converted into xylulose by xylose isomerase and then phosphorylated by xylulose kinase to form xylulose-5-phosphate. Xyulose-5-phosphate may then be incorporated into the bifidus pathway, although at a cost of ATP and one acetate when compared with glucose (Bezkorovainy, 1989). This may explain the higher cell density obtained on glucose and FOS compared with the xylose containing carbohydrates. Fructose can be incorporated into the bifidus pathway by fructokinase yielding the same levels of ATP and acetate as glucose. In this context, it is interesting to note that *B. catenulatum* grew to a higher cell density on xylose than glucose and on XOS than FOS. This could be due to *B. catenulatum* possessing a more efficient transport mechanism for xylose than for glucose. Alternatively, it is known that the enzymes of the bifidus pathway are not expressed to the same levels in all bifidobacteria species (Bezkorovainy 1989). This could also account for differences in carbohydrate preferences within the genus.

The current paradigm for prebiotic action is that probiotics possess cell-associated glycosidases which hydrolyse oligosaccharides prior to uptake of the monosaccharide (Perrin *et al.,* 2001). In the case of FOS, this is a fructofuranosidase (Imamura *et al.,* 1994) By this mechanism, XOS and xylan fermentation would be dependent upon xylanases and/or β-xylosidases. An interesting observation was that *B. bifidum* gave a higher cell density when grown on XOS than xylose. In addition, *B. bifidum* cultures displayed no extracellular and/or cell associated β-xylosidase activity when grown upon XOS. This suggests that the oligosaccharides were being fermented internally and therefore transported into the cell by specific oligosaccharide transport mechanisms. If the transport mechanism for XOS is more efficient than for xylose, then XOS will be fermented more readily than xylose, as observed in this study. *B. longum* also demonstrated a lack of β-xylosidase activity, whereas all other XOS utilisers showed varying levels of activity. These results indicate two strategies for bifidobacteria to ferment XOS, and possibly other oligosaccharides. The first being that displayed by *B. bifidum* and *B. longum* in this study, where the oligosaccharide was transported into the cell and fermented internally. The second route involves the cleavage of the oligosaccharides externally and uptake of the subsequent monomers as described previously (Perrin *et al.,* 2001).

Preferences in oligosaccharide chain length by bifidobacteria have been demonstrated in recent studies. In one study of the metabolism of fractionated galacto-oligosaccharides (GOS), *B. lactis* was found to prefer the trisaccharide and tetrasaccharide fractions and displayed a preference for these over glucose (Gopal *et al.,* 2001). The metabolism of individual FOS oligosaccharides by species of *Lactobacillus* has also been studied (Kaplan and Hutkins 2000). *L. plantarum* and *L. rhamnosus* only metabolised trisaccharides and tetrasaccharides and these strains could not metabolise the pentasaccharide, suggesting that they may possess transport systems specific for tri- and tetrasaccharides.

Consideration of the ratios of SCFA produced allows speculation on the different pathways used in the fermentation of the particular carbohydrates. Theoretically the bifidus pathway will ferment hexose sugars to give a molar ratio for acetate: lactate of 1.5 : 1, and the pentose monomers will give a ratio of 1 : 1. However these ratios are rarely achieved in practice (Bezkorovainy, 1989) and were not seen in this study. Some bifidobacteria convert pyruvic acid into formic acid and ethanol, rather than into lactic acid thereby yielding an extra ATP. From the data presented here, each of the *Bifidobacterium* species tested could produce both formic and lactic acids, with the pathway followed being regulated by growth substrate.

The current generation of prebiotic oligosaccharides are characterised as acting at the genus level. It is known, however, that not all bifidobacteria have the same health benefits. For example Gibson and Wang (1994) demonstrated that the anti-pathogen activity of a number of bifidobacteria species differed. It is, therefore, highly desirable to characterise the species-specificity of prebiotic oligosaccharides. Knowledge of the fermentation pathways of such oligosaccharides by specific bifidobacteria may allow the rational design of carbohydrates targeted at particular species of probiotic microorganism.

References

Bezkorovainy, A. 1989. Nutrition and metabolism of *Bifidobacteria*. In: Biochemistry and Physiology of *Bifidobacteria*. Bezkorovainy, A., Miller-Catchpole, R., eds. CRC Press, Boca Raton.

De Vriers, W., Gerbrandy, J., and Stouthamer, A.H. 1967. Carbohydrate Metabolism in *Bifidobacterium bifidum*. Biochem. Biophys. Acta. 136: 415-42

Fandi, K.G., Ghazali, H.M., Yazid, A.M. and Raha, A.R. 2001. Purification and N-terminal amino acid sequence of fructose-6-phosphate phosphoketolase from *Bifidobacterium longum* BB536. Lett. Appl. Microbiol. 32: 235-239.

Gibson, G.R. 1999. Dietary modulation of the human gut microbiota using the prebiotics oligofructose and inulin. J. Nutrition. 1438S

Gibson, G.R., and Roberfroid, M.B. 1995. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. J. Nutrition. 125: 1401-1412.

Gibson, G.R., Beatty, E.R., Wang, X., and Cummings, J.H. 1995. Selective stimulation of bifidobacteria in the human colon by oligofructose and inulin. Gastroenterol. 108: 975-982.

Gibson, G.R., and Wang, X. 1994. Regulatory effects of *bifidobacteria* on the growth of other colonic bacteria. J. Appl. Microbiol. 77:412-420.

Gopal P.K., Sullivan, P.A., and Smart, J.B. 2001. Utilisation of galacto-oligosaccharides as selective substrates for growth by lactic acid bacteria including *Bifidobacterium lactis* DR10 and *Lactobacillus rhamnosus* DR20. Intnl. Dairy J.11: 19-25.

Imamura, L., Hisamitsu, K., and Kobashi, K. 1994. Purification and characterisation of β– fructofuranosidase from *Bifidobacterium infantis*. Biol. Pharm. Bull. 17: 596-602.

Kaplan, H., and Hutkins, R.W. 2000. Fermentation of fructo-oligosaccharides by lactic acid bacteria and bifidobacteria. Appli. Environ. Microbiol. 66: 2682-2684.

Lokman, B.C., Heerikhuisen, M., Leer, R.J., van den Broek, A., Borsboom, Y., Chaillou, S., Postma, P.W., and Pouwels, P.H. 1997. Regulation and expression of the *Lactobacillus pentosus xyl*AB operon. J. Bacteriol. 179: 5391-5397.

Perrin S., Warchol M., Grill J.P., and Schneider F. 2001. Fermentations of fructo-oligosaccharides and their components by *Bifidobacterium infantis* ATCC 15697 on batch culture in semi-synthetic medium. J. Appli. Microbiol. 90: 859-865.

Roberfroid, M.B., Bornet, F., Bouley, C. and Cummings, J.H. 1995. Colonic microflora: Nutrition and health. Nutrition Rev. 53: 127-130.

Song, S., and Park, C. 1997 Organisation and regulation of the D-Xylose operons in *Escherichia coli* K-12: *Xyl*R acts as a transcriptional activator. J. Bacteriol. 179: 7025-7032.

Vazquez, M.J., Alonso, J.L., Dominguez, H., and Parajo, J.C. 2000. Xylooligosaccharides: manufacture and applications. Trends Food Sci. Technol. 11: 387-393.