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Assessment on the Fermentability of Xylooligosaccharides from Rice Husks by Probiotic Bacteria

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Liquors from rice husk autohydrolysis, containing xylooligosaccharides (XOS), other saccharides, and nonsaccharide compounds, were refined by membrane processing to increase the proportion of substituted XOS in refined liquors. XOS were assayed for composition and degree of polymerization (DP) distribution and hydrolyzed with commercial enzymes for obtaining XOS with DP in the range of 2−6. Nanofiltered, hydrolyzed liquors were subjected to ion exchange processing to yield a final product containing monosaccharides, XOS (accounting for 55.6% of the nonvolatile solutes), and other nonvolatile compounds. The solution obtained after enzymatic hydrolysis with commercial xylanases (in which 82.8% of XOS were in the DP range of 2−6) was examined as a medium for promoting the growth of *Bifidobacterium adolescentis* CECT 5781, *B. longum* CECT 4503, *B. infantis* CECT 4551, and *B. breve* CECT 4839. The growth rate of *B. adolescentis* (0.58 h^{-1}) was higher than the ones determined for *B. longum*, *B. infantis*, and *B. breve* (0.37, 0.30, and 0.40 h^{-1}, respectively). The percentage of total XOS consumption by *B. adolescentis* was 77% after 24 h, the highest percentage of utilization corresponding to xylotriose (90%), followed by xylobiose (84%), xylotetraose (83%), and xylopentaose (71%).

KEYWORDS: Autohydrolysis; fermentation; xylooligosaccharides; probiotic bacteria

INTRODUCTION

Xylooligosaccharides (XOS) are sugar oligomers made up of xylose units, which appear naturally in bamboo shoots, fruits, vegetables, milk, and honey. They are made up of a main backbone of xylose linked by β 1→4 bonds, where the structural units are often substituted at position C2 or C3 with arabinofuranosyl, 4-*O*-methylglucuronic acid, acetyl, or phenolic substituents (*1*). Typical materials for XOS production are lignocellulosic, xylan-containing materials such as hardwoods, corn cobs, straws, rice husks, or bran. XOS have been referred to as promising emergent prebiotics (*2*).

A prebiotic is a nonviable food component that confers a health benefit on the host associated with modulation of the microbiota (*3*). This definition does not emphasize a specific bacterial group, but it is assumed that a prebiotic should increase the number and/or activity of bifidobacteria and/or lactic acid bacteria, as these groups of microorganisms are claimed to cause several beneficial effects on the host.

To be defined as a prebiotic, a food ingredient should fulfill the following criteria: (1) resistance to gastric acidity, hydrolysis by digestive enzymes, and gastrointestinal absorption; (2) fermentability by intestinal microbiota; and (3) ability to enable a selective stimulation of the growth and/or activity of intestinal bacteria that contribute to health and well-being (*4*).

From a nutritional point of view, XOS are usually considered to be nondigestible oligosaccharides (NDO). In the past few years, the popularity of NDO as food ingredients has increased markedly. As a consequence, several studies have been performed to discover new NDO and to develop new products containing these compounds (*5*).

One of the most important features of XOS as food ingredients is their ability to stimulate the growth of intestinal bifidobacteria. In vitro assays proved that *Bifidobacterium* spp. and *B. adolescentis* are able to utilize both xylobiose and xylotriose, whereas a mixture containing xylobiose as the major component was utilized by *B. adolescentis*, *B. infantis*, and *B. longum*. In comparison with other NDO, *Bifidobacterium* spp. preferred xylooligomers, raffinose, and fructooligomers to

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hexoses. Xylooligomers were almost as effective as raffinose and better than FOS to enhance the in vitro growth of these bacteria (*6*).

Numerous studies on prebiotics have been carried out during the past few years, owing to the growing awareness of consumers for healthy foods. Okazaki et al. (*7*) employed a mixture of xylose (22%), xylobiose (58%), xylotriose (13%), and other saccharides (7%) as a carbon source for in vitro fermentations with *B. adolescentis*, *B. longum*, and *B. infantis* and assessed the degree of utilization of the various saccharides by these microorganisms. In this study, *B. adolescentis* showed a remarkable ability to use both xylobiose and xylotriose. Hopkins et al. (*8*) carried out fermentations with commercial XOS (from Suntory, Japan) with 70% purity and degree of polymerization (DP) 2−4, finding that the ability of bifidobacteria for growing on XOS depended on the considered strain. Some strains used XOS with DP 2, 3, and 4 preferentially, whereas other strains preferred xylose. Van Laere et al. (*9*) studied the fermentation of arabinoxylooligosaccharides from wheat meal and reported that branched structures show increased selectivity for bifidobacteria with respect to linear structures. According to the information systematized by Moure et al. (*2*), administration of 0.12 g/kg of body weight to male Japanese adults has resulted in beneficial effect, such as help to restrain the growth of pathogenic bacteria, to retard disorders caused by imbalanced fermentation in colon, and to avoid intestinal disorders such as constipation, inflammatory bowel disease, diarrhea, and gastritis. Moreover, XOS intake has been found to be highly effective for the reduction of severe constipation in pregnant women without adverse effects, and nutritional infant formulas containing XOS have been claimed to have synergistic effects all along the intestinal tract, improving gut barrier maturation. In comparison with other prebiotic oligosaccharides, the slower fermentation of branched XOS led to higher butyric acid production, which may result in even more advantageous effects, whereas the presence of feruloyl substituents may promote the growth of beneficial bacteria.

On the other hand, the high selling price of XOS, the fast growth of the functional food market, and the increasing number of medical, pharmaceutical, and cosmetic applications reported recently contribute to the interest in developing new technologies for manufacturing high-purity XOS with a defined distribution of DP (*4*).

Commercial XOS (for example, the ones manufactured by Suntory Ltd., Osaka, Japan) are mixtures of compounds with various DP, mainly xylobiose and xylotriose. XOS can be prepared by enzymatic treatment of xylan-rich materials from agricultural byproducts, such as corn cobs, cotton husk bran, or rice husks. Alternatively, XOS can be produced from xylan-rich materials by treatments in aqueous media. The use of water as the only chemical provides comparative advantages to the latter process, including low byproduct generation, limited corrosion owing to the mild pH of the reaction media, and reduction of operational costs (*10*).

Food applications require high-purity XOS, which are already commercial products with market prices higher than other NDO. To use autohydrolysis-derived XOS as food ingredients, non-desired compounds must be removed from autohydrolysis liquors, a field in which membrane technologies have potential applications (*11*). Low-DP XOS, the products preferred for food applications (*6*), can be obtained from rice husks by autohydrolysis (performed under optimal conditions to achieve xylan solubilization without significant saccharide degradation), and further DP reduction can be obtained with endo-1−4-β-xylanases.

The main objective of this study was to assess the suitability of rice husk-derived XOS (obtained by autohydrolysis and subjected to further purification and DP reduction) as carbon sources for the in vitro growth of four selected bifidobacteria strains (*B. adolescentis* CECT 5781, *B. longum* CECT 4503, *B. infantis* CECT 4551, and *B. breve* CECT 4839). Their comparative ability for XOS utilization was tested in terms of growth and specific growth rate. The time course of oligomers with different DP in fermentations with *B. adolescentis* CECT 5781 was assessed in additional experiments.

MATERIALS AND METHODS

Raw Material and Autohydrolysis Conditions. Rice husks from a local factory (Procesadora Gallega de Alimentos, Lalín, Pontevedra, Spain) were air-dried, homogenized in a single lot to avoid differences in composition among aliquots, and stored. Aliquots from the above homogenized lot were mixed with water at the desired proportions (8 kg/kg of oven-dry solid) and reacted in a Parr reactor fitted with double six-blade turbine impellers. The vessel was heated with external fabric mantles and cooled with an internal stainless steel loop. Temperature was monitored using an inner thermocouple and controlled by a PID module. Non-isothermal reaction was carried out using the fastest heating profile of the reactor to achieve 205 °C, conditions under which the XOS concentration was maximal (*12*).

Refining of Autohydrolysis Liquors. *Membrane Processing of Autohydrolysis Liquors.* Raw autohydrolysis liquors were nanofiltered using an Osmonics GE2540 DESAL membrane, with 1.77 m^2 and cutoff of 1000 Da, as given by the manufacturer. The maximum allowed transmembrane pressure (TMP) was 20 bar. The membrane can operate at temperatures up to 50 °C and pH in the range of 2−11. A diaphragm pump (Hydra-Cell, Wanner Engineering Inc., Minneapolis, MN) was used to feed liquors to the membrane module. Pressure was monitored at the entrance and exit of the membrane module, and a needle valve located after the membrane module was used to achieve the desired TMP. The feed flow rate was measured using a rotameter. Temperature was measured using a thermometer and controlled by flushing tap water through a refrigeration coil placed in the 25 L feed tank. Preliminary experiments were carried out in full recycle mode operating at TMP in the range of 3−15 bar, and further experiments were carried out in concentration mode at the optimal TMP (13 bar). Retentates from the concentration experiments were subjected to further enzymatic processing and purification, as described below.

Enzymatic Processing. Retentates from the membrane concentration assays were treated with commercial endoxylanases (Shearzyme 2×) kindly provided by Novozymes-Spain. The endoxylanase activity of the commercial concentrate was measured by the Megazyme assay (Megazyme International Ireland Ltd., Wicklow, Ireland), based on the depolymerization of Remazol Brilliant Blue (RBB), and converted into xylanase units (XU) using the method provided by the manufacturer. The activity of Shearzyme 2× was 1500 XU/mL. Commercial xylanases were added to the concentrate at the desired enzyme loading (200 XU/kg of liquor), and the solution was set at pH 5 and shaken at 120 rpm and 40 °C for 96 h.

Ion Exchange Processing of Liquors. Processed liquors were treated with Amberlite IRA 96 (a weak anion exchange resin) for removing undesired, noncarbohydrate compounds. Liquors and resin were contacted overnight with gentle agitation at room temperature using a mass ratio of resin/liquor of 1:15. The resulting liquors were freeze-dried before storage and diluted at the desired proportions for manufacturing the culture media.

Fermentation Experiments. *Bacterial Strains and Growth Media.* The bacterial strains used in this study were *B. adolescentis* CECT 5781 (corresponding to ATCC 15703), *B. longum* CECT 4503 (ATCC 15707), *B. infantis* CECT 4551 (ATCC 15697), and *B. breve* CECT 4839 (ATCC 15700), all obtained from the Colección Española de Cultivos Tipo (CECT, Valencia, Spain). Before fermentation, the strains

were precultured in Wilkins−Chalgren broth (Cultimed Panreac, Barcelona, Spain). The media were prepared under anaerobic conditions, replacing the gas phase by a mixture of nitrogen and carbon dioxide using a gassing manifold system. Before autoclave sterilization, the media were distributed into airtight serum bottles (in which the anaerobic conditions were obtained by displacing the inner space with a N_2/CO_2 mixture), closed with butyl rubber septum-type stoppers, and sealed with aluminum caps (Bellco Biotechnology Inc., Vineland, NJ). The nutrient base medium used in fermentations was modified from that of Jaskari et al. (*13*) as follows: 5.0 g/L trypticase soy broth (TSB) without dextrose (BBL, Lockeysville), 5.0 g/L bactopeptone (Amersham, Buckinghamshire, U.K.), 5.0 g/L yeast nitrogen base (YNB, Difco, Detroit, MI) prepared separately as described below, 0.5 g/L cysteine hydrochloride (Merck, Darmstadt, Germany), 1.0% (v/v) of salt solution A (100.0 g/L NH_4Cl, 10.0 g/L $MgCl_2 \cdot 6H_2O$, 10.0 g/L $CaCl_2 \cdot 2H_2O$), and trace minerals solution, 0.2% (v/v) of salt solution B (200.0 g/L $K_2HPO_4 \cdot 3H_2O$) and 0.2% (v/v) of 0.5 g/L resazurin solution, in distilled water. The final pH of the medium was adjusted at 6.8. The medium was deoxygenated as described above, and 9.0 mL aliquots were dispensed into airtight anaerobic culture tubes, which were sealed with aluminum caps before autoclave sterilization. Stock solutions of YNB, XOS, and glucose (Sigma, St. Louis, MO) were filter-sterilized (0.2 μm, Chromafils, Macherey-Nagel, Düren, Germany) into sterile airtight serum bottles. These solutions were aseptically made anaerobic by repeated substitutions of the headspace gas phase with nitrogen through the butyl rubber septum-type stoppers, using a gassing manifold.

Before inoculation, both YNB and carbohydrate solutions were aseptically added to the anaerobic culture tubes with nutrient base medium (using syringes and needles in which the anaerobic conditions were obtained by displacing their headspace with nitrogen), to achieve a final concentration of 7.0 g of freeze-dried carbohydrates/L.

Batch Fermentation Experiments. Bacteria (*B. adolescentis*, *B. longum*, *B.breve*, and *B. infantis*) were precultured at 37 °C for 16 h without shaking. Anaerobic culture tubes containing nutrient base medium were inoculated (2% v/v) with the considered carbon source. Three replicates were prepared for each combination of strain/carbohydrate/fermentation time. Fermentations were carried out at 37 °C for *B. adolescentis*, *B. longum*, *B. breve*, and *B. infantis* under static conditions. Bacterial growth was monitored at 600 nm using a spectrophotometer (Thermo Spectronic Genesys 20, Garforth, U.K.). Growth rates were calculated by linear regression of data. In experiments with *B. adolescentis*, fermentation was stopped after the desired times, the media were centrifuged at 9000 rpm for 10 min, and the supernatants were filtered through 0.2 μm cellulose acetate filters (Sartorius, Goettingen, Germany) before high-performance liquid chromatography (HPLC) and high-performance anion exchange chromatography−pulsed amperometric detection (HPAEC-PAD) analyses. Acid production during the incubation period was checked by pH measurements.

Analytical Methods. *Analysis of Liquors from Hydrothermal Treatments.* Samples of liquors were filtered through 0.45 μm cellulose acetate membranes and assayed by HPLC for glucose, xylose, arabinose, and acetic acid, using a 1100 series Hewlett-Packard chromatograph fitted with a refractive index detector (temperature, 50 °C). Other analysis conditions were as follows: Aminex HPX-87H column (Transgenomic Inc., Omaha, NE); mobile phase, 0.003 M H_2SO_4; flow, 0.6 mL/min. A second sample of liquors was subjected to quantitative posthydrolysis (with 4% sulfuric acid at 121 °C for 20 min) before duplicate HPLC analysis. The increase in the concentrations of xylose and glucose caused by posthydrolysis provided a measure of the oligomers (xylooligomers and glucooligomers) present in the media. In the same way, the increase in acetic acid concentration measured the amount of acetyl groups present in the reaction products, and the increase in arabinose concentration measured the amount of arabinosyl moieties present as substituents in the reaction products. Uronic acids were determined according to the method of Blumenkrantz and Asboe-Hansen (*14*), using galacturonic acid as a standard for quantification. All of the analyses were made in triplicate.

HPAEC-PAD. HPAEC analysis of sugar oligomers was performed using a Dionex instrument (Dionex, Sunnyvale, CA). Separation of carbohydrates was carried out with a CarboPac PA-1 (4 mm × 250 mm) in combination with a CarboPac PA-1 guard column (4 mm × 50 mm) maintained at 30 °C, and PAD. The mobile phases were degassed with helium. Samples were filtered using 0.22 μm cellulose acetate filters (Sartorius, Germany) and diluted 1:7. Analyses were performed using a gradient prepared from deionized water (eluent A), 200 mM sodium hydroxide (eluent B), and 2 M sodium acetate in 200 mM sodium hydroxide (eluent C). The total analysis time was 115 min. Xylobiose, xylotriose, xylotetraose, xylopentaose and xylohexaose were used as external standards for quantification. All of the analyses were made in triplicate.

Determination of Carbohydrates and Fermentation Products in Cell-free Supernatants. Filtered supernatants from the anaerobic culture tubes inoculated with *B. adolescentis* were analyzed by HPLC for monosaccharides, total oligosaccharides, and fermentation products (formic, lactic, and acetic acid), using the method described above, as well as by HPAEC-PAD to quantify the concentrations of the various oligosaccharides during fermentations.

Table 1. Composition of Streams A, B, and H in **Figure 1** Expressed as Kilograms of Component per 100 kg of NVC

component	stream A	stream B	stream H
glucose	0.54 ± 0.01	0.17 ± 0.00	0.70 ± 0.01
xylose	3.12 ± 0.02	0.87 ± 0.00	1.17 ± 0.00
arabinose	1.89 ± 0.01	0.48 ± 0.00	0.59 ± 0.01
glucooligosaccharides	5.66 ± 0.03	7.44 ± 0.07	8.96 ± 0.01
xylooligosaccharides	39.93 ± 0.08	47.77 ± 0.03	55.62 ± 0.07
arabinooligosaccharides	2.26 ± 0.09	1.56 ± 0.01	1.46 ± 0.05
acetyl groups linked to oligosaccharides	3.26 ± 0.04	4.17 ± 0.02	3.92 ± 0.04
uronic acids	4.01 ± 0.07	3.50 ± 0.09	4.90 ± 0.09
ONCV	39.32 ± 0.29	34.03 ± 0.09	22.70 ± 0.13

RESULTS AND DISCUSSION

Autohydrolysis and Refining of XOS. Autohydrolysis of rice husks under suitable operational conditions leads to the depolymerization of hemicelluloses, resulting in the formation of oligosaccharides (*12, 15, 16*). During treatments, other side processes occur, including extractive removal from solid phase, solubilization of acid-soluble lignin, dissolution of inorganic components, and saccharide conversion into dehydration products. Because of this, crude liquors have to be refined to obtain food-grade XOS.

Table 1 lists compositional data of autohydrolysis liquors. The nonvolatile compounds (NVC, measured by oven-drying at 105 °C) are mainly made up of oligosaccharides, including XOS, glucooligosaccharides (GlucOS), arabinooligosaccharides (ArOS), and oligosaccharide substituents. For calculation purposes, oligosaccharides are expressed as monosaccharide equivalents, and the acetyl and uronic groups are expressed as acetic acid and galacturonic acid equivalents, respectively. Other components of liquors include monosaccharides (xylose, glucose, and arabinose, which are undesired compounds for the purposes of this work), and other nonsaccharide, nonvolatile compounds (ONVC), which have to be removed in further refining steps.

The scheme of the process followed for purification of raw autohydrolysis liquors is shown in **Figure 1** and included sequential steps of nanofiltration and ion exchange with an anionic resin (*11*), with an inserted endoxylanase hydrolysis step to keep the DP distribution within the desired limits. Finally, the refined liquors (stream H in **Figure 1**) were freeze-dried for conservation.

Preliminary nanofiltration assays of liquors were carried out operating in full recycle mode (data not shown) to assess the effects of TMP along the whole experimental range (3−15 bar).

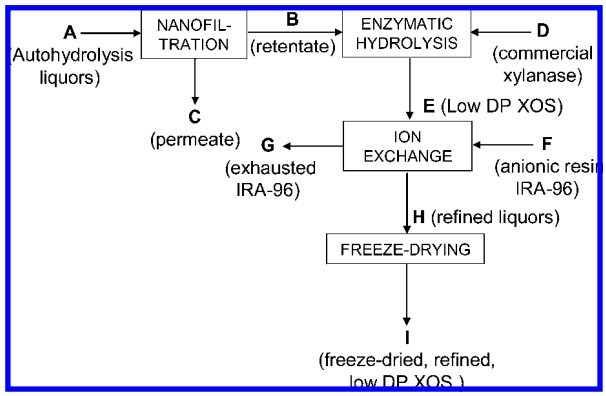

Figure 1. Scheme of the process considered for XOS manufacture.

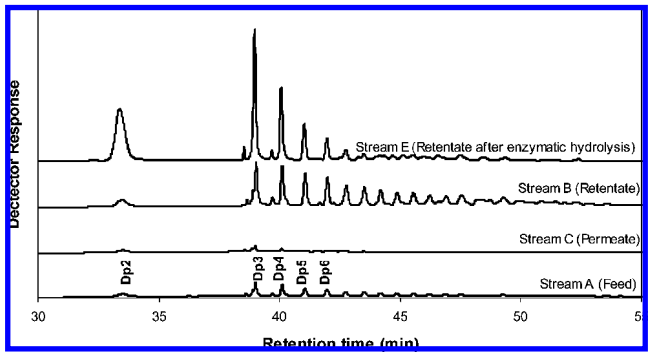

Figure 2. HPAEC-PAD chromatograms of feed, permeate, retentate as collected, and retentate after enzymatic hydrolysis.

On the basis of this information, the TMP selected for operation in concentration mode was 13 bar. Concentration was stopped when a volume reduction factor (VRF, defined as the volume ratio feed/retentate) of 6 was achieved. Owing to the different densities of raw autohydrolysis liquors and retentate, VCR = 6 corresponded to a mass concentration ratio (kg of feed/kg of retentate) of 5.12.

Material balances showed that the percentages of recovery in concentrate were 17.5−22.2% for monosaccharides, in comparison with 47.7% for ArOS, 82.6% for XOS, 90.9% for GlucOS, and 88.2% for acetyl groups linked to oligosaccharides (denoted AcOS). Just 59.8% of the initial ONVC were kept in the retentate, leading to a decreased ONVC mass fraction in stream B of **Figure 1** (0.3403) in comparison with stream A (0.3932). These data confirmed that nanofiltration resulted in purification owing to the preferential removal of both monosaccharides and ONVC. The effects obtained for individual components can be evaluated by comparing their respective compositions in streams A and B of **Figure 1** (see **Table 1** for experimental data).

As the DP distribution of XOS is an important factor affecting their biological properties, **Figure 2** shows HPAEC-PAD chromatograms of feed, permeate, crude retentate, and retentate after enzymatic hydrolysis. Both raw autohydrolysis liquors (stream A in **Figure 1**) and retentate (stream B in **Figure 1**) presented a wide distribution of molecular weights. In the case of retentate, just 35.6% of the total oligosaccharides corresponded to DP in the range of 2−6 (the ones preferred for use as prebiotics), whereas permeate lacked compounds with DP > 3−4.

To convert high molecular weight compounds into low-DP oligomers, the retentate was subjected to the action of a commercial endoxylanase (Shearzyme 2×) under reported operational conditions (*17*). In the hydrolyzed retentate (stream E in **Figure 1**), the fraction of oligosaccharides with DP in the range of 2−6 was 82.7 wt %. **Table 2** shows the distribution of the oligomers with DP within the range of 2−6 and DP > 6 for streams A, B, and E.

Table 2. DP Distribution of Rice Husks XOS Present in Raw Autohydrolysis Liquors

	wt %		
	feed	retentate	retentate after enzymatic hydrolysis
DP 2	5.1	1.6	13.6
DP 3	10.6	7.7	37.3
DP 4	7.7	6.3	13.4
DP 5	8.5	7.7	9.1
DP 6	12.1	12.3	9.4
DP > 6	56	64.4	17.3

As shown in **Figure 1**, stream E (hydrolyzed retentate) was treated with an anionic resin (IRA-96) to yield stream H. The purification effects achieved in this stage can be assessed from the compositional data listed in **Table 1**. The most noticeable changes corresponded to a decrease in the mass fraction of ONVC (from 0.3403 to 0.2270) and an increase in the mass fraction of XOS (from 0.4777 to 0.5562). Finally, the hydrolyzed retentate was freeze-dried to give the refined product (stream I).

Fermentation of XOS by Bifidobacteria. To assess the suitability of the purified XOS obtained in this work (stream I in **Figure 1**) as carbon sources for probiotic bacteria, in vitro fermentation assays were carried out with four bifidobacteria strains (*B. adolescentis* CECT 5781, *B. longum* CECT 4503, *B. breve* CECT 4839, and *B. infantis* CECT 4551). These microorganisms were selected as reference intestinal bacteria responsible for beneficial health effects.

Figure 3 shows the growth curves determined for the various microorganisms growing in media made with XOS. The experiments lasted 24 h. The maximum growth rates (μ, determined by regression of experimental data) are listed in **Table 3**, as well as the maximum absorbances (A_{max}) corresponding to the various experiments. To provide a basis for comparison, **Table 3** also includes the data determined under similar operational conditions for culture media containing glucose instead of XOS. A comparison among the specific growth rates (μ) obtained in the two types of media confirms the particular ability of *B. adolescentis* CECT 5781 for utilizing XOS (with $\mu = 0.58\ h^{-1}$ in XOS-containing media and $0.59\ h^{-1}$ in glucose-containing media). *B. breve* CECT 4839 showed

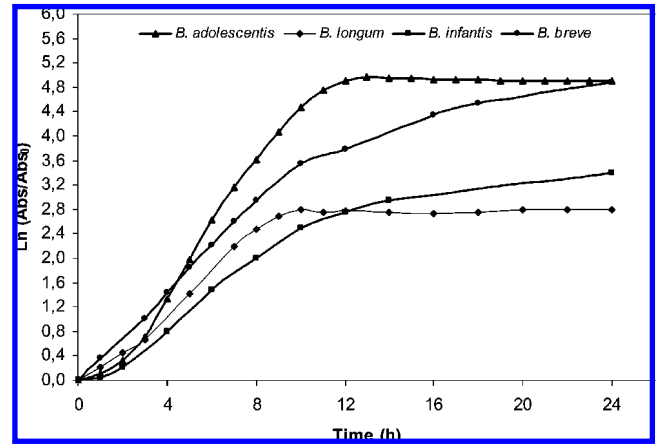

Figure 3. Growth of *B. adolescentis*, *B. longum*, *B. breve*, and *B. infantis* in nutrient base medium supplemented with xylooligosaccharides from rice husk autohydrolysis.

Table 3. Maximum Specific Growth Rates (μ) and Maximum Absorbances (A_{max})Determined for *B. adolescentis*, *B. longum*, *B. breve*, and *B. infantis* Growing in Media Containing Glucose or Rice Husk Xylooligosaccharides (Average Values ± Standard Deviations)

		B. adolescentis[a]	*B. longum*[a]	*B. breve*[a]	*B. infantis*[a]
μ (h^{-1})	glucose	0.59 ± 0.00	0.45 ± 0.01	0.38 ± 0.00	0.37 ± 0.00
	XOS	0.58 ± 0.00	0.37 ± 0.00	0.40 ± 0.01	0.30 ± 0.00
A_{max}	glucose	1.65 ± 0.01	1.29 ± 0.00	1.61 ± 0.01	1.68 ± 0.02
	XOS	1.27 ± 0.00	0.17 ± 0.00	0.47 ± 0.00	0.17 ± 0.00

[a] The nutrient base media were supplemented with 7.0 g/L of each carbon source in experiments with *B. adolescentis* and with 5.0 g/L in experiments with *B. longum*, *B. breve*, and *B. infantis*.

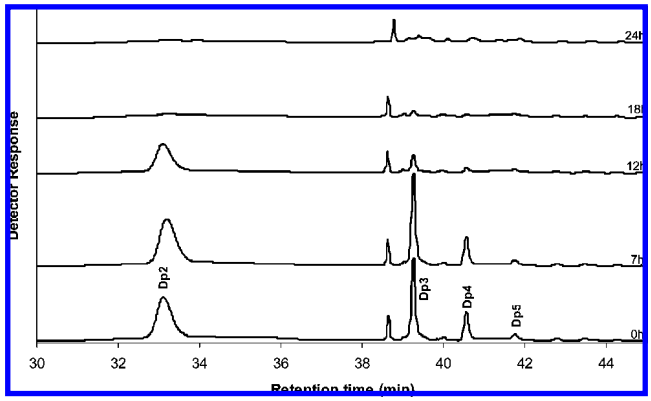

Figure 4. HPAEC-PAD chromatograms of XOS-containing media fermented with *B. adolescentis* CECT 5781.

higher μ growing on XOS than on glucose (0.40 h^{-1} in comparison with 0.38 h^{-1}). *B. longum* CECT 4503 and *B. infantis* CECT 4551 showed higher μ in glucose-containing media, but their specific growth rates on XOS media (0.37 and 0.30 h^{-1}, respectively) were also remarkable.

The results determined in this work can be justified on the basis of the information reported by Amaretti et al. (*18*), who found differences in the transport systems of di- and oligosaccharides for different bifidobacteria. Among the tested microorganisms, *B. adolescentis* MB 239 showed the highest efficiency in using dimeric and oligomeric carbon sources. This special ability was ascribed to the coexistence of two ways for oligosaccharide utilization: (a) oligosaccharide transport to the interior of the cell with further hydrolysis−fermentation by intracellular enzymes and (b) splitting of oligosaccharides by extracellular enzymes with further transport and metabolism of monosaccharides. Perrin et al. (*19*) identified the extracellular enzymes as cell-associated glycosidases (xylanases and/or β-xylosidases in the case of XOS hydrolysis). Oppositely, no extracellular β-xylosidase activity was found for *B. longum* DSM 20219 growing in XOS-containing media, suggesting that the products were transported across the cell membrane by specific mechanisms and then fermented (*20*).

Due to the special ability of *B. adolescentis* CECT 5781 for XOS utilization, and to assess the degradation of XOS with various degrees of polymerization by this strain, samples corresponding to different fermentation times were analyzed by HPAEC-PAD. The experimental results (see **Figure 4**) have to be interpreted with the consideration that the concentrations of low- and intermediate-DP oligosaccharides can be affected in two ways: consumption by bacteria and generation from XOS of higher DP. Keeping this idea in mind, the fast decrease of the concentrations of XOS with DP 2 and 3 confirms that these compounds are the preferred substrates. Compounds with DP 4 and 5 were also suitable carbon sources, but showed a slower

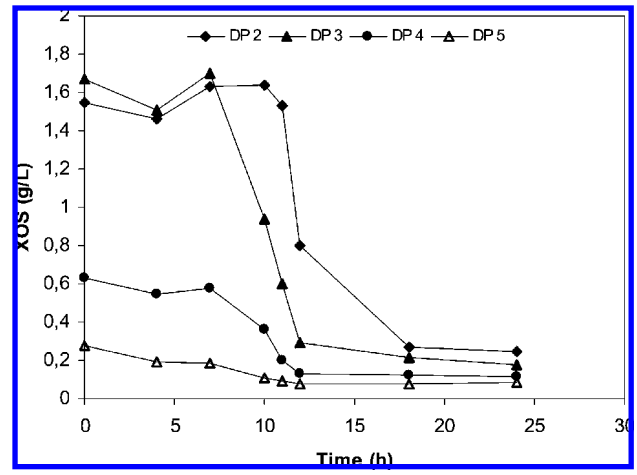

Figure 5. Time course of the concentrations of XOS during the fermentation with *B. adolescentis* CECT 5781.

utilization kinetics. These findings are in agreement with literature reported for the fermentability of corn cob-derived XOS with defined DP distribution (*21*). The increase observed in xylose concentration confirms the results reported by Crittenden et al. (*22*) about the inability of *B. adolescentis* for consuming this sugar. This behavior can be ascribed to the adaptation of this strain to the special environment in the colon, where monosaccharides are naturally absent, but can be reached by unaltered NDO. The ability of this bacterium to produce glycosyl-hydrolases suitable for NDO degradation can be considered to be a comparative advantage with respect to other colonic bacteria (*18*).

The XOS consumed at the end of the fermentation with *B. adolescentis* CECT 5781 accounted by 77% of their initial amount. As the assay was carried out without pH control, the progress of fermentation resulted in a continuous pH decrease (to 4.92), suggesting that acidification could have hindered further substrate conversion. In the literature, the presence of XOS fractions resistant to fermentation (owing to increased molecular weight or to the type and amount of XOS substituents) has been reported (*23*). The HPAEC-PAD data in **Figure 4** show a single peak of significant area. This peak corresponded to the salt of the mobile phase, and not to a xylooligomer, as can be seen by comparing the retention time with the ones of standard compounds. The major metabolic products were acetate (3.46 g/L), lactate (2.62 g/L), and formate (0.64 g/L).

Figure 5 shows the concentration profiles determined for the XOS of various degress of polymerization in the fermentation with *B. adolescentis* CECT 5781. The percentages of total XOS consumption were 46% after 12 h and 77% after 24 h. At the latter fermentation time, the highest percentage of utilization corresponded to xylotriose (90%), followed by xylobiose (84%), xylotetraose (83%), and xylopentaose (71%). The utilization kinetics was also faster for oligosaccharides with DP 2 and 3. These results are in agreement with the findings of Moura et al. (*21*) in their study with corn cob-derived XOS.

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