Available online at www.sciencedirect.com
ScienceDirect
Food Chemistry 101 (2007) 1172–1177
Food Chemistry
www.elsevier.com/locate/foodchem

Properties of xylanolytic enzyme system in bifidobacteria and their effects on the utilization of xylooligosaccharides

Hongyan Zeng, Yemin Xue, Tingting Peng, Weilan Shao *

Jiangsu Key Laboratory for Biodiversity and Bio-resources, Nanjing, China
The Key Laboratory of Microbial Engineering, Nanjing Normal University, 122 Ninghai Road, Nanjing 210097, China

Received 25 December 2005; received in revised form 11 March 2006; accepted 13 March 2006

Abstract

The xylanolytic enzyme system was examined in *Bifidobacterium adolescentis*, *B. infantis* and *B. bifidum* to determine their ability to utilize xylooligosaccharides (XOSs). All these species produced only xylosidase and arabinosidase, and no xylanase, α-glucuronidase and acetyl xylan esterase were found. The optimal activity of β-D-xylosidase from *B. adolescentis* was at pH 5.6 and 45 °C, and α-L-arabinoside was at pH 5.0 and 40 °C. The degradation products of cell-free extracts and the growth rate of *B. adolescentis* were tested over XOSs and XOSs de-branched by recombinant α-glucuronidase. The results showed that de-branching α-glucuronidase increased the production of xylose and the total cell density by 10%, and accelerated the growth of *B. adolescentis* by 20%.

Keywords: *Bifidobacterium*; Xylanolytic enzyme system; Xylooligosaccharides; Degradation

1. Introduction

Intestinal microflora play significant roles in the metabolism of non-digestible oligosaccharides. Bifidobacteria are anaerobic bacteria in the human large intestine, which are capable of using oligosaccharides as carbon sources. Due to the health-promoting effects on humans, they are generally considered as probiotic and important components of the human intestinal microbiota. Xylooligosaccharides (XOSs) have emerged in recent years as valuable prebiotics (health-promoting non-digestible food ingredients) because of their beneficial effects as *Bifidobacterium* growth-promoting factors (Crittenden & Playne, 1996; Vásquez, Alonso, Domínguez, & Parajó, 2000). Among the non-digestible oligosaccharides, XOSs are one of the effective prebiotics on the bifidobacteria population of intestinal microbiota (Hsu, Liao, Chung, Hsieh, & Chan, 2004; Rycroft, Jones, Gibson, & Rastall, 2001).

XOSs are usually produced by xylanase hydrolysis of xylan extracted from plants, and therefore many fragments in XOSs have side residues because most xylans are branched, and usually have side residues of 1,2-linked-α-D-glucuronic acid or its 4-*O*-methyl ethers, 1,3-linked or 1,2-linked α-L-arabinose or *O*-acetyl groups on its β-1,4-linked xylopyransoyl backbones. The side residues can block xylanase and xylosidase reactions around branched area, and cause slow and incomplete digestion (Sunna & Autranikian, 1997; Puls & Schusel, 1992; Shao & Wiegel, 1995). Thus, efficient and complete degradation of XOSs requires the cooperation of different enzymes, including β-xylosidase, α-glucuronidase, α-L-arabinosidase or acetyl xylan esterase. Therefore, the ability of bifidobacteria to metabolise XOSs depends on the efficiency of their xylanolytic enzyme systems. A xylosidase and a few arabinosidases have been purified and characterized from bifidobacteria. They are β-D-xylosidase from *B. breve* K-110 (Shin, Lee, Han, Han, & Kim, 2003a), and arabinosidases from *B. adolescentis* DSM20083 (Van Laere, Beldman,

* Corresponding author. Fax: +86 25 83598838.
E-mail addresses: Zeng-hongyan@hotmail.com (H. Zeng), wlshao@js-mail.com.cn (W. Shao).

0308-8146/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2006.03.019

& Voragen, 1997, 1999), arabinosidase (Shin, Lee, Han, Han, & Kim, 2003b). The arabinosidase genes from *B. longum* B667 (Margolles & De Los Reyes-Gavila'n, 2003) and *B. adolescentis* (Van den Broek et al., 2005) have been cloned and sequenced. However, no report has focused on the whole xylanolytic enzyme system in bifidobacteria.

In this work, we examined the xylanolytic enzyme systems in *B. adolescentis*, *B. bifidum* and *B. infantis*, and their activities on the degradation of XOSs. We also estimated the ability of *B. adolescentis* to utilize branched XOSs with and without addition of α-glucuronidase, which is an important de-branching enzyme not detectable in the xylanolytic enzyme systems of bifidobacteria.

2. Materials and methods

2.1. Bacterial strains and culture conditions

B. adolescentis, *B. infantis* and *B. bifidum* used in this study were obtained from China Center of Industrial Culture Collection (CICC). The organisms were grown at 37 °C statically in media supplemented with 0.1% (w/v) L-cysteine (Merck). The anaerobic media could be prepared in Hungate tubes (5% H_2: 95% N_2 using 0.1% (w/v) resazurin as the oxygen indicator) or outside employing the Hungate technique (Ljungdahl & Wiegel, 1986). The routine culture medium contained the following compounds per liter: 2 g soya peptone, 5 g urea, 5 g XOSs, 0.4 g $NaHCO_3$, 8 mg $CaCl_2$, 20 mg $MgSO_4$, 80 mg NaCl, 40 mg KH_2PO_4, and 40 mg K_2HPO_4. XOSs used in the media were prepared from alkali-extracted straw xylan pretreated with recombinant xylanase (rXyn) of *Thermomyces lanuginosus* (Schlacher, Holzmann, Hayn, Steiner, & Schwab, 1996). rXyn was over-expressed in *Escherichia coli* in this lab, and the amount used was 20 units (U) per gram xylan for 16 h at 65 °C. The XOSs we obtained contained mainly xylobiose, xylotriose and xylotetraose. In the tests for the induction of xylosidase and arabinosidase activities in *B. adolescentis*, 0.5% (w/v) glucose and XOSs were used as carbon sources, and the amount of soya peptone was reduced to 0.1% (w/v).

2.2. Preparation of crude enzymes

Bifidobacteria were cultivated to late log phase, and the cells were separated from culture broth by centrifugation at 5000*g* for 10 min. Crude enzymes included preparations of cell-free extract from cells, and extra-cellular protein from culture broth.

Extra-cellular protein was precipitated from culture broth by 70% saturated $(NH_4)_2SO_4$, dissolved in and dialyzed against the buffer composed of 25 mM imidazole and 25 mM potassium phthalate (IP buffer). Cells were washed three times with distilled water, and re-suspended in IP buffer, pH 6.0. Cell-free extracts were prepared at 4 °C by disrupting cells with a French press at 7.1×10^4 kPa, and centrifuged for 30 min at 20,000*g*.

Protein concentration in the preparations was measured by the Bradford method (Bradford, 1976) using bovine serum albumin (Sigma) as a standard.

2.3. Enzymes assays

2.3.1. α-L-Arabinosidase and β-xylosidase assays

p-Nitrophenyl β-D-xyloside (*p*NPX), and *p*-nitrophenyl α-L-arabinofuranoside (*p*NPA) (Sigma, St. Louis, MO, USA) were used as substrates for β-xylosidase and α-L-arabinosidase activity assay. One unit of activity was defined as the amount of enzyme that produced 1 μmol of *p*-nitrophenol per min at 40 °C. The reaction mixture contained 10 μl of 10 mM of substrates, 180 μl of IP buffer (pH 6.0 at 40 °C) and 10 μl of properly diluted crude enzymes. After incubation for 10 min at 40 °C, the reaction was stopped by adding 0.6 ml of 1 M Na_2CO_3, and the amount of *p*-nitrophenol released from substrates was measured by spectrophotometer at λ_{405}. A standard curve was prepared with *p*-nitrophenol (Sigma).

2.3.2. Xylanase assay

Xylan from oat spelt (Sigma) was used as the substrate. Reaction mixture contained 100 μl of 0.5% (w/v) xylan in water, 80 μl of IP buffer (pH 4.0–8.0), and 20 μl properly diluted crude enzyme. Reaction was performed by incubating for 10 min at 40–60 °C, and stopped by adding 600 μl NaOH/4-hydroxybenzoic acid hydrazide solution (Lever, 1972). The mixture was boiled for 10 min, cooled down in an ice bath, and the absorbance was read at 410 nm. One unit of xylanase activity was defined as the amount of enzyme releasing 1 μmol of reducing sugar per min. A standard curve was prepared with xylose (Sigma).

2.3.3. Acetyl xylan esterase assay

Assays were performed for 10 min at 40–60 °C, pH 5.8–8.0, using 4-methylumbelliferyl acetate (4-MUA) (Sigma) as substrate as described elsewhere (Shao & Wiegel, 1995). An enzyme unit was defined as the amount of enzyme that produced 1 μmol of 4-methylumbelliferone per min.

2.3.4. α-Glucuronidase assay

The substrate for α-glucuronidase was 4-O-methyl-D-glucurono-D-xylan (Sigma) pretreated with purified rXyn (10 U/g) for 16 h at 65 °C. α-Glucuronidase activity was determined by measuring the release of 4-*O*-methyl-α-D-glucuronic acid from the substrate. The amount of enzyme to produce 1 μmol of glucuronic acid per min was defined as 1 U. The reaction mixture contained 10 μl of substrate (2% w/v), 70 μl of IP buffer (pH 4.0–8.0), and 20 μl of crude enzymes. After 30 min of incubation at 40–60 °C, the reaction was stopped by adding 0.3 ml of copper reagent (Milner & Avigad, 1967). Then the reaction mixture was boiled for 10 min and cooled in an ice bath and centrifuged (10,000*g*) for 1 min. The supernatant was mixed up with 0.2 ml of arsenomolybdate reagent (Nelson, 1944), and

the absorbance at 620 nm was measured after 0.6 ml of H_2O was complemented.

2.4. Analysis of hydrolysis products

2.4.1. Preparation of analytical XOSs and de-branched XOSs

Birch wood xylan (Sigma) of 5% (w/v) in IP buffer was hydrolyzed by adding purified rXyn to a concentration of 20 U per gram xylan, and incubated at 65 °C for 10 h. Then hydrolysates were mixed with three volumes of 95% (v/v) ethanol, and centrifuged to remove precipitated polymers. The XOSs in the supernatant was lyophilized, re-dissolved in distilled water, and divided into two tubes. Into one of the tubes, purified recombinant α-glucuronidase of *Thermotoga maritima* (rAgu, prepared in this lab) (Xue, Mao, & Shao, 2004) was added to 6 U per gram xylan, and a reacted for 16 h at 80 °C to produce de-branched XOSs (dXOSs).

2.4.2. The degradation and utilization of XOSs and dXOSs by B. adolescentis

The ability of *B. adolescentis* to degrade XOSs or dXOSs was determined by incubating cell-free extract with XOSs or dXOSs at a ratio of about xylosidase 25 U per gram XOSs, and then the degradation products were subjected to the analysis of high performance liquid chromatography (HPLC, Waters 600, Waters Co., USA) on a carbohydrate analysis column (Waters Sugar-Pak I, USA) with water as a mobile phase. The xylooligosaccharides were quantified by a refractive-index detector (Waters Co., USA). The growth rates were determined during the time when organism was growing in the media with 0.5% (w/v) XOSs and dXOSs as main carbon source.

3. Results

3.1. Xylanolytic enzyme systems in Bifidobacteria

The whole xylanolytic enzymes were examined in the cell-free extract and extracellular proteins of *B. adolescentis*, *B. bifidum* and *B. infantis*. β-D-Xylosidase and α-L-arabinosidase activities were found in cell-free extracts from all these strains, no activity was detected in the extracellular fraction, and no xylanase, α-glucuronidase and acetyl esterase activities were detectable in any crude enzyme preparations. When the bacteria were grown in routine medium, xylosidase and arabinosidase activities (U mg^{-1}) determined at 45 and 37 °C were 0.993 and 0.084 in *B. adolescentis*, 0.787 and 0.025 in *B. infantis*, and 0.644 and 0.022 in *B. bifidum*, respectively. The xylosidase and arabinosidase activities in *B. adolescentis* differed when the organism grown in the media (0.1%, w/v soya peptone) with glucose, xylose or XOSs as main carbon sources (Table 1). The organism produced more xylosidase when was grown on xylose and XOSs than on glucose. The highest level of arabinosidase was produced when the organism was grown on XOSs.

Table 1
Effect of different carbon sources on the xylosidase and arabinosidase production of *B. adolescentis*

Carbon sources[a]	Xylosidase activity (U mg^{-1})	Arabinosidase activity (U mg^{-1})
Glucose	0.060	0.014
Xylose	0.110	0.014
XOSs	0.106	0.020

[a] Carbon sources were added 1% (w/v) to the routine culture medium and incubated for 24 h at 37 °C and pH 7.0. These data were only of the cell free extract activity.

3.2. Effects of temperature and pH on enzyme activities and stabilities

The optimal temperature of β-D-xylosidase and α-L-arabinosidase were at 45 and 40 °C, respectively (Fig. 1a). The α-L-arabinosidase and β-D-xylosidase activities decreased at temperatures below 35 °C and above 60 °C, and had the half-life of 1 h at 50 °C (Fig. 1b).

The crude enzyme from *B. adolescentis* showed high β-D-xylosidase activity in a pH range of 4.8-6.0 with the optimum pH at 5.6, and high α-L-arabinosidase activity in the pH range of 4.4–5.6 with the optimum activity at pH 5.0 (Fig. 1c). Low β-D-xylosidase and α-L-arabinosidase activity were detected at pH 4.0, and about 40% of the maximum activities were observed at pH 6.8. The α-L-arabinosidase and β-D-xylosidase were stable for 1 and 2 h at 37 °C over the pH range 4.9–6.4, respectively (Fig. 1d).

3.3. Hydrolysis products from birch wood xylan

The amount of cell-free extract affected the hydrolysis of XOSs. When the amount of the xylosidase from *B. adolescentis* was over 15 U per gram XOSs, the amount of reducing sugars increased slowly (data not shown), and thus the following data represented the final products, in which cell-free extracts were used as 25 U xylosidase per gram XOSs. The result of HPLC showed that the preparation of XOSs contained xylobiose, xylotriose and xylotetraose (Fig. 2a); the main products were xylose and a small amount of xylobiose after XOSs incubated with cell-free extract of *B. adolescentis* were (Fig. 2b); and only xylose was observed after dXOSs were treated in the same way (Fig. 2c). HPLC profiles revealed that 10% more xylose was obtained by adding α-glucuronidase (Fig. 2).

3.4. Growth of B. adolescentis on XOSs and dXOSs

When *B. adolescentis* was grown on XOSs and dXOSs, the doubling times were 12 and 10 h, and the final cell densities, OD_{600} reached 0.379 and 0.415, respectively (Fig. 3).

4. Discussion

Complete digestion of xylan need a whole set of xylanolytic enzymes. We examined all these enzymes in three selected strains of bifidobacteria, which were grown under

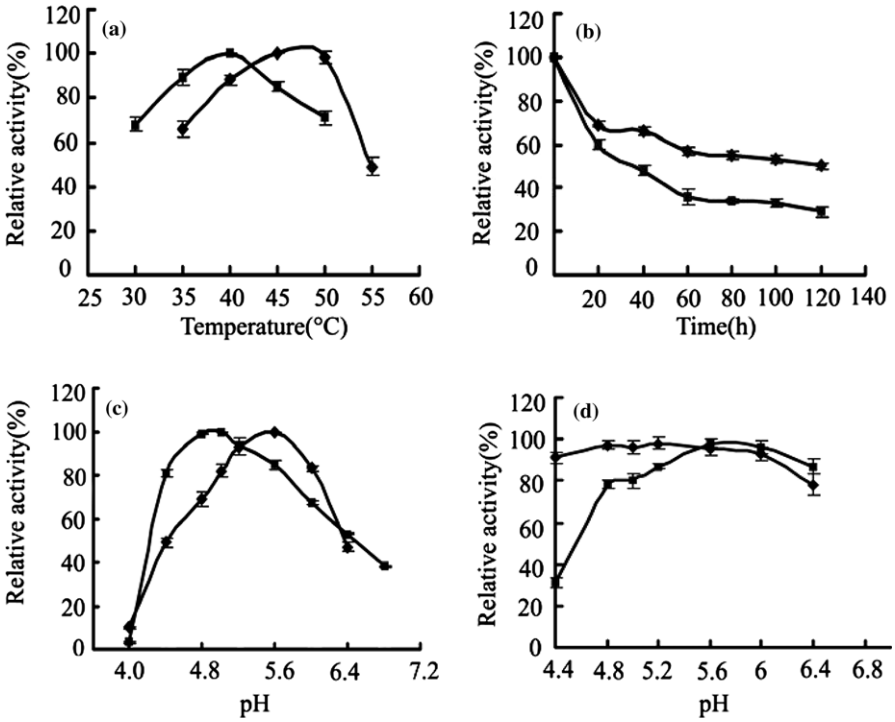

Fig. 1. Effects of temperature and pH on the activity of β-D-xylosidase (□) and α-L-arabinosidase (■). (a) Determination of temperature optimum at pH 6.0 with a 10-min assay. The highest activity determined for each enzyme was set to 100%. (b) Temperature stability of β-D-xylosidase (■) and α-L-arabinosidase (■). Residual enzymes activities were monitored after different times of incubation at 50 °C. The initial activity was defined as 100%. (c) Effects of pH on the activity of β-D-xylosidase (□) and α-L-arabinosidase (■) in 50 mM IP buffer (pH 4.0–7.0) at their optimal temperatures. The highest level of activity determined for each enzyme was set to 100%. (d) pH stability of β-D-xylosidase (□) and α-L-arabinosidase (■). β-D-xylosidase and α-L-arabinosidase were incubated for 2 and 1 h at 37 °C, respectively. The remaining activities of two enzymes were determined at their optimal temperatures. The activities at each pH value without preincubation of the enzymes represent 100% activities. Data points are means of two replicates of each experiment.

enzyme-inducing conditions. The results showed that all tested strains had only intracellular β-xylosidase and α-arabinosidase, but no xylanase, α-glucuronidase, and acetyl xylan esterase detectible under these assay conditions.

In this work, the T_{opt} and pH_{opt} for the β-D-xylosidase from *B. adolescentis*, were 45 °C and pH 5.6, which were higher than those of β-D-xylosidase (37 °C, pH 5.0) from *B. breve* K-110 (Shin et al., 2003a). The T_{opt} for α-L-arabinosidase activity (40 °C) was higher, and the pH_{opt} was lower than that of those for arabinosidases (30–37 °C, pH 6.0, using Wheat-flour arabinoxylan as substrate) from *B. adolescentis* DSM 20083 (Van Laere et al., 1997, 1999), while they were in agreement as found for the arabinosidases from *B. breve* K-110 (Shin et al., 2003b). The different enzyme incubation conditions may be responsibly for the differences obtained.

Xylanase gene *xylA* has been identified in *B. adolescentis* DSM 20083, but the putative gene does not contain a sequence encoding signal peptide (Van den Broek et al., 2005), implying that the xylanase will not be secreted if it is expressed. In this work, xylanase activity was found in neither cell-free extract nor extra-cellular protein, indicating that *xylA* was not expressed, or its gene product did not have xylanase activity. Fortunately, xylanase is no more an essential enzyme when XOSs are used as bifidobacteria growth-promoting factors.

The cost is quite high for the extraction of xylan from plant materials, and the isolation of XOSs from xylanase hydrolates. It is speculated elsewhere that XOS can be transported into certain bacteria for further digestion. The genome sequence of *B. longum* NCC2705 also revealed the presence of eight high-affinity MalEFG-type oligosaccharide transporters (Schell et al., 2002). Thus, it is necessary to examine how efficient XOSs are consumed by bifidobacteria, and how the utilization can be improved.

α-Glucuronidase is very important for xylan degradation because glucuronic acid residues exist in almost all xylans from grass, soft wood and hard wood. Only a few α-glucuronidases have been found in the nature, and their activities are low. We used recombinant α-glucuronidase to treat XOSs, and obtained dXOSs. The HPLC profiles indicated that more xylose was produced from dXOSs than from XOSs when they were used as substrates of enzymes in cell-free extracts of *B. adolescentis*, implying that some xylose was not released in XOSs. The faster growth and higher final cell density of the organism on dXOSs than on XOSs also supported the hypothesis that more xylose was available as carbon source for growth.

In most cases, the side chains protect the linkages around branched xylose residues from the attack of xylanase and xylosidase, and thus synergistic reactions have been observed betwen α-glucuronidase and xylanase or xylosidase

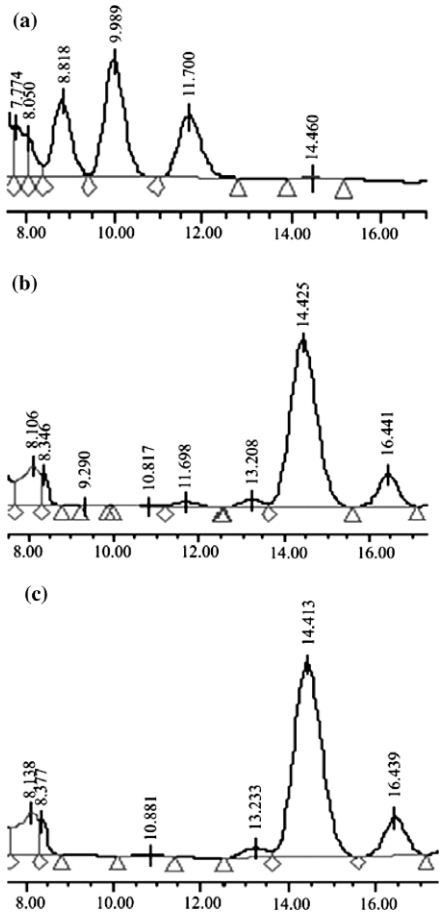

Fig. 2. High performance liquid chromatographic profiles (HPLC) of products from birch wood xylan hydrolyzed by the purified xylanase (a), xylan hydrolyzed by xylanase and enzymes in cell-free extract of *B. adolescentis* (b), xylan hydrolyzed by xylanase, α-glucuronidase and cell-free extract of *B. adolescentis* (c). A 10 μl sample was injected into the carbohydrate analysis column with water as mobile phase. Xylose, xylobiose and xylotriose (Suntory Ltd., Kyoto, Japan) were used as markers with retention times of 14.395, 11.674, and 9.975 min, respectively.

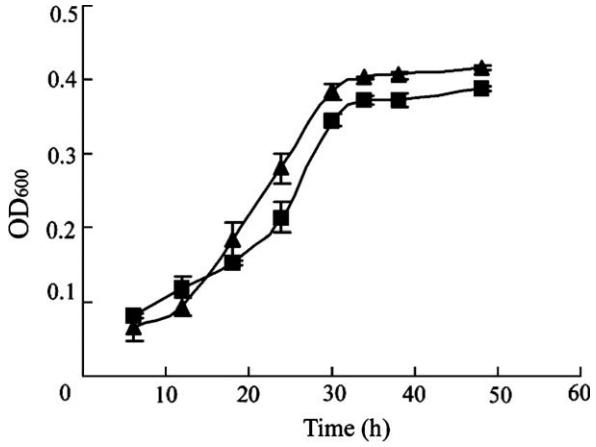

Fig. 3. The growth of *B. adolescentis* in the media containing 1% (w/v) XOSs (■), or dXOSs (▲). In the basal medium, other components were constant to the routine culture medium except that soya peptone was replaced with 0.05% (w/v) yeast extract. *B. adolescentis* was cultured at 37 °C. These data are representative of the mean of two experiments.

(Shao & Wiegel, 1995; Sunna & Autranikian, 1996). The xylosidase from *Thermoanaerobacterium* sp. JW/SL-YS485 exhibited high activity to glucuronosyl xylotetraose, low activity to glucuronosyl xylotriose, and almost no activity to glucuronosyl xylobiose (Shao & Wiegel, 1995). The molar ratio of xlose to glucuronic acid is about 10–1 in hardwood such as birch xylan. In this work, de-branching of α-glucuronidase caused about 10% increase of xylose (Fig. 2) as well as 10% more cells produced in the culture (Fig. 3), indicating that xylosidase of bifidobacteria was active over glucuronosyl xylobiose. But, the doubling time of the bacterium grown on dXOSs was about 20% shorter than that on XOSs, implying that the xylosidase activity was low on glucuronosyl xylobiose although the final level could be reached.

In our lab, the α-glucuronidase gene from *T. maritima* was cloned and over-expressed in *E. coli*. Previously we found that the addition of the recombinant α-glucuronidase to xylanase could significantly increase the production of xylobiose from xylan (Xue et al., 2004, Chinese patent CN1584040A). From this work, we conclude that de-branching of α-glucuronidase can enhance the degradation and utilization of XOSs by bifidobacteria, and significantly accelerate the growth of bifidobacteria.

Acknowledgements

This work was supported by grants from the High Technology Foundation of Jiangsu, China (BG2005326), the Nature Science Foundation of higher school of Jiangsu, China (05KJB180059).

References

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry, 72*, 248–254.

Crittenden, R. G., & Playne, M. J. (1996). Production, properties and application of food-grade oligosaccharides. *Trends in Food Science and Technology, 7*, 353–361.

Hsu, C. K., Liao, J. W., Chung, Y. C., Hsieh, C. P., & Chan, Y. C. (2004). Xylooligosaccharides and Fructooligosaccharides affect the intestinal microbiota and precancerous colonic lesion development in rats. *Journal of Nutrition, 134*, 1523–1528.

Lever, M. A. (1972). New reaction for folorimetric determination of carbohydrates. *Analytical Biochemistry, 47*, 27–34.

Ljungdahl, L. G., & Wiegel, J. (1986). Work with anaerobic bacteria. In A. L. Demain & J. E. Davis (Eds.), *Manual of industrial microbiology and biotechnology* (pp. 84–96). Washington, DC: American Society for Microbiology.

Margolles, A., & De Los Reyes-Gavila'n, G. C. (2003). Purification and functional characterization of a novel α-L-arabinofuranosidase from *Bifidobacterium longum* B667. *Applied and Environment Microbiology, 69*, 5096–5103.

Milner, Y., & Avigad, G. (1967). A copper reagent for the determination of hexuronic acid and certain ketohexoses. *Carbohydrate Research, 4*, 359–361.

Nelson, N. (1944). A photometric adaptation of the Somogyi method for determination of glucose. *Biological Chemistry, 153*, 75–380.

Puls, J., & Schusel, J. (1992). Chemistry of hemicellulose: relationship between hemicellulose structure and enzyme required for hydrolysis. In M. P. Coughlan & G. P. Hazlewood (Eds.), *Hemicellulose and hemicellulases* (pp. 1–28). London: Portland Press.

Rycroft, C. E., Jones, M. R. G., Gibson, R., & Rastall, R. A. (2001). A comparative in vitro evaluation of the fermentation properties of prebiotic oligosaccharides. *Journal of Applied Microbiology, 91*, 878–887.

Schell, M. A., Karmirantzou, M., Snel, B., Vilanova, D., Berger, B., Pessi, G., et al. (2002). The genome sequence of *Bifidobacterium longum* reflects its adaptation to the human gastrointestinal tract. *Proceedings of the National Academy of Sciences of the United States of America, 99*, 14422–14427.

Schlacher, A., Holzmann, K., Hayn, M., Steiner, W., & Schwab, H. (1996). Cloning and characterization of the gene for the thermostable xylanase XynA from *Thermomyces lanuginosus*. *Journal of Biotechnology, 49*, 211–218.

Shao, W. L., & Wiegel, J. (1995). Purification and characterization of two acetyl xylan esterases from *Thermoanaerobacterium* sp. strain JW/SL-YS485. *Applied and Environment Microbiology, 61*, 729–733.

Shin, H. Y., Lee, J. H., Han, Y. O., Han, M. J., & Kim, D. H. (2003a). Purification and characterization of ginsenoside Ra-hydrolyzing β-D-xylosidase from *Bifidobacterium breve* k-110, a human intestinal anaerobic bacterium. *Biological Pharmaceutical Bulletin, 26*, 1170–1173.

Shin, H. Y., Lee, J. H., Han, Y. O., Han, M. J., & Kim, D. H. (2003b). Purification and characterization of α-L-arabinopyranosidase and α-L-arabinofuranosidase from *Bifidobacterium breve* K-110, a human intestinal anaerobic bacterium metabolizing ginsenoside Rb2 and Rc. *Applied Environmental Microbiology, 69*, 7116–7123.

Sunna, A., & Autranikian, G. (1997). Xylanolytic enzymes from fungi and bacteria. *Critical Reviews in Biotechology, 17*, 39–67.

Van den Broek, L. A. M., Lloyd, R. M., Beldman, G., Verdoes, J. C., McCleary, B. V., & Voragen, A. G. J. (2005). Cloning and characterization of arabinoxylan arabinofuranohydrolase-D3 (AXHd3) from *Bifidobacterium adolescentis* DSM20083. *Applied Microbiology and Biotechnology, 67*, 641–647.

Van Laere, K. M., Beldman, G., & Voragen, A. G. J. (1997). A new arabinofuranohydrolase from *Bifidobacterium adolescentis* able to remove arabinosyl residues from double-substituted xylose units in arabinoxylan. *Applied Microbiology, 47*, 231–235.

Van Laere, K. M., Voragen, C. H. L., Kroef, T., Van den Broek, L. A. M., Beldman, G., & Voragen, A. G. J. (1999). Purification and mode of action of two different arabinoxylan arabinofuranohydrolases from *Bifidobacterium adolescentis* DSM 20083. *Applied Microbiology, 51*, 606–613.

Vásquez, M. J., Alonso, J. L., Domínguez, H., & Parajó, J. C. (2000). Xylooligosaccharides: manufacture and applications. *Trends in Food Science and Technology, 11*, 387–393.

Xue, Y. M., Mao, Z. G., & Shao, W. L. (2004). Expression and purification of thermostable α-glucuronidase from *Thermotoga maritime*. *Chinese Journal of Biotechnology, 20*, 554–560.