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ORIGINAL ARTICLE

Fermentation of xylo-oligosaccharides obtained from wheat bran and Bengal gram husk by lactic acid bacteria and bifidobacteria

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Abstract Different strains of Bifidobacteria, Lactobacilli and Pediococci spp. were evaluated for their utilization of xylo-oligosaccharides derived from Bengal gram husk and wheat bran water extractable polysaccharides. The fermentation pattern of xylo-oligosaccharides by bacteria depends on the nature of xylo-oligosaccharides i.e. degree of polymerization and arabinose to xylose ratio as well as the bacterial strain tested, which inturn are very important for designing species-specific prebiotic xylo-oligosaccharides and synbiotic preparations for incorporation in various health foods. All the bacterial strains tested readily utilized xylo-oligosaccharides derived from bengal gram husk and wheat bran as indicated by the increase in (a) turbidity of the culture broth (b) xylanase, xylosidase and arabinosidase activities (c) dry cell mass and (d) the liberation of short chain fatty acids (SCFA). Acetate was found to be the major SCFA produced as the end product of fermentation and its amount varied from 75.4 to 100 mol%. Xylo-oligosaccharides derived from wheat bran were found to have better prebiotic activity compared to the one derived from Bengal gram husk and this can be ascribed to relatively high amount of arabinose.

Keywords Water extractable polysaccharides · Bengal gram husk · Wheat bran · Xylo-oligosaccharides · Prebiotic activity

The gastrointestinal tract of human adult contains a vast and complex consortium of more than 500 different species of bacteria that play a major role in colonic function, present to the extent of up to 10^{12} cells/g of luminal contents, and these affect the host homeostasis (Guarner and Malagelada 2003). Some of these microbes are potential pathogens, while others are largely commensals that coexist peacefully with their host and remain harmless. They may even confer health benefits by helping to digest dietary complex carbohydrates and by maintaining the appropriate balance among the different types of gut bacteria (Kraehenbuhl and Corbett 2004). They also produce vitamins, short chain fatty acids (SCFA) and other nutrients for their hosts, providing upto 15% of the total caloric intake. It has been reported that a balanced microflora is essential for healthy intestinal function including resistance to infection by pathogenic bacteria (Berg 1996). The gut microflora is affected by many factors such as age, drug therapy, diet, host physiology, peristalsis, local immunity and *in situ* bacterial metabolism, of which diet is probably the most significant factor determining the gut flora since foodstuffs provide the main nutrient sources for colonic bacteria (Berg 1996).

There is currently enhanced interest in the concept of actively managing the colonic microflora with the aim of improving host health by the dietary intervention, through incorporation of prebiotics such as non-digestible oligosaccharides (NDOs). A prebiotic is defined as "a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of beneficial bacteria in the colon, and thus improves the host health" (Gibson and Roberfroid 1995). Many studies have now confirmed that the incorporation of prebiotics in the diet is a valid approach to the dietary manipulation of the colonic microflora (Gibson and Roberfroid 1995; Kleesen et al 1997). NDOs resist digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine. Apart from their prebiotic effect, NDOs which come under the broad definition of dietary fibres are believed to alleviate

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disease symptoms such as diabetes, arteriosclerosis and colon cancer (Hsu et al 2004; Swennen et al 2006). Many of the health effects are believed to be related to the microbial fermentation of NDOs in the large intestine and the extent of fermentation depends on the nature of the substrates.

Bifidobacteria and lactobacilli are the most important beneficial microbes inhabiting the human gut and thus they are the usual target species for dietary intervention studies (Gibson 1999; Laparra and Sanz 2010). *Bifidobacterium* strains are capable of efficiently fermenting xylose-based oligo and polysaccharides (Crittenden et al 2002; Okazaki et al 1990). Beneficial colonic bacteria such as lactic acid bacteria and bifidobacteria produce carbohydrate degrading enzymes which ferment the NDOs and produce SCFA such as acetate, propionate and butyrate which provide metabolic energy for the host and help in the acidification of the bowel (Swennen et al 2006). Acidification can affect the balance of the bacterial species, bacterial metabolic activity and product formation. Probiotic bacteria are also shown to exhibit pronounced antibacterial activity against human enteropathogenic bacterial strains (Borpuzari et al 2007). Acetate is mainly metabolized in human muscle, kidney, heart and brain, whereas propionate acts as a possible gluconeogenic precursor suppressing the cholesterol synthesis (Gibson 1999). Butyrate is known to have pro-differentiation, anti-proliferation and anti-angiogenic effects on colonocytes (Mai and Morris 2004). Moreover, decrease in pH due to production of SCFA as a result of fermentation correlates with the population growth of the beneficial microbes (Berggren et al 1993) and inturn inhibits the growth of the undesirable pathogenic bacteria (Gibson and Wang 1994).

Acid hydrolysis of polysaccharides to obtain oligosaccharides is nonspecific and restricts its wider application. Utilization of enzymes might improve the technical and economic feasibility of biotechnological processes for arabinoxylan hydrolysis. Endoxylanase hydrolyzes the arabinoxylan randomly producing xylo-oligosaccharides (Chithra and Muralikrishna 2010) of varying degree of polymerization (DP 2–10). Enzymatic conversion of natural resources is one of the important approach having wide applications in functional food research. Recent study indicated replacement of sucrose with 7.5% xylooligosaccharides obtained from almond shells imparted desirable colour, taste, texture, crispness and surface smoothness to cookies (Rehman et al 2008). Prebiotic effect of xylo-oligosaccharides has not yet been effectively exploited compared to fructo-oligosaccharides (FOS) and galacto-oligosaccharides (GOS) (Delzenne and Kok 2001). Monitoring the extent of fermentation of oligosaccharides by the intestinal bacteria as well as identification of SCFA production under in vivo condition is cumbersome. The fermentation of polysaccharides/oligosaccharides can be measured from faeces, but SCFA are readily absorbed in the colon and the type as well as amount found in faeces does not describe the true situation.

Cereals and pulses are cultivated in almost all parts of the world, and their processing wastes such as cereal brans and pulse husks (agro wastes), which are rich sources of arabinoxylans, are abundantly available in India. Prebiotic xylo-oligosaccharides derived from such agro wastes holds great promise for the whole grain processing industry. The pattern of fermentation of oligosaccharides by beneficial bacteria depends on the nature of the oligosaccharides as well as the bacterial strain present in the large intestine which might be of relevance for the design of future species-specific prebiotic NDOs and synbiotic preparations. Hence, a study has been carried out using various strains of Bifidobacteria, Lactobacilli and Pediococci spp. to determine the prebiotic potency of xylo-oligosaccharides derived from Bengal gram husk and wheat bran water extractable polysaccharides (WEP) by xylanase treatment.

Materials and methods

Materials Wheat bran (*Triticum aestivum*) was obtained from local market. Bengal gram (*Cicer arietinum* L.) was dehusked in the GST department of C.F.T.R.I., Mysore, India. HPLC (μ-Bondapak-NH_2 carbohydrate) and GLC (OV-225 and PEG 20 M) columns were obtained from Shimadzu Corporation, Kyoto, Japan. Sugars, enzymes (Driselase, EC 3.2.1.8), substrates and other fine chemicals were purchased from Sigma Chemical Company, MO, USA. ATCC, NCDO and NDRI strains of microorganisms were obtained from National Dairy Research Institute (NDRI), Karnal, India. Microbiological culture media and media ingredients were obtained from HiMedia Laboratories Pvt. Ltd., Mumbai, India. All other chemicals and solvents were of analytical grade.

Liberation of oligosaccharides from WEP The WEP were isolated from Bengal gram husk and wheat bran as described earlier (Madhukumar and Muralikrishna 2010). WEP of wheat bran and Bengal gram husk (500 mg each) were dissolved in acetate buffer (pH 4.8, 0.1 M, 10 ml) and incubated with Driselase (0.28 U/mg protein) in a constant shaking water bath at 50°C for 2 h. Subsequently the reaction was stopped by adding three volumes of ethanol and the precipitated material was removed by centrifugation (3000×g for 15 min). The resultant supernatant consisting of oligosaccharides was concentrated and used for further analysis.

Separation of oligosaccharides from monosaccharides The crude oligosaccharides obtained from WEP of wheat bran

and Bengal gram husk were passed through charcoal-Celite column (3.0×20 cm) (Whistler and Durso 1950). Samples containing Driselase hydrolyzate in 15 ml water was applied to the column and the components were eluted with 700 ml of 1% (v/v) ethanol in water to elute monosaccharides followed by oligosaccharides elution with 600 ml of 50% (v/v) ethanol in water.

Determination of neutral sugar composition of poly/oligosaccharides by GLC The polysaccharides (10 mg) were suspended in water and were completely hydrolyzed by prior solubilization with 72% sulphuric acid at ice cold temperature followed by diluting to 8% acid and heating at 100°C for 10–12 h (Rao and Muralikrishna 2004). The oligosaccharides (10 mg) were hydrolyzed with sulphuric acid (2 N) for 8 h. The resultant monosaccharides were converted into alditol acetates as described earlier (Rao and Muralikrishna 2004). The alditol acetates were extracted with chloroform, filtered and analyzed by GLC (Sawardekar et al 1965).

Microorganism and culture conditions *Lactobacillus brevis* NDRI strain RTS, *Lactobacillus plantarum* NDRI strain 184, *Pediococcus pentosaceus* ATCC 8081, *Pediococcus pentosaceus* NCDO 813, *Bifidobacterium adolescentis* NDRI 236, *Bifidobacterium bifidum* ATCC 29521 *and Bifidobacterium bifidum* NCDO 2715 were the microorganisms used to study the prebiotic activity of xylo-oligosaccharides. These microorganisms were selected as reference intestinal bacteria responsible for beneficial health effects. The culture was maintained at 6°C in lactobacillus MRS broth medium and sub cultured at regular intervals of 30 days. With respect to *Bifidobacterium* cultures, Cysteine-HCl (0.05%) was added to the culture broth and incubation was carried out in a Whitley anaerobic jar (stainless steel, with Whitley Schrader valve system).

Inoculum The cultures were grown (24 h) in lactobacillus MRS broth medium and subjected to centrifugation (8000× g for 10 min at 4°C) and the resultant cells were suspended in 0.85% normal saline. Serial dilutions were prepared to get the requisite cell population. Lactobacillus MRS broth medium and all the glassware, centrifuge tubes were sterilized (autoclaved at 15 lbs pressure, 121°C for 15 min) and used for the microbiological experiments.

In vitro fermentation experiments Membrane filter (0.22 mμ, Millipore) sterilized wheat bran oligosaccharides (WBO) and Bengal gram oligosaccharides (BGO) in 10× concentration were incorporated at 0.5% level (final concentration) into 2 ml MRS broth medium (formulated without beef extract, yeast extract, sodium acetate and dextrose and replaced protease peptone to tryptone) and inoculated with 100 μl of culture suspension giving 200 cfu (colony-forming unit) and incubated at 37°C for different time intervals (0 h, 24 h and 48 h). The final pH of the fermentation medium was adjusted to 6.5±0.2. The MRS broth medium was modified in such a way that the xylo-oligosaccharide is the only carbon and energy source available for the bacteria. All the bacterial strains were inoculated at the same level. Three replicates were prepared for each combination of strain/carbohydrate/fermentation time. Control represents the culture broth without xylo-oligosaccharides, which were inoculated with specific microorganism.

A change in the broth color from colorless to yellow was considered as positive test. Growth of bacteria and utilization of oligosaccharides by the bacteria were monitored by measuring pH and absorbance of the culture broth. Turbidity was monitored spectrophotometrically with an UV-visible spectrophotometer at 600 nm. Initial optical density values of all the culture broths were negligible and varied from 0.000 to 0.003. After 48 h of incubation, cultures were centrifuged (3000×g for 20 min) and bacterial cells were oven dried (for constant weight, at 80°C) to determine the dry cell mass. Resultant supernatant was analyzed for SCFA.

Enzyme assays Culture broth (24 h old) was assayed for the presence of various enzyme activities. For determining the xylanase activity, larch wood xylan (1 ml, 0.5% in sodium acetate buffer, 0.1 M, pH 4.8) was incubated with sample (0.1 ml of culture broth) for 30 min at 50°C. Reaction was stopped by adding 1 ml of dinitrosalycilic acid reagent and the reducing sugar was quantified (Miller 1959). One unit of activity was defined as the amount of enzyme required to liberate 1 μmol of xylose/min under assay conditions. β-D-xylopyranosidase (Beldman et al 1996), α-L-arabinofuranosidase (Beldman et al 1996), α-D-galactopyranosidase and β-D-galactopyranosidase activities were determined by monitoring the release of p-nitro phenol from respective substrates i.e. p-nitro phenol glycosides (0.5 ml of 2 mM substrate in sodium phosphate buffer, 0.1 M, pH 5.7) by incubating them with sample (0.1 ml of culture broth) for 1 h at 37°C. For determining acetyl esterase activity, substrate (1 ml of saturated solution of p-nitro phenyl acetate in sodium potassium phosphate buffer, 0.2 M, pH 6.5) was incubated with sample (0.1 ml of culture broth) for 30 min at 25°C. Reactions in the above enzyme assays were stopped by adding saturated solution of sodium tetra borate (0.5 ml). Absorbance was read at 400 nm. One unit of activity was defined as the amount of enzyme required to liberate 1 μmol of p-nitro phenol/min under assay conditions.

SCFA analysis The culture supernatant was acidified with sulphuric acid (50%) and extracted with diethyl ether (Karppinen et al 2000) and analyzed for SCFA by GLC

on PEG-20 M by maintaining, column, injector and detector temperatures at 120°C, 220°C and 230°C respectively (Silvi et al 1999), using nitrogen as the carrier gas (40 ml/min). Acetate, propionate and butyrate (10 μmol each/ml in diethyl ether) were used as standards. Individual SCFA in the sample was quantified by using peak area obtained for the standards.

Statistical analysis All the values are expressed as mean ± standard deviation (SD). Statistical analysis was performed using one way ANOVA followed by Duncan multiple comparison test using the software-statistical package for social sciences (SPSS Inc, Version 10.0.5) to obtain the significant difference between microorganisms, carbon sources and fermentation period (24 h and 48 h). A value of $P<0.05$ was considered to indicate a significant difference between the above parameters.

Results and discussion

Liberation of oligosaccharides from WEP The WEP from Bengal gram husk and wheat bran (Madhukumar and Muralikrishna 2010), when subjected to commercial Driselase hydrolysis resulted in xylo-oligosaccharides in 5.8% and 14.4% yields respectively. Driselase from *Basidiomycetes sp*. is an endoxylanase, which cleaves arabinoxylans randomly (unbranched regions of both internal and external chains), resulting in the liberation of arabinose substituted xylo-oligosaccharides with varying degree of polymerization. Purified endoxylanase from finger millet (Chithra and Muralikrishna 2008) differed with respect to the mode of action compared to the Driselase from *Basidiomycetes sp*. as evident from the nature of the xylo-oligosaccharides liberated (Madhukumar and Muralikrishna 2010; Chithra and Muralikrishna 2010).

Separation of xylo-oligosaccharides from monosaccharides The crude hydrolysates obtained by enzymatic hydrolysis of WEP of Bengal gram husk and wheat bran consisted significant amount of monosaccharides in addition to xylo-oligosaccharides. In order to study the prebiotic effect of xylo-oligosaccharides, monosaccharides were removed from the crude hydrolysates by charcoal-Celite chromatography. The use of different ethanol concentrations allows the selective extraction of the carbohydrates previously adsorbed onto the charcoal column, depending on their degree of polymerization (Whistler and Durso 1950). In the present study, 1% ethanol elutes monosaccharide components i.e. free arabinose and xylose present in crude hydrolysates. The yields of monosaccharides were 11% and 12.6% with respect to Bengal gram husk and wheat bran respectively. The complete removal of monosaccharides from crude hydrolysates was confirmed by gel permeation chromatography on Biogel P-2 column as well as by HPLC (results not shown). Ethanol (50%) was used for the elution of oligosaccharides previously adsorbed onto the charcoal column and these xylo-oligosaccharide fractions devoid of monosaccharides were used for the prebiotic activity experiments.

In vitro fermentation of xylo-oligosaccharides The growth characteristics pattern shown by different strains of Bifidobacteria, Lactobacilli and Pediococci spp. on BGO and WBO proved their prebiotic nature in vitro compared to control fermentation media (i.e. media without sugar supplementation). The prebiotic potency of xylo-oligosaccharides varied significantly with respect to nature of oligosaccharides, individual bacterial strain tested as well as fermentation time (0 h, 24 h and 48 h). Oligosaccharide mixture used in the present study was arabinose containing xylo-oligosaccharides with varying degree of polymerization (BGO: DP 2–5 and WBO: DP 2–7) (Madhukumar and Muralikrishna 2010). In vitro experiments allow the comparison of the rates at which oligosaccharides are broken down and consumed in fermentation experiments. A decrease in the pH of the culture broth due to production of SCFA and increase in optical density of the culture broth (turbidity) were indicative of extensive microbial fermentation by all the strains inoculated (Table 1). A parallel experiment was done by inoculating glucose as carbon source at 0.5% level. All the bacterial strains grew well in glucose supplemented media. The prebiotic effect of the xylo-oligosaccharides was further confirmed by an increase in the dry cell mass of the bacteria after 48 h of total fermentation period compared to the control value (0.04 mg/ml). Decrease in pH can also be used as an indication of the prebiotic effect of the oligosaccharides incorporated into the culture broth (Berggren et al. 1993).

BGO and WBO were utilized effectively by all the microorganisms except *L. plantarum* NDRI strain 184 which could not utilize BGO even after 48 h of fermentation (Table 1). The growth of the microorganisms were varied with the total fermentation period and also with the oligosaccharides incorporated. All the microbial strains tested grew well on WBO as compared to BGO after 48 h of incubation. *L. brevis* NDRI strain RTS, *P. pentosaceus* NCDO 813, *B. adolescentis* NDRI 236, *B. bifidum* ATCC 29521 *B. bifidum* NCDO 2715 utilized the BGO completely within 24 h of incubation and reached the stationary phase, where as they required more than 24 h to completely utilize WBO (Table 1). This may be due to presence of higher DP (greater than 5) xylo-oligosaccharides in WBO, which requires more fermentation period to completely hydrolyze the same and for subsequent utilization. Growth of *L. plantarum* NDRI strain 184 towards BGO was poor

Table 1 Growth characteristics of lactic acid bacteria grown on 0.5% BGO and WBO

Microorganism	Carbon source (0.5%)	Optical density at 600 nm		pH	Dry cell mass (mg/ml broth)
		24 h	48 h		
Lactobacillus brevis NDRI strain RTS	Control	0.003±0.001	0.067±0.005	6.6±0.53	0.09±0.003
	BGO	1.305±0.04^{d}	1.305±0.05^{e}	5.5±0.40	4.5±0.32
	WBO	1.432±0.05^{C}	1.927±0.06^{CD}	5.5±0.21	5.6±0.47
Lactobacillus plantarum NDRI strain 184	Control	0.006±0.001	0.028±0.002	6.5±0.43	0.03±0.001
	BGO	0.296±0.01^{a}	0.453±0.02^{a}	5.3±0.26	2.3±0.15
	WBO	0.604±0.03^{A}	1.868±0.03^{C}	5.6±0.32	4.8±0.41
Pediococcus pentosaceus ATCC 8081	Control	0.004±0.002	0.021±0.002	6.5±0.43	0.03±0.002
	BGO	0.973±0.03^{c}	1.113±0.02^{d}	5.1±0.22	4.1±0.25
	WBO	0.658±0.02^{A}	1.896±0.05^{C}	5.8±0.25	4.9±0.32
Pediococcus pentosaceus NCDO 813	Control	0.009±0.001	0.053±0.004	6.4±0.53	0.07±0.002
	BGO	1.399±0.04^{e}	1.391±0.03^{f}	5.2±0.34	4.5±0.37
	WBO	1.372±0.05^{C}	2.016±0.05^{D}	5.3±0.25	5.7±0.41
Bifidobacterium adolescentis NDRI 236	Control	0.027±0.002	0.026±0.003	6.5±0.31	0.04±0.001
	BGO	0.842±0.03^{b}	0.864±0.04^{b}	5.1±0.24	3.9±0.21
	WBO	1.294±0.05^{B}	1.764±0.06^{B}	5.3±0.35	4.5±0.34
Bifidobacterium bifidum ATCC 29521	Control	0.025±0.003	0.026±0.002	6.4±0.43	0.04±0.002
	BGO	0.870±0.03^{b}	0.888±0.04^{bc}	5.1±0.25	3.9±0.22
	WBO	1.274±0.05^{B}	1.602±0.05^{A}	5.2±0.12	4.4±0.36
Bifidobacterium bifidum NCDO 2715	Control	0.029±0.002	0.025±0.003	6.5±0.24	0.03±0.001
	BGO	0.946±0.04^{c}	0.948±0.05^{c}	5.1±0.13	4.0±0.32
	WBO	1.279±0.05^{B}	1.753±0.06^{B}	5.3±0.12	4.8±0.43

BG—Bengal gram husk xylooligosaccharides, WBO—Wheat bran xylooligosaccharides. Control represents media without sugar supplementation and inoculated with bacteria. Given pH and dry cell mass values were for 48 h of fermentation. The values are represented as mean ± SD, n=3. Reported values were corrected for the 0 h values and blank tube values. The level of significance was tested by Duncan multiple range test at $P<0.05$. Values were significantly different between microorganisms and the letters a–f (BGO) and A–D (WBO) in the same column indicates the level of significant difference in increasing order at $P<0.05$. The data showed statistically significant difference when compared between carbon sources. The effect of carbon source at different time intervals were compared and the growth significantly increased from 24 h to 48 h for WBO at $P<0.05$, whereas BGO induces significant growth in *L. plantarum* NDRI strain 184 and *P. pentosaceus* ATCC 8081

($OD_{600\ nm}$ 0.45) even after 48 h of incubation (Table 1) but it grew rapidly on WBO and reached stationary phase at 48 h ($OD_{600\ nm}$ 1.86). Similar growth pattern was observed for *P. pentosaceus* ATCC 8081. This may be due to diauxic growth behavior exhibited by the particular strain of bacteria towards xylo-oligosaccharide substrates. Similar results were reported with respect to fermentation of GOS by *B. lactis* and *L. rhamnosus* (Gopal et al 2001).

B. adolescentis NDRI 236, *B. bifidum* ATCC 29521 *B. bifidum* NCDO 2715 exhibited almost similar growth pattern towards both BGO and WBO. All the three bacteria ferment BGO completely within 24 h of fermentation, where as they require more than 24 h to completely ferment the WBO (Table 1). These results are in concurrence with earlier studies on bifidobacteria grown on xylo-oligosaccharides, which indicate the relatively higher capacity of *B. adolescentis* to utilize xylobiose and xylotriose obtained by enzymatic hydrolysis of birch wood xylan (Okazaki et al 1990). It was observed that the growth of the microorganisms on particular oligosaccharide might be strain specific (Holtl et al 2005). *P. pentosaceus* NCDO 813 showed increased growth towards both BGO and WBO compared to *P. pentosaceus* ATCC 8081 (Table 1). Particularly *P. pentosaceus* NCDO 813 reached relatively higher growth (OD 1.372) at 24 h of fermentation period compared to *P. pentosaceus* ATCC 8081 (OD 0.658). After extended time of fermentation (48 h of incubation), the OD values remained higher for *P. pentosaceus* NCDO 813 compared to that of *P. pentosaceus* ATCC 8081 strain. Similar results were obtained for the microorganisms *B. bifidum* NCDO 2715 and *B. bifidum* ATCC 29521, wherein *B. bifidum* NCDO 2715 was more effectively grown on both BGO and WBO compared to that of *B. bifidum* ATCC 29521 (Table 1).

There are different mechanisms, which explain the fermentations of xylan and/or xylo-oligosaccharides by lactic acid bacteria and bifidobacteria i.e. (a) splitting of oligosaccharides by extracellular enzymes (xylanases and/or β-xylosidases) with further transport and metabolism of monosaccharides (Perrin et al 2001), (b) transport of oligosaccharides into the cell by specific oligosaccharide transport mechanisms for further hydrolysis by intracellular enzymes. In bifidobacteria 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, at the cost of ATP and one molecule of acetate as compared with glucose (Bezkorovainy and Miller-Catchpole 1989).

Enzyme activities in the culture broth Xylanase, xylopyranosidase and arabinofuranosidase were the enzymes responsible for the breakdown of arabino-xylooligo saccharides. The presence of these enzyme activities in the culture broths inoculated with the bacteria indicates the fermentation of the substrate (xylo-oligosaccharides) and their subsequent utilization by the bacteria. The 24 h old culture broths showed the presence of various carbohydrate degrading enzyme activities such as xylanase, β-D-xylopyranosidase, α-L-arabinofuranosidase, α-D-galactopyranosidase, β-D-galactopyranosidase and acetyl esterase activities. High activity of xylanase was detected in the culture broth of all the tested microorganisms grown on BGO (200–373 mU/ml) and WBO (631–726 mU/ml), but for the culture broth of *L. brevis* NDRI strain RTS and *P. pentosaceus* NCDO 813 the activity of xylopyranosidase was maximum (Table 2). Xylanase activity was maximum in the culture broth inoculated with WBO compared to that of BGO; this might be due to the presence of higher DP xylo-oligosaccharides in the WBO. Higher activities of xylopyranosidase and arabinfuranosidase were observed in all the culture broths (Table 2), which indicated the breakdown of arabinose and xylose from xylo-oligosaccharides and their subsequent utilization by the bacteria for their growth. However the enzyme activities differed substantially in the present study compared to the earlier ones and this is perhaps due to the difference in degree of polymerization of xylo-oligosaccharides and their extent of purity (Chithra and Muralikrishna 2010). Bifidobacteria and lactic acid bacteria are shown to poorly utilize arabinoxylans, however they could utilize arabinoxylan hydrolysates much more effectively (Jaskari et al 1998). The hydrolytic enzymes produced by the microorganisms help in the breakdown of the xylo-oligosaccharides (NDOs), which resist digestion by the gastrointestinal enzymes. The activities of α-D-galactopyranosidase and β-D-galactopyranosidase were lower compared to xylanase, xylopyranosidase and arabinofuranosidase (Table 2). Acetyl esterase activity was found to be negligible (8–18 mU/ml).

SCFA analysis in the culture broth In vitro methods are also valuable to measure the production of SCFA such as acetate, propionate and butyrate. Acetate was found to be the major SCFA produced as the end product of fermentation and its amount varied from 75.4 mol% to 100 mol% (Table 3). This is in accordance with the earlier reports (Ruppin et al 1980; Smiricky-Tjardes et al 2003). Acetogenic potential of xylo-oligosaccharides observed in this present study is in agreement with previous data from (a) in vivo experiments conducted involving rats by feeding diets supplemented with commercial xylo-oligosaccharides (Smiricky-Tjardes et al 2003) and (b) in vitro fermentations of commercial xylo-oligosaccharides using pig faecal contents (Imaizumi et al 1991). Lactate concentration was not determined in the present study. Major SCFA resulting from fermentation in mammalian intestinal tract are acetate, propionate and butyrate, whereas lactate is an intermediate metabolite from the fermentation process (Macfarlane and Macfarlane 2003). The amount of total SCFA produced varied for individual oligosaccharides inoculated (Table 3). Acetate concentrations were very high compared to propionate and butyrate in the culture broths. However significant amount of butyrate was detected in the culture broth of *B. bifidum* ATCC 29521 grown on WBO as oligosaccharide substrate (19.9 mol%). The amount of propionate produced is very low and its concentration varied from 0.31 to 4.7 mol%. Similar results were obtained for in vitro fermentation of xylo-oligosaccharides by piglet intestinal microbiota (Moura et al 2008).

Conclusion

The ability of fermentation of xylo-oligosaccharides by the bacteria varied with respect to the nature of xylo-oligosaccharides, individual bacterial strain tested as well as total fermentation time. Out of all the microorganisms tested *Pediococcus pentosaceus* NCDO 813 and *Lactobacillus brevis* NDRI strain RTS utilized more effectively both WBO and BGO followed by *Pediococcus pentosaceus* ATCC 8081, *Bifidobacterium adolescentis* NDRI 236, *Bifidobacterium bifidum* NCDO 2715, *Bifidobacterium bifidum* ATCC 29521 and *Lactobacillus plantarum* NDRI strain 184. Acetate was found to be the major SCFA produced as the end product of xylo-oligosaccharides fermentation. Xylo-oligosaccharides from wheat bran are having more prebiotic activity than Bengal gram husk xylo-oligosaccharides as indicated by their prebiotic activity

Table 2 Enzyme activities (mU/mL) in 24 h old lactic acid bacterial culture broths grown on 0.5% xylo-oligosaccharides

Microorganism	Carbon source (5.0%)	Xylanase	Xylosidase	Arabinosidase	α-Galactosidase	β-Galactosidase	Acetyl esterase
Lactobacillus brevis NDRI strain RTS	BGO	373.3 ± 13.23^{c}	673.0 ± 15.62^{d}	143.8 ± 5.23^{c}	44.6±2.21	27.5±0.87	14.5±0.43
	WBO	673.4 ± 15.21^{C}	705.0 ± 16.41^{E}	174.8 ± 4.71^{E}	64.7±3.14	36.2±1.24	18.3±0.82
Lactobacillus plantarum NDRI strain 184	BGO	273.2 ± 12.52^{b}	62.6 ± 3.63^{ab}	81.3 ± 2.92^{b}	40.3±2.42	13.4±0.26	8.3±0.24
	WBO	753.3 ± 17.24^{D}	86.3 ± 4.81^{CD}	99.3 ± 3.74^{B}	46.7±2.13	22.1±0.59	10.0±0.32
Pediococcus pentosaceus ATCC 8081	BGO	200.0 ± 12.04^{a}	78.4 ± 4.24^{b}	86.3 ± 3.23^{b}	41.0±1.81	14.7±0.32	12.2±0.36
	WBO	726.6 ± 15.63^{D}	90.6 ± 5.32^{D}	101.4 ± 4.82^{C}	51.1±2.52	23.4±0.29	14.9±0.48
Pediococcus pentosaceus NCDO 813	BGO	209.3 ± 13.23^{a}	647.0 ± 16.31^{c}	129.5 ± 5.81^{c}	44.6±3.43	29.6±0.41	12.8±0.33
	WBO	637.2 ± 16.12^{AB}	701.0 ± 15.23^{E}	151.8 ± 7.64^{D}	69.0±4.62	37.8±0.37	19.6±0.75
Bifidobacterium adolescentis NDRI 236	BGO	358.0 ± 14.03^{c}	66.9 ± 2.91^{ab}	90.6 ± 3.63^{b} *	40.3±2.81	14.4±0.35	13.7±0.35
	WBO	661.3 ± 16.31^{B}	75.5 ± 3.53^{AB}	99.3 ± 4.32^{B} *	45.3±1.73	29.3±0.44	21.5±0.92
Bifidobacterium bifidum ATCC 29521	BGO	270.6 ± 13.52^{b}	59.7 ± 2.72^{a} *	68.3 ± 2.41^{a}	56.1±3.14	36.2±0.52	14.9±0.36
	WBO	631.3 ± 15.54^{A}	63.3 ± 2.54^{A} *	74.1 ± 3.82^{A}	57.5±4.25	38.9±0.48	22.9±0.72
Bifidobacterium bifidum NCDO 2715	BGO	276.0 ± 12.63^{b}	63.3 ± 2.32^{ab} *	73.4 ± 4.14^{b} *	59.0±4.62	39.5±0.67	13.6±0.56
	WBO	652.0 ± 15.04^{AB}	70.5 ± 3.14^{AB} *	77.7 ± 3.53^{AB}*	62.6±5.23	44.0±0.47	18.6±0.65

BGO—Bengal gram husk xylooligosaccharides, WBO—Wheat bran xylooligosaccharides. The values are represented as mean ± SD, n=3. The level of significance was tested by Duncan multiple range test at $P<0.05$. The control (media without sugar supplement) did not give any enzyme activity, hence not shown in the table. Values were significantly different between microorganisms and the letters a–d (BGO) and A–E (WBO) in the same column indicates the level of significant difference in increasing order at $P<0.05$. * indicates statistically not significant difference between microorganisms when compared between carbon sources at $P<0.05$. Statistical analysis provided for the major enzymes

Table 3 Short chain fatty acid composition (mol%) of lactic acid bacterial culture broths grown on 0.5% xylo-oligosaccharides

Microorganism	Carbon source (5.0%)	Acetate	Propionate	Butyrate
Lactobacillus brevis NDRI strain RTS	BGO	98.4±4.84	0.41 ± 0.02^{a}	1.2 ± 0.04^{b}
	WBO	89.2 ± 3.23^{B}	2.9 ± 0.23^{CD}	7.9 ± 0.62^{BC}
Lactobacillus plantarum NDRI strain 184	BGO	99.7±5.14 *	0.31 ± 0.01^{a}	0.98 ± 0.03^{b}
	WBO	91.1 ± 3.60^{B} *	2.1 ± 0.14^{A}	6.8 ± 0.34^{AB}
Pediococcus pentosaceus ATCC 8081	BGO	94.9±3.81 *	1.4 ± 0.05^{c}	3.7 ± 0.23^{d}
	WBO	87.7 ± 2.92^{B} *	3.2 ± 0.21^{D}	9.1 ± 0.60^{C}
Pediococcus pentosaceus NCDO 813	BGO	96.9±4.72 *	0.91 ± 0.02^{b}	2.2 ± 0.13^{c}
	WBO	92.0 ± 4.10^{B} *	2.3 ± 0.41^{AB}	5.7 ± 0.42^{A}
Bifidobacterium adolescentis NDRI 236	BGO	92.1±4.31 *	2.2 ± 0.32^{d}	5.7 ± 0.34^{e}
	WBO	86.9 ± 3.64^{B} *	3.9 ± 0.53^{E}	9.2 ± 0.73^{C}
Bifidobacterium bifidum ATCC 29521	BGO	99.2±4.73	0.41 ± 0.03^{a}	0.66 ± 0.03^{a}
	WBO	75.4 ± 2.72^{A}	4.7 ± 0.61^{F}	19.9 ± 1.92^{D}
Bifidobacterium bifidum NCDO 2715	BGO	100±0.00	ND	ND
	WBO	100 ± 0.00^{C}	ND	ND

BGO—Bengal gram husk xylooligosaccharides, WBO—Wheat bran xylooligosaccharides. ND—Not detected

The values are represented as mean values mol % ± SD, n=3. The level of significance was tested by Duncan multiple range test at $P<0.05$. Values were significantly different between microorganisms and the letters a–e (BGO) and A–F (WBO) in the same column indicates the level of significant difference in increasing order at $P<0.05$. There was no significant difference between microorganisms in acetate production inoculated with 0.5% BGO as carbon source. * indicates statistically not significant difference between microorganisms when compared between carbon sources at $P<0.05$

experiments, which may be due to their relatively higher arabinose content.

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