Appl Microbiol Biotechnol (2002) 58:439–445
DOI 10.1007/s00253-001-0911-6

ORIGINAL PAPER

M.-N. Hung · B. H. Lee

Purification and characterization of a recombinant β-galactosidase with transgalactosylation activity from *Bifidobacterium infantis* HL96

Received: 21 October 2001 / Accepted: 16 November 2001 / Published online: 1 February 2002

Abstract A β-galactosidase isoenzyme, β-GalI, from *Bifidobacterium infantis* HL96, was expressed in *Escherichia coli* and purified to homogeneity. The molecular mass of the β-GalI subunit was estimated to be 115 kDa by SDS-PAGE. The enzyme appeared to be a tetramer, with a molecular weight of about 470 kDa by native PAGE. The optimum temperature and pH for *o*-nitrophenyl-β-D-galactopyranoside (ONPG) and lactose were 60°C, pH 7.5, and 50°C, pH 7.5, respectively. The enzyme was stable over a pH range of 5.0–8.5, and remained active for more than 80 min at pH 7.0, 50°C. The enzyme activity was significantly increased by reducing agents. Maximum activity required the presence of both Na^+ and K^+, at a concentration of 10 mM. The enzyme was strongly inhibited by *p*-chloromercuribenzoic acid, divalent metal cations, and Cr^{3+}, and to a lesser extent by EDTA and urea. The hydrolytic activity using lactose as a substrate was significantly inhibited by galactose. The K_m and V_{max} values for ONPG and lactose were 2.6 mM, 262 U/mg, and 73.8 mM, 1.28 U/mg, respectively. β-GalI possesses strong transgalactosylation activity. The production rate of galactooligosaccharides from 20% lactose at 30 and 60°C was 120 mg/ml, and this rate increased to 190 mg/ml when 30% lactose was used.

Introduction

Bifidobacteria constitute one of the major classes of organisms in the intestine of healthy humans, and are helpful in maintaining a normal intestinal flora (Mitsuoka 1990). The reduction or disappearance of bifidobacteria in the intestine often coincides with poor health status. In order to maintain high bifidobacteria counts and protect the intestinal tract from the proliferation of harmful bacteria, live bifidobacteria or certain selective compounds defined as "prebiotics" (Gibson and Roberfroid 1995) have been used as dietary supplements. For instance, prebiotics such as galactooligosaccharides (GaOS), when administered orally, were proved to result in increases of bifidobacteria counts in human fecal flora (Ohtsuka et al. 1989; Ito et al. 1993).

GaOS can be synthesized either by classical organic methods, by glycosyltransferases or by β-galactosidases (Schmidt 1986; Ichikawa et al. 1992; Farkas and Thiem 1999). Using the transgalactosylation activity of β-galactosidase to synthesize GaOS from lactose could be very efficient and inexpensive if the enzyme chosen possesses strong transgalactosylation activity. The formation of GaOS by β-galactosidases from yeast, bacteria, and fungi has been reported by several investigators (Burvall et al. 1979; Asp et al. 1980; Greenberg and Mahoney 1983; Mozaffar et al. 1985; Toba et al. 1985; Onishi and Tanaka 1995, 1996). However, yields of product were less than 80 mg/ml. β-GalI, one of the three β-galactosidase isoenzymes (β-Gal-I, -II, and -III) in *Bifidobacterium infantis* HL96, has revealed higher GaOS production activity in our previous studies, with a major trisaccharide identified as 3′-galactosyl-lactose (Hung et al. 2001). Two β-galactosidase isoenzyme genes, *β-galI* and *β-galIII* from this strain have been cloned in *Escherichia coli*, and their nucleotide sequences determined (Hung and Lee 1998; Hung et al. 2001). In this paper, we describe the purification and characterization of β-GalI.

M.-N. Hung · B.H. Lee (✉)
Department of Food Science and Agricultural Chemistry,
McGill University, 21,111 Lakeshore Road,
Ste-Anne-de-Bellevue, Quebec, H9X 3V9 Canada
e-mail: blee@macdonald.mcgill.ca
Tel.: +1-514-3987979, Fax: +1-514-3987977

B.H. Lee
Food Research and Development Center,
Agriculture and Agri-Food Canada, Ste-Hyacinthe,
Quebec, Canada

Materials and methods

Chemicals and reagents

All chemicals used in this study were of analytical reagent grade and were purchased from Sigma or from other suppliers as specified. Bacto-tryptone, yeast extract and agar were from Difco.

Over-expression of β-GalI and preparation of cell extracts

The gene encoding β-GalI from *B. infantis* HL96, a strain isolated at the Food Research and Development Center of Agriculture and Agri-Food Canada (Ste-Hyacinthe, Quebec), was cloned into pET24(+) (Novagen) by PCR using pBIG1 (Hung et al. 2001) as template, and the overexpression plasmid, pEBIG1, was introduced into *E. coli* ER2566. ER2566 (pEBIG1), ER2566 (pET24), and JM109 (pBIG1) cell extracts were prepared for SDS-PAGE analysis by the method described in Hung et al. (2001). For purification of β-GalI, the cell extract of ER2566 (pEBIG1) was prepared by the same method, except that the cells were washed twice and suspended in Z buffer (100 mM Na_2HPO_4, 100 mM NaH_2PO_4, 10 mM KCl, 1 mM $MgSO_4$, 50 mM 2-mercaptoethanol, pH 7.5) before sonication treatment.

Enzyme purification

The cell extract was precipitated at 4°C with ammonium sulfate (20–50%), the pellet was resuspended in 20 ml of Z buffer and dialyzed overnight against the same buffer. Portions (500 µl) of the concentrated sample were applied to an anion-exchange column (Mono Q HR 5/5, Pharmacia) using a FPLC system (Pharmacia) equilibrated with Z buffer. Elution was performed using a linear gradient of 0.5 M salt in Z buffer at a flow rate of 0.5 ml/min, and 1 ml fractions were collected. Active β-galactosidase fractions collected from several chromatographic runs were pooled, desalted and further concentrated using the Centriplus system (Millipore). The active fraction (200 µl) obtained from ion-exchange chromatography was applied to a Superose-12 HR 10/30 column (Pharmacia) equilibrated with Z buffer. Elution was performed at a flow rate of 0.2 ml/min, and 1 ml fractions were collected. Fractions exhibiting β-galactosidase activity were pooled and assayed for protein and enzymatic activity.

Enzyme and protein assays

β-Galactosidase activity was measured and one unit of activity was defined by previously described methods (Hung et al. 2001) with slight modification. Enzyme samples were incubated with *o*-nitrophenyl-β-D-galactopyranoside (ONPG) in Z buffer (pH 7.5), at 60°C for 10 min; the *o*-nitrophenol released was subsequently quantitatively determined using a spectrophotometer. The specific activity of the enzyme was expressed as units per milligram of protein. Protein concentration was determined by the method of Bradford (1970) using bovine serum albumin as standard.

Gel electrophoresis and activity staining

To study the over-expression and the molecular mass of β-GalI, 10% SDS-PAGE was carried out following the method of Laemmli (1970). To determine the purity and the molecular mass of native β-GalI, 8% non-denaturing PAGE was performed using marker proteins [thyroglobulin (669 kDa), ferritin (440 kDa), catalase (232 kDa), and lactate dehydrogenase (140 kDa)] (High Molecular Weight Calibration Kit, Pharmacia), and the gels were stained with Coomassie Brilliant Blue R-250. Activity staining was carried out as previously described (Hung and Lee 1998).

Enzyme kinetics

The K_m and V_{max} of the purified enzyme were determined using the substrate ONPG at 60°C in Z buffer without $MgSO_4$. Initial reaction rates (U/ml of enzyme) were measured at a series of ONPG concentrations between 0.1 and 10.0 mM. The kinetics with lactose as substrate were studied by measuring released glucose using HPLC chromatography. Initial rates of lactose hydrolysis were determined at 50°C with lactose concentrations in the range 5.0–50.0 mM in Z buffer without $MgSO_4$. After adding an equal volume of cold H_2SO_4 (1.2 M) to stop the reaction, each sample was filtered and injected onto the HPLC column. The kinetic constants were computed from the slope and intercept of the regression line on Lineweaver-Burk plots.

Effect of pH and temperature

Three buffer systems, citrate buffer (50 mM, pH 4.5–6.0), sodium phosphate buffer (50 mM, pH 6.5–7.5), and boric acid–borax buffer (50 mM, pH 8.0–9.0) were used for measuring the optimal pH for enzyme activity. To estimate the pH stability, enzyme samples were preincubated in different buffers at room temperature for 3 h before adding the substrate. Temperature optimum was determined over the range of 28–70°C by incubating for 10 min in Z buffer. Thermal stability was estimated by incubating the enzyme at the desired temperature, and residual activity was measured at 60°C under standard assay conditions.

Effects of inhibitors and activators

Enzyme samples were incubated with 10 mM of various carbohydrates and 1 mM and 10 mM of reducing agents, a thio-binding agent, EDTA, and urea, individually, in 10 mM ONPG solution (Na-phosphate buffer, pH 7.5) at 60°C for 10 min. Enzyme activity without effectors was used as control. The effect of carbohydrates on the rate of lactose hydrolysis was determined by incubating the enzyme with 10 mM of carbohydrate and 20 mM of lactose in Na-phosphate buffer at 50°C for 10 min; the extent of lactose hydrolysis was analyzed by HPLC. To study the effect of various cations, the enzyme was dialyzed against 10 mM Tris-HCl, pH 7.5 for 24 h, concentrated by Centriplus (Millipore), and cations (final concentration 1.0, 10, or 100 mM; chloride or sulfate form) were then incubated with enzyme and 10 mM ONPG in 10 mM Tris-HCl buffer (pH 7.5, with or without 10 mM Na^+) at 60°C for 10 min. Enzyme activity measured without added cations was used as control.

Transgalactosylation activity assay and HPLC

The time-course synthesis of GaOS carried out under specified conditions was used to measure transgalactosylation activity. β-GalI enzyme (25 U) was incubated with 10 ml of either 20% or 30% (w/v) lactose solution in 50 mM Na-phosphate buffer (pH 7.5) at 30°C or 60°C for 30 h under constant agitation (100 rpm) in a shaker incubator. Samples were withdrawn at 5 h intervals and analyzed by HPLC following the method previously described (Hung et al. 2001). The GaOS yield (mg/ml) was calculated from the total amount of saccharides eluted at the retention times between standard sugars, lactose and stachyose.

Results

Purification

The recombinant plasmid pEBIG1 significantly increased the expression level of β-GalI. Figure 1 shows that a protein band corresponding to β-GalI was apparent in the lane containing the sample of ER2566(pEBIG1), whereas this band was invisible in lanes corresponding to pET24 and pBIG1. The cell extract from IPTG-induced ER2566 (pEBIG1) cells was purified to homogeneity, and the results are summarized in Table 1. The specific activity of purified β-GalI was 568.7 U/mg, which is about 15.5-fold higher than that of the crude ex-

Table 1 Purification of β-galactosidase-I (β-GalI) from ER2566 (pEBIG1)

Purification step	Total protein (mg)	Total activity (units)	Specific activity (units/mg)	Purification	Recovery factor (%)
Crude extract	219.0	8,035	36.7	1.0	100
Ammonium sulfate precipitation	87.6	6,200	70.8	1.9	77.2
Mono Q ion-exchange	13.2	5,231	396.3	10.8	65.1
Superose-12 gel filtration	6.5	3,697	568.7	15.5	46.0

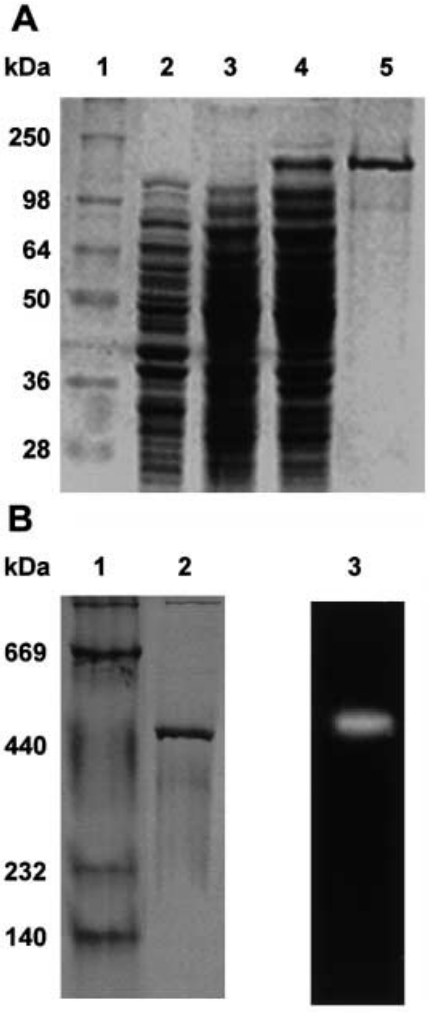

Fig. 1 **A** SDS-PAGE analysis of the over-expressed β-galactosidase-I (β-GalI) in *Escherichia coli* ER2566, and purified β-GalI. Lanes: *1* marker proteins (molecular masses are indicted); *2*, *3*, *4* cell extracts of JM109(pBIG1), ER2566(pET24), and ER2566(pEBIG1), respectively; *5* purified β-GalI. **B** Native PAGE analysis and activity staining of the purified β-GalI. Lanes: *1* marker proteins, *2* purified β-GalI, *3* activity staining

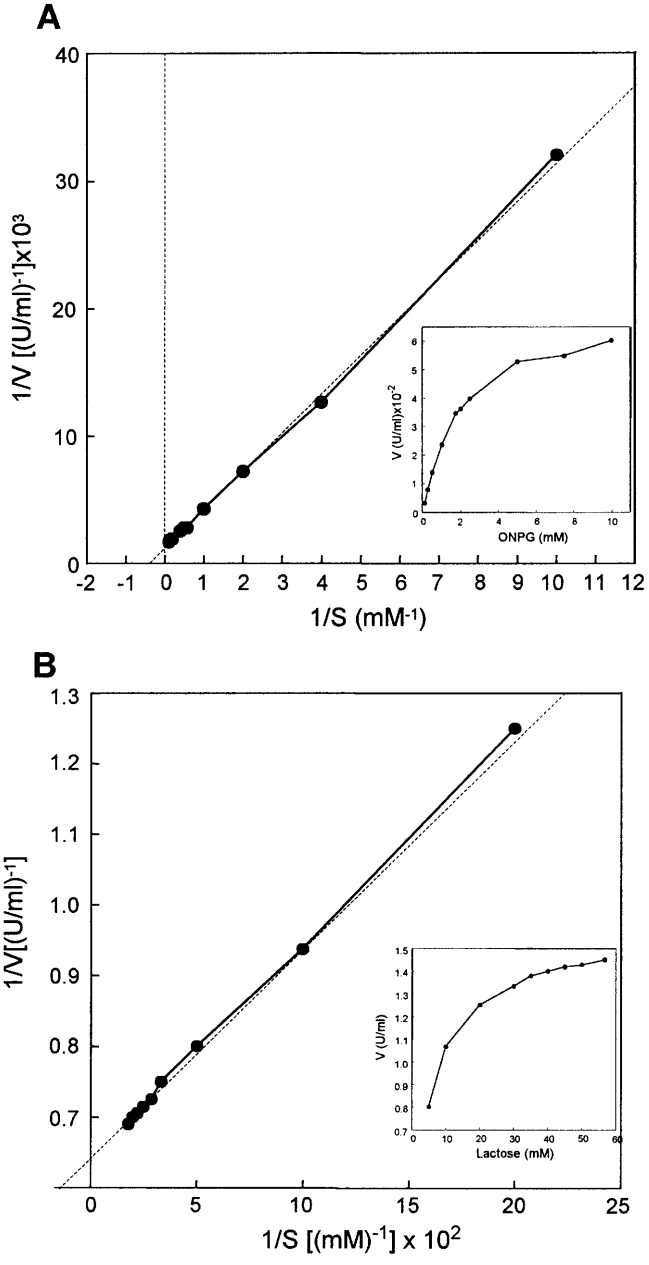

Fig. 2 Lineweaver-Burk plots of β-GalI activity with **A** *o*-nitrophenyl-β-D-galactopyranoside (*ONPG*) or **B** lactose

tract. The purification sequence allowed for the recovery of 46% of the original crude enzyme activity. The homogeneity of purified β-GalI was verified on SDS-PAGE and native PAGE, which showed a single band coinciding with the activity band developed by activity staining (Fig. 1).

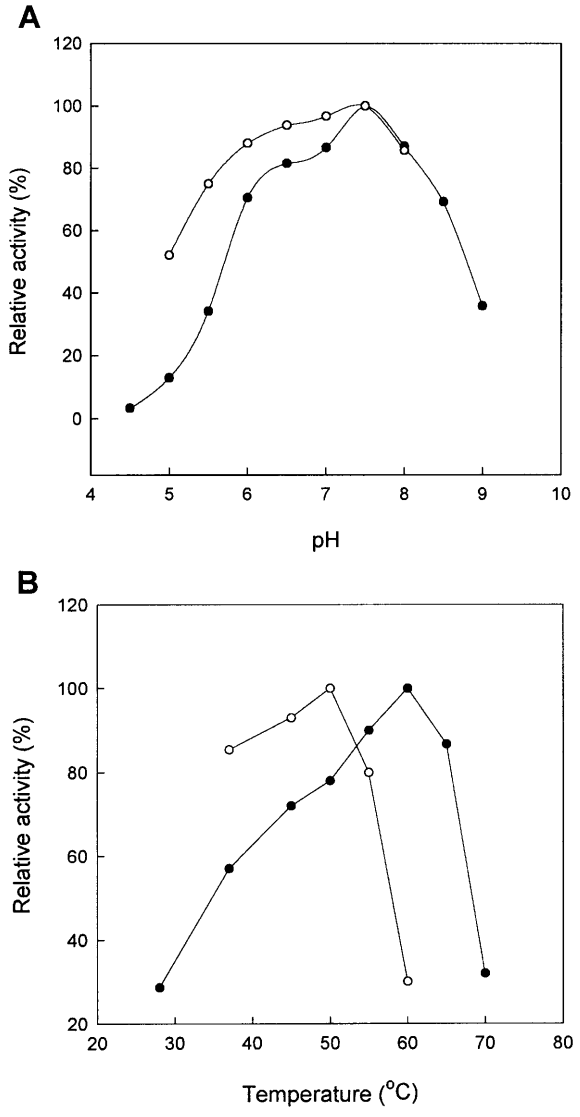

Fig. 3 Optimal pH **(A)** and temperature **(B)** of the purified β-GalI using ONPG (*black circles*) or lactose (*open circles*) as substrate. The buffers used are citrate buffer (pH 4.5–6.0), Na-phosphate buffer (pH 6.5–7.5) and boric acid-borax buffer (pH 8.0–9.0)

Molecular mass

The molecular mass of the denatured β-GalI was estimated to be 115 kDa by SDS-PAGE, as shown for the purified protein or the over-expressed protein from the cell extract (Fig. 1A). The molecular mass of native β-GalI was estimated by using gel filtration chromatography on a Superose-12 (HR 12/30) column. Thyroglobulin, ferritin, catalase (Pharmacia), and *E. coli* β-galactosidase (Boehringer Mannheim) were used as reference proteins. β-GalI eluted at a retention time similar to that of *E. coli* β-galactosidase, corresponding to the 450–500 kDa range. The molecular mass of the native enzyme was also estimated to be about 470 kDa on native PAGE (Fig. 1B).

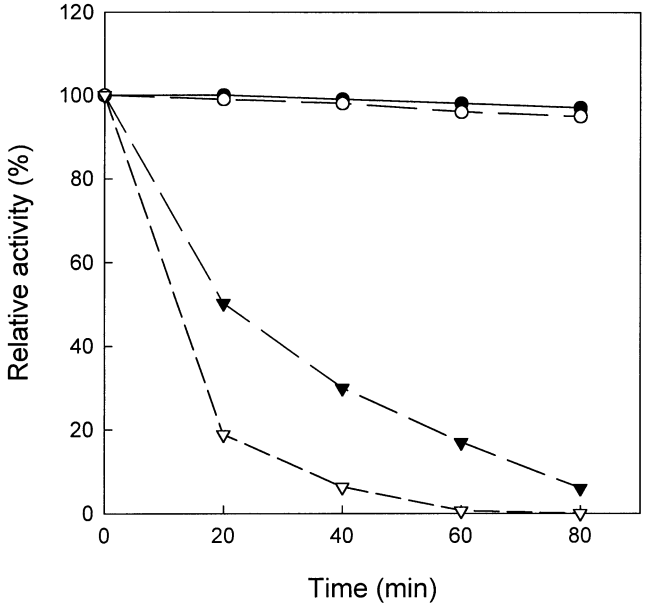

Fig. 4 Stability of β-GalI at different temperatures: *black circles* 37°C, *open circles* 50°C, *black triangles* 60°C, *open triangles* 70°C

Enzyme kinetics

The kinetic constant (K_m) and maximum reaction velocity (V_{max}) of β-GalI were calculated to be 2.6 mM and 262 U/mg for ONPG, and 73.8 mM and 1.28 U/mg for lactose, respectively (Fig. 2).

Effect of pH and temperature

The optimal pH of β-GalI was determined to be pH 7.5 for both ONPG and lactose hydrolysis (Fig. 3A). Preincubation of the enzyme in different buffers (pH 4.5–9.0) had no effect on the pH optimum profile. The enzyme retained more than 90% of its activity in the range of pH 5.0–8.5 after 3 h incubation (data not shown). Optimum temperature for the activity with ONPG was 60°C (Fig. 3B), and 50°C for lactose hydrolysis. After pre-incubation in Z buffer at 37°C or 50°C for 80 min, the enzyme retained over 90% of its activity, whereas only about 50% of the activity remained after pre-incubation at 60°C for 20 min (Fig. 4).

Effect of cations, carbohydrates and other reagents

The enzyme was significantly activated by Na^+ and K^+, and required both ions at 10 mM for maximum activity, which was 25 times higher than the control (Table 2). All the divalent cations tested, as well as Cr^{3+}, markedly inhibited the enzyme activity. In the presence of 10 mM Mg^{2+}, the activation by 10 mM Na^+ dropped by about 55%, and complete inhibition was observed when 10 mM of Zn^{2+}, Hg^{2+}, or Cu^{2+} were added along

Table 2 Effects of metallic cations on the activity of the purified β-GalI

Cations[a]	Relative activity (%)		
	1 mM	10 mM	100 mM
None	100	–	–
Na^{+}	1,050	1,900	1,900
K^{+}	1,300	1,825	1,825
K^{+} [c]	1,574	2,500	2,500
Mg^{2+} [c]	1,220	850	–
Fe^{2+} [c]	1,750	1,200	–
Mn^{2+} [c]	300	225	–
Ca^{2+} [c]	279	75	–
Co^{2+} [c]	250	50	–
Zn^{2+} [c]	75	0	–
Hg^{2+} [c]	20	0	–
Cu^{2+} [b, c]	50	0	–
Cr^{3+} [c]	260	50	–

[a] Added as chloride
[b] Added as sulfate
[c] Tested in the presence of 10 mM Na+

Table 3 Effect of carbohydrates on the activity of the purified β-GalI

Carbohydrate (10 mM)	Relative activity (%)	
	o-nitrophenyl-β-D-galactopyranoside (ONPG)	lactose
None	100	–
Glucose	105	98
Galactose	100	45
Maltose	105	107
Lactose	100	100
Raffinose	108	101
Fructose	83	94

Table 4 Effect of various reagents on the activity of the purified β-GalI

Reagent	Relative activity (%)	
	1 mM	10 mM
Control	100	100
Cysteine-HCl	315	747
Mercaptoethanol	310	815
Glutathione	374	925
p-Chloromercuribenzoic acid (PCMB)	0	0
EDTA	54	50
Urea	86	60

with Na^{+}. The inhibitory effects of 1 mM of either Mn^{2+}, Ca^{2+}, Co^{2+}, and Cr^{3+} were similar in that more than 84% of the activity was inhibited. Glucose, galactose, lactose, maltose and raffinose showed no effect on ONPG hydrolysis activity (Table 3). With respect to lactose hydrolysis, 10 mM galactose in 20 mM lactose was found to significantly inhibit the activity at 50°C during a 10 min incubation. The enzyme activity was markedly enhanced by 1 mM or 10 mM of either cysteine, glutathione or mercaptoethanol, while no β-GalI activity was detected when the thio-binding agent, *p*-chloromercuribenzoic acid (PCMB) was added (Table 4). This enzyme activity was moderately inhibited by EDTA and urea.

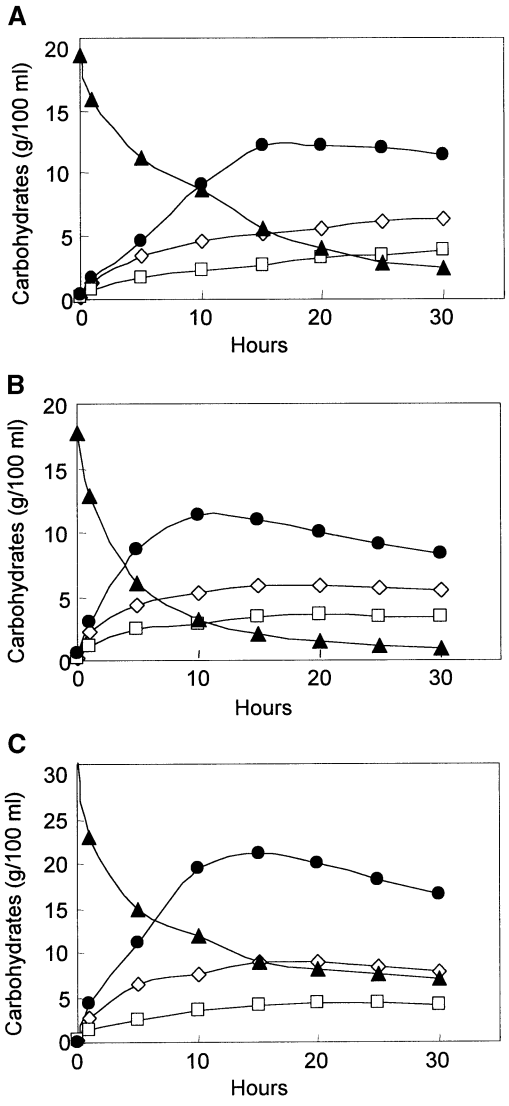

Fig. 5A–C Time course of production of galactooligosaccharides (GaOS) by purified β-GalI. A solution containing β-GalI and 20% (**A**, **B**) or 30% (**C**) lactose in Na-phosphate buffer was incubated at 30°C (**A**) or 60°C (**B**, **C**); *open diamonds* glucose, *open squares* galactose, *black triangles* lactose, *black circles* GaOS

GaOS synthesis

Three oligosaccharides were produced besides the expected glucose and galactose from the hydrolysis of lactose by the purified enzyme. Figure 5A shows the time-course of GaOS production from 20% lactose by β-GalI at 30°C in Na-phosphate buffer. The maximum amount of GaOS obtained after 15 h incubation was 120 mg/ml, and this amount was maintained until the end of the incubation period. The amount of glucose was twice that of ga-

lactose. Increasing the temperature to 60°C accelerated lactose hydrolysis, as well as GaOS synthesis (Fig. 5B). In this case, the maximal amount of GaOS was reached after 10 h incubation and was identical to that produced during incubation at 30°C. The effect of lactose concentration on the transgalactosylation activity was significant. A yield of 190 mg/ml was obtained from a 30% lactose solution (Na-phosphate buffer) after 15 h incubation, and a 83% rate of lactose hydrolysis was noted (Fig. 5C).

Discussion

The cloning of *β-galI* into pET24 significantly increased the expression level, which facilitates the protein purification steps. Our previous studies with crude preparations of the β-GalI and β-GalIII enzymes revealed that β-GalI exhibited both higher lactose hydrolysis and transgalactosylation activity than β-GalIII. Therefore, we purified and characterized β-GalI, and compared it with other microbial β-galactosidases.

Although β-galactosidases from *B. bifidum* had temperature and pH optima for lactose of 37°C and pH 6.5, respectively (Dumortier et al. 1994), β-GalI of *B. infantis* presents different characteristics. β-GalI showed a relatively high temperature optimum (50–60°C) that was similar to that of *Pediococcus pentosaceus* (45°C, Bhowmik and Marth 1990), and of *Bacillus stearothermophilus* (65°C, Griffiths and Muir 1978). High temperature optima were also noted for β-galactosidases from fungi (50–55°C, Park et al. 1979; Ozbaas and Kutsal 1990) and some lactic acid bacteria such as *Lactobacillus thermophilus* and *Leuconostoc citrovorum* (55°C and 60°C, respectively; Greenberg and Mahoney 1981). Most β-galactosidases hydrolyzed ONPG and lactose with identical optimum temperature, but β-GalI showed high ONPG hydrolysis activity at 60°C, although at this temperature the enzyme appeared unstable. This suggests that the binding of ONPG is less affected by change of structure than the binding of lactose.

The optimum pH (7.5) was slightly higher than for general microbial β-galactosidases, which range around pH 3.0–7.2. β-GalI was very stable within a broad range of pHs at temperatures up to 50°C, but the activity decreased by 50% during 20 min of incubation at 60°C, and complete loss of activity was observed at 70°C. Similar features were reported for the enzymes of *Bifidobacterium bifidum* (Dumortier et al. 1994), *Bacillus circulans* (Mozaffar et al. 1984), and *Cryptococcus laurentii* (Ohtsuka et al. 1990).

β-GalI was strongly stimulated by monovalent ions and was inhibited by various divalent metallic cations, including Mg^{2+}, Mn^{2+}, and Ca^{2+}. In contrast, Mg^{2+} or/and Mn^{2+} were found to enhance β-galactosidase activity in *E. coli* (Huber et al. 1976), *Arthrobacter* B7 (Trimbur et al. 1994), *Bifidobacterium bifidum* 1901 (Garman et al. 1996), *Kluyveromyces lactis* (Cavaille and Combes 1995), and *Bacillus* sp. MTCC 3088 (Chakraborti et al. 2000). β-Galactosidases of *Lactobacillus casei* 20094, *Bifidobacterium bifidum* 1901, and *Streptococcus thermophilus* were also activated by Na^+ and K^+ (Smart and Richardson 1987; Garman et al. 1996).

The pattern of activation by reducing agents and inhibition by heavy metals and PCMB suggests a sulfhydryl enzyme. The sulfhydryl group has been proposed as important for enzyme catalysis in *Kluyveromyces fragilis* β-galactosidase (Wendorff and Admundson 1971). On the other hand, PCMB showed no effect towards β-galactosidases from *Bacillus circulans* (Mozaffar et al. 1984), *Cryptococcus laurentii* (Ohtsuka et al. 1990) and *Sterigmatomyces elviae* (Onishi and Tanaka 1995). Galactose is known to be a competitive inhibitor of β-galactosidase. In the present case, the inhibitory effect was observed only during lactose hydrolysis, not with ONPG as substrate. On the contrary, for the β-galactosidase of *Streptococcus thermophilus*, galactose was a stronger inhibitor with ONPG than with lactose (Greenberg and Mahoney 1982; Smart and Richardson 1987). The β-galactosidase of *Crytococcus laurentii* was not inhibited by galactose when ONPG was used as substrate (Ohtsuka et al. 1990).

For β-GalI in this study, K_ms of 2.6 and 73.8 mM, respectively, were calculated for ONPG and lactose, and these constants are similar to those (2.4 and 85 mM) of the β-galactosidase of *Aspergillus niger* (Widmer and Leuba 1979), while the *B. bifidum* enzyme had a K_m of 2.0 (with ONPG) and 13 mM (with lactose) (Dumortier et al. 1994). β-Galactosidases generally have higher K_m values with lactose as substrate than with ONPG.

A high level of GaOS was formed in the reaction with purified β-GalI, the yield being similar to that obtained with the crude enzyme (Hung et al. 2001). A maximum yield of 190 mg/ml (comprising 95 mg/ml of tri- and tetra-saccharide, data not shown) was obtained using a 30% lactose solution during a 15 h incubation period. Approximately 83% of the lactose was hydrolyzed when the GaOS yield reached its maximum. The yield was higher (around 78 mg/ml) than those reported for *Sterigmatomyces elviae* CBS8119 and *Rhodotorula minuta* IFO879 (Onishi and Tanaka 1995, 1996), and comparable to the yield of 136 mg/ml obtained with toluene-treated cells of *Sirobasidium magnum* CBS6803 (Onishi et al. 1996), the composition of which was mainly tri- and tetra-saccharides. This study showed that high lactose concentrations and high temperatures were preferable for GaOS synthesis: high temperature favored reaction velocity and substrate solubility while helping to prevent contamination. Such high substrate concentrations also appeared to protect β-GalI from denaturation at temperatures as high as 60°C. The accumulation of GaOS was observed as early as 1 h into the incubation period. At the early stage of the reaction (10 min of incubation) or at low initial lactose concentration (less than 50 mM), no oligosaccharides were detected. Work done with *E. coli* β-galactosidase suggested that allolactose, a product of transgalactosylation, was produced along with glucose and galactose at the early stage of the reaction, and also when low initial lactose concentrations were used (Huber

et al. 1976). The relationship between the transgalactosylation activity and the enzyme K_m with lactose is not clear. β-Galactosidases with low lactose K_m (<3 mM) were found to feature high transgalactosylation activity (Nakao et al. 1994; Onishi and Tanaka 1996).

This study demonstrated that a recombinant β-galactosidase, cloned from *B. infantis* HL96 possesses several interesting properties for industrial applications. The GaOS yield in this report is the highest obtained by purified β-galactosidases. The possibility of increasing the GaOS yield, as well as the biochemical importance of the β-galactosidase isoenzyme with high transgalactosylation activity are currently under investigation. Further studies on the β-galactosidase from Bifidobacteria are needed in order to expand the knowledge on β-galactosidase enzymes as well on the enzymatic properties of probiotic strains.

Acknowledgement This work was supported by an NSERC Strategic Grant for Research and Doctorate Fellowship awarded to M.-N. Hung

References

Asp NG, Burvall A, Dahlqvist A, Hallgren P, Lundblad A (1980) Oligosaccharide formation during hydrolysis of lactose with *Saccharomyces lactis* lactase (Maxilact^R): part 2-oligosaccharide structures. Food Chem 5:147–153

Bhowmik T, Marth EH (1990) β-Galactosidase of *Pediococcus* species: induction, purification and partial characterization. Appl Microbiol Biotechnol 33:317–323

Bradford MM (1970) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254

Burvall A, Asp NG, Dahlqvist A (1979) Oligosaccharide formation during hydrolysis of lactose with *Saccharomyces lactis* lactase (Maxilact^R): part 1-quantitative aspects. Food Chem 4:243–250

Cavaille D, Combes D (1995) Characterization of β-galactosidase from *Kluyveromyces lactis*. Biotechnol Appl Biochem 22:55–64

Chakraborti S, Sani RK, Banerjee UC, Sobti RC (2000) Purification and characterization of a novel β-galactosidase from *Bacillus* sp MTCC 3088. J Ind Microbiol Biotechnol 24:58–63

Dumortier V, Brassart C, Bouquelet S (1994) Purification and properties of a β-D-galactosidase from *Bifidobacterium bifidum* exhibiting a transgalactosylation reaction. Biotechnol Appl Biochem 19:341–354

Farkas E, Thiem J (1999) Enzymatic synthesis of galactose-containing disaccharides employing β-galactosidase from *Bacillus circulans*. Eur J Org Chem 11:3073–3078

Garman J, Coolbear T, Smart J (1996) The effect of cations on the hydrolysis of lactose and the transferase reactions catalysed by β-galactosidase from six strains of lactic acid bacteria. Appl Microbiol Biotechnol 46:22–27

Gibson GR, Roberfroid MB (1995) Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. J Nutr 125:1401–1412

Greenberg NA, Mahoney RR (1981) Immobilization of lactase (β-galactosidase) for use in dairy processing: a review. Process Biochem 16:2–8

Greenberg NA, Mahoney RR (1982) Production and characterization of β-galactosidase from *Streptococcus thermophilus*. J Food Sci 37:248–254

Greenberg NA, Mahoney RR (1983) Formation of oligosaccharides by β-galactosidase from *Streptococcus thermophilus*. Food Chem 10:195–204

Griffiths MW, Muir DD (1978) Properties of a thermostable beta-galactosidase from a thermophilic bacillus: comparison of the enzyme activity of whole cells, purified enzyme and immobilized whole cell. J Sci Food Agric 29:753–761

Huber RE, Kurz G, Wallenfels K (1976) A quantitation of the factors which affect the hydrolase and transgalactosylase activities of β-galactosidase (*E. coli*) on lactose. Biochemistry 15:1994–2001

Hung MN, Lee BH (1998) Cloning and expression of β-galactosidase genes from *Bifidobacterium infantis* into *Escherichia coli*. Biotechnol Lett 20:659–662

Hung MN, Xia Z, Hu NT, Lee BH (2001) Molecular and biochemical analysis of two β-galactosidases from *Bifidobacterium infantis* HL96. Appl Environ Microbiol 67:4256–4263

Ichikawa Y, Look GC, Wong C-H (1992) Review: enzyme-catalyzed oligosaccharide synthesis. Anal Biochem 202:215–238

Ito M, Deguchi Y, Matsumoto K, Kimura M, Onodera N, Yajima T (1993) Influence of galactooligosaccharides on the human fecal microflora. J Nutr Sci Vitaminol 39:635–640

Laemmli LK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680–685

Mitsuoka T (1990) Bifidobacteria and their role in human health. J Ind Microbiol 6:263–268

Mozaffar Z, Nakanishi K, Matsuno R, Kamikubo T (1984) Purification and properties of β-galactosidases from *Bacillus circulans*. Agric Biol Chem 48:3053–3061

Mozaffar Z, Nakanishi K, Matsuno R (1985) Formation of oligosaccharides during hydrolysis of lactose in milk using β-galactosidase from *Bacillus circulans*. J Food Sci 50:1602–1606

Nakao M, Harada M, Kodama Y, Nakayama T, Shibano Y, Amachi T (1994) Purification and characterization of a thermostable β-galactosidase with high transgalactosylation activity from *Saccharopolyspora rectivirgula*. Appl Microbiol Biotechnol 40:657–663

Ohtsuka K, Benno Y, Endo K, Ozawa O, Ueda H, Uchida T, Mitsuoka T (1989) Effects of 4′galactosyllactose intake on human fecal microflora. Bifidus 2:143–149

Ohtsuka K, Tanoh A, Ozawa O, Kanematsu T, Uchida T, Shinke R (1990) Purification and properties of a β-galactosidase with high galactosyl transfer activity from *Cryptococcus laurentii*. J Ferment Bioeng 70:301–307

Onishi N, Tanaka T (1995) Purification and properties of a novel thermostable galacto-oligosaccharide-producing β-galactosidase from *Sterigmatomyces elviae* CBS8119. Appl Environ Microbiol 61:4026–4030

Onishi N, Tanaka T (1996) Purification and properties of a galacto- and gluco-oligosaccharide-producing β-glycosidase from *Rhodotorula minuta* IFO879. J Ferment Bioeng 82:439–443

Onishi N, Kira I, Yokozeki K (1996) Galacto-oligosaccharide production from lactose by *Sirobasidium magnum* CBS6803. Lett Appl Microbiol 23:253–256

Ozbaas T, Kutsal T (1990) Hydrolysis of lactose in whey powder solutions by *Aspergillus oryzae* lactase. J Food Sci Technol 27:195–197

Park YK, DeSanti MSS, Pastore GM (1979) Production and characterization of β-galactosidase from *Aspergillus oryzae*. J Food Sci 44:100–103

Schmidt RR (1986) New methods for the synthesis of glycosides and oligosaccharides. Are there alternatives to the Koenigs-Knorr methods? Angew Chem Int Ed Engl 25:212–235

Smart J, Richardson B (1987) Molecular properties and sensitivity to cations of β-galactosidase from *Streptococcus thermophilus* with four enzyme substrates. Appl Microbiol Biotechnol 26:177–185

Toba T, Yokota A, Adachi S (1985) Oligosaccharide structures formed during the hydrolysis of lactose by *Aspergillus oryzae* β-galactosidase. Food Chem 16:147–162

Trimbur DE, Gutshall KR, Prema P, Brenchley JE (1994) Characterization of a psychrotrophic arthrobacter gene and its cold-active β-galactosidase. Appl Environ Microbiol 60:4544–4552

Wendorff WL, Amundson CH (1971) Characterization of β-galactosidase from *Saccharomyces fragilis*. J Milk Food Technol 34:300–306

Widmer F, Leuba J-L (1979) Galactosidase from *Aspergillus niger*. Eur J Biochem 100:559–567