한 국 축 산 식 품 학 회 지
Korean J. Food Sci. Ani. Resour.
Vol. 27, No. 1, pp. 110~116(2007)

Properties of β-Galactosidase from *Lactobacillus salivarius* subsp. *salivarius* Nam27

Hyoung Churl Bae, Gereltuya Renchinkhand, and Myoung Soo Nam*
Division of Animal Science & Resources, Chungnam National University, Daejeon 305-764, Korea

ABSTRACT

Lactobacillus salivarius subsp. *salivarius* Nam27 with a high β-galactosidase activity was selected for enzymatic characterization. For purification, cell pellet was disrupted by Bead Beater, by DEAE-Sepharose and Mono-Q chromatography. The specific activity of the purified enzyme was 5,312 units/mg. The molecular weight of native monomeric β-galactosidase was estimated to be 30,000 dalton (monomer) by the SDS-PAGE. The optimum temperature and optimum pH were 50°C and 5.0, respectively. This enzyme was stable between 35 and 55°C. β-Galactosidase activity was lost rapidly above pH 7.0. But β-galactosidase was more stable at pH 4.0 (acidic conditions). And β-galactosidase activity was lost rapidly above 65°C after 10 min incubation. Ca^{2+} and Zn^{2+} metal ions enhanced β-galactosidase activity by 164.09% and 127.37%, while Cu^{2+}, Fe^{3+} and Mn^{2+} lowered β-galactosidase activity by 58.29%, 85.10% and 77.66%, respectively. Other metal ions didn't affect β-galactosidase activity significantly.

Key words : *Lactobacillus salivarius*, β-galactosidase, purification, enzyme

INTRODUCTION

Lactobacillus salivarius are present in common intestinal flora in human and animals. This species is resembles the *streptobacteria* or *bifidobacteria* in having a wide spectrum of fermentable carbohydrate and elevated colonies, and in morphology. *L. salivarius* subsp. *salivarius* Nam27 is a newly isolated stain isolated from human feces, which is have a Gram-positive, non-spore forming rod that is member of the resident microflora of the human colon with growing in both anaerobic and aerobic conditions. This bacteria have several probiotic functions, including its antimicrobial activity, cholesterol lowering effect, specifically through the production of a- and β-galactosidase strongly (Balotescu and Petrache, 2004; Bae *et al.*, 2001; Bae *et al.*, 2002; Woo *et al.*, 2002). It is known that *Lactobacillus* sp. produces several enzymes that hydrolyze non-digestible oligosaccharides, including prebiotics, which cannot be digested in the upper part of the gastrointestinal tract.

The β-galactosidase catalyzes the breakdown of the substrate lactose into two monosaccharides galactose and glucose, which readily feed into the glycolytic pathway. β-Galactosidase deficiency due to biochemical or genetic aberrations leads to flatulence, diarrhea, and bleeding. Various populations, particularly those of East Asia and Africa, suffer from lactase deficiency (Hoyoux *et al.*, 2001).

The enzyme β-galactosidase has two main biotechnological uses in the dairy industry ;- the removal of lactose from milk for lactose tolerant persons and the production of galactooligosaccharides for use in probiotic foodstuffs (Boon *et al.*, 1999). The main reasons for diarrhea of lactose intolerant persons straight after bovine milk is consumed are an inability to digest lactose. In normal healthy people β-galactosidase held in the brush border of the mucosal cells of the small intestine breaks down lactose, which is absorbed into the blood as monosaccharides, so that blood sugar increases and lactose is not passed in urine, in lactose intolerant people lack β-galactosidase, and lactose passed in urine, because some of the lactose in absorbed intact, and there is no increases in blood sugar. Unabsorbed lactose is hydrolyzed enzymatically by bacteria of intestine and converted to organic acids of low molecular weight. These organic acids, together with the increased osmotic pressure of unhydrolyzed lactose, result in diarrhea. Galactooligosaccharides are health-promoting; they can prevent caries, because the causative bacterium *Streptococcus mutans* is unable to utilize them, they enhance the growth of beneficial *bifidobacteria*

*Corresponding author : M. S. Nam, Division of Animal Science & Resources, College of Agriculture & Life Sciences, Chungnam National University, 220 Gung-dong, Yuseong-gu, Daejeon 305-764, Korea. Tel: +82-42-821-5782, Fax: +82-42-823-2766, E-mail: namsoo@cnu.ac.kr

in the large intestine, and they maybe used low-calorie sweeteners since they are not metabolized in the small intestine (Petra *et al.,* 2003). The source of the enzyme and the operating conditions regulate the type and amount of oligosaccharides produced (Garman *et al.,* 1996). The ingestion of these oligosaccharides encourages the proliferation of bifidobacteria, which are considered to be beneficial for the human health (Boon *et al.,* 1999; Meller *et al.,* 2001). Some studies indicated that oligosaccharides are often not hydrolyzed by human digestive enzymes but are utilized by some human intestinal bacteria such as *bifidobacteria, lactobacillus*. Hydrolysis of galactooligosaccharides may alleviate gastric distress caused by fermentation of carbohydrate in the large intestine, thereby the improvement of the nutritional quality of legume-based foods such as soybean milk and cowpea meal can be achieved (Ibrahim and O'Sullivan, 2000; Park *et al.,* 2001; Bae *et al.,* 2002). The enzyme is intracellular in bacteria and fungi, but many fungi excrete it. The β-galactosidase has molecular mass weight ranging from 86,000 to 540,000 dalton and consist several identical subunits. β-Galactosidase have different properties because they isolated from different resources such as bacteria, fungi, yeast, animal and plants (Kang *et al.,* 1991; Kang and Park, 1989; Park *et al.,* 2001; Leila and Maria, 2002).

In this paper we purified an β-galactosidase associated with *L. salivarius* subsp. *salivarius* Nam27 from human feces, and identified some of its biochemical characteristics.

MATERIALS AND METHODS

Microorganism and Media

L. salivarius subsp. *salivarius* Nam27 was identified by 16S rDNA sequencing (Bae *et al.,* 2001) and was maintained as frozen stocks in 20% glycerol (Sigma Chemical Co., St. Louis, Mo, U.S.A.) at -70°C. Before use, the bacterium was propagated twice in MRS broth (Difco Laboratories, Detroit, MI, U.S.A.) at 37°C.

Preparation of Cell Extracts from *L. salivarius* subsp. *salivarius* Nam27

Four liters of MRS broth (Difco) was inoculated with 40 mL *L. salivarius* subsp. *salivarius* Nam27 and grown for 24 hr at 37°C. The cells were harvested by centrifugation at 10,800 g for 15 min at 4°C. Cells were broken with a Bead Beater (Biospec Products, Barlesville, OK, U.S.A.). A 350 mL container was filled with 200 mL of 0.1 mm diameter glass beads that had been previously washed with 25 mM Bis-tris propane buffer (pH 6.9). The cells were disrupted by operating a Bead-Beater for 6 cycles of 3 min followed by 2 min chilling on ice. The glass beads were allowed to settle and the supernatant collected. Cellular debris was separated from the crude enzyme by centrifugation at 10,800 g for 15 min. The total volume, total protein concentration, and total activity of the crude supernatant were determined.

Purification Protocol of β-Galactosidase by Chromatography

The crude supernatant was collected 200 mL by centrifugation. The first step in the purification procedure used DEAE-Sephadex A-50 chromatography (6 × 12 cm, 130 mL volume; Sigma Chemical Co., St. Louis, Mo, U.S.A.). Samples were eluted with segmented gradient of 0.1, 0.2, 0.25 and 1.0 M NaCl in 5 mM sodium phosphate buffer (pH 7.0) with a flow rate of 0.5 mL per min at 4°C. OD value of the fractions were determined and assayed for β-galactosidase activity, using o-nitrophenyl-β-D-galactosidase (ONPG) as a substrate. The sample was thrice dialyzed with distilled water for 2 days at 4°C using Dialysis Tubing (cellulose membrane cutoff M.W. 12,400 Dalton; Sigma Chemical Co.). This sample was freeze-dried, and used to make a solution with 50 mM sodium phosphate buffer. Prior to use, all buffers were filtered a 0.45 μm pore size filter. The sample was further purified by loading onto an Mono-Q HR 5/5 column (Amersham Pharmacia Biotech, Uppsala, Sweden) mounted on a FPLC (Amersham Pharmacia Biotech, Uppsala, Sweden). The sample was eluted with a gradient of 0.2, 0.25, 0.3, and 1.0 M NaCl over 85 min with a flow rate of 1 mL per one minute. Fractions were collected and assayed for activity. Active fractions were pooled for further analysis. The purified enzyme was stored at -20°C until use.

Electrophoresis of Purified Enzyme

Purified enzyme was analyzed using sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). The separation condition consisted of a 10 × 8 cm, 1.5 mm thick, 10 well, 12% separating gel containing acrylamide and bisacrylamide and the unit was a Mighty Small Mini-Vertical Electrophoresis system (Hoefer Scientific Instruments, San Francisco, CA, USA). The lyophilized samples were dissolved in sample buffer. The electrode chamber buffer consisted of 0.025 M Tris-base and 0.192 M of glycine with pH 8.3. After polymerization of the stacking gel for 1 hr, 10 μL of sample were loaded into each well. Moleular weight standards in the range of 14,400 to 94,000 dalton (Bio-Rad Laboratories, Inc., Hercules, CA, USA) were used for identification.

Enzyme Activity

β-galactosidase activity was determined by the rate of

hydrolysis of 5 mM ONPG at 40°C and pH 6.5 (50 mM K-phosphate buffer). The reaction mixture was incubated at 40°C for a fixed length of time, usually 10 min. The reaction was stopped by adding 500 mL of 5% (w/v) Na_2CO_3. The increase in absorbance at 420 nm was measured spectrophotometrically. One unit of β-galactosidase was defined as the amount of enzyme which released 1 mmol of ONP from ONPG per min under the standard assay conditions. The specific activity was expressed as units per mg protein.

Protein Concentration

Protein concentration was determined by the Bradford method (Bradford, 1976) using bovine serum albumin as a protein standard.

Optimum pH and Stability

The effect of pH on β-galactosidase activity was measured over a pH range from 4.0 to 9.0 by using 50 mM acetate buffer (pH 4.0-6.0), 50 mM K-phosphate buffer (pH 6.0-8.0) and 50 mM Tris-HCl (pH 8.0-9.0). The pH effect was examined using ONPG as substrate. The effect of pH on β-galactosidase stability was determined by using the same buffer system in the range from pH 4.0 to pH 9.0. After incubation of the enzyme for 1 hour, the pH was adjusted to 6.5 with 50 mM K-phosphate buffer (pH 6.5) containing 5 mM (final concentration) ONPG.

Optimum Temperature and Stability

The enzyme activity at various temperatures will be determined by assaying the enzyme at various incubation temperatures using ONPG as a substrate. The optimum temperature was determined by performing the standard assay at temperature ranging from 5 to 100°C. Thermal stability was determined by assaying for residual β-galactosidase activity after incubation of the enzyme in 50 mM K-phosphate buffer (pH 6.5) at 40, 45, 50, 55, 60 and 65°C, and residual enzyme activity determined from 0 to 60 min at 10 min intervals.

Effect of Metal Ions for β-Galactosidase Activity

The effect of metal ions on the β-galactosidase activity was examined by adding 1 mM of metal salts (Ca^{2+}, Cu^{2+}, Fe^{3+}, K^+, Li^+, Mg^{2+}, Mn^{2+}, Zn^{2+}) and enzyme activity was determined after incubation of the reaction mixture at 40°C for 1 hr.

RESULTS AND DISCUSSION

Purification of β-Galactosidase from *L. salivarius* subsp. *salivarius* Nam27

The β-galactosidase was bound to a DEAE-Sepharose chromatography column, which had been equilibrated with 5 mM sodium phosphate buffer pH 7.0 and segment eluted at NaCl concentration of 0.1, 0.2, 0.25, 0.3 and 1.0 M NaCl (Fig. 1). The fractions having the highest activity were collected and further purified on a Mono-Q HR 5/5 column of FPLC (Fig. 2). The high activity of β-galactosidase showed 0.25 M NaCl gradient at DEAE-Sepharose chromatography and Mono-Q HR 5/5 column of FPLC.

L. salivarius subsp. *salivarius* Nam27 was grown on MRS broth until stationary phase and cells were harvested by centrifugation after washing the cells and sonication with bead beater. We obtained 230 mL of enzyme crude extract and

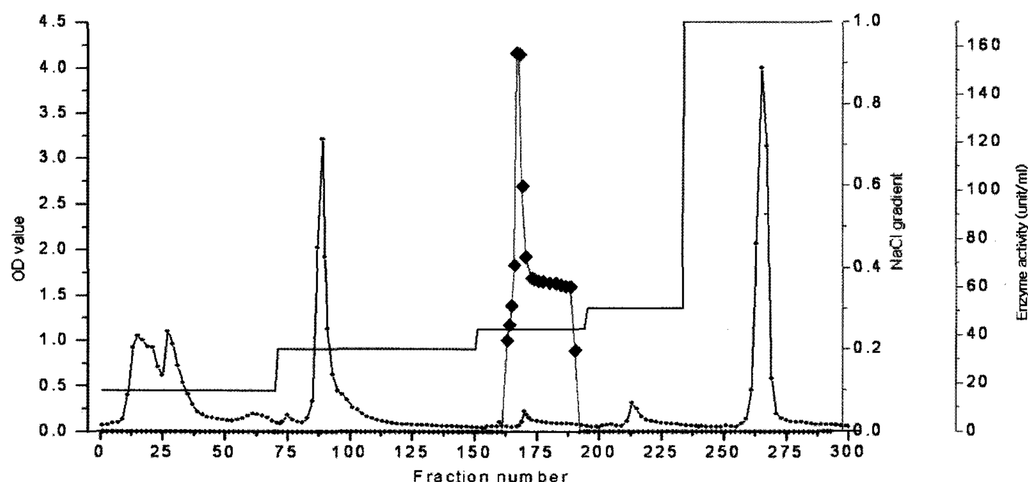

Fig. 1. DEAE-Sepharose chromatography of crude enzyme from *L. salivarius* subsp. *salivarius* Nam27. The samples was eluted with a gradient of 0.1, 0.2, 0.25, 0.3 and 1.0 M NaCl in 5 mM sodium phosphate buffer (pH 7.0) with a flow rate of 0.5 mL per min at 4°C. Proteins were eluted using the same buffer along with measurement of absorbance at 280 nm (- ● -). The β-galactosidase activity (unit/mL, - ◆ -) was monitored using ONPG as a substrate. Zero activity points are not shown.

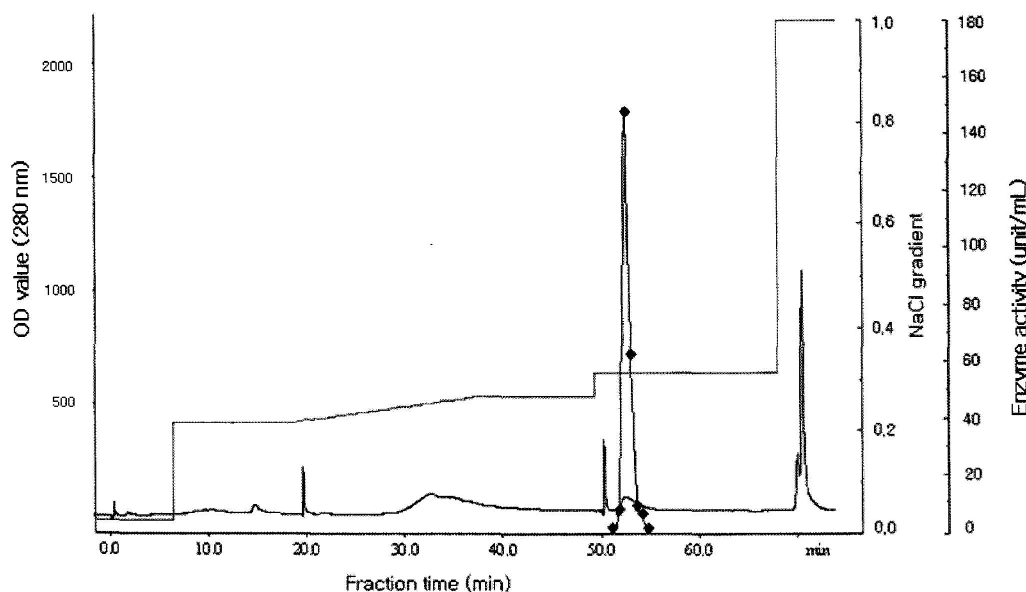

Fig. 2. Mono-Q anion exchange chromatography of β-galactosidase of DEAE-Sepharose fractions with enzyme activity. The sample was eluted with a gradient of 0.2, 0.25, 0.3 and 1.0 M NaCl in 5 mM sodium phosphate buffer (pH 7.0) over 85 min with a flow rate of 1.0 mL per min. Proteins were eluted using the same buffer along with measurement of absorbance at 280 nm (—). The β-galactosidase activity (unit/mL, - ◆ -) was monitored using ONPG as a substrate. One fraction volume was 0.5 mL. Zero activity points are not shown.

enzyme extract was found contain 4.78 mg/mL protein and had a β-galactosidase activity of 2,218.4 unit/mg with ONPG as substrate. Approximately 1,100 mg of the enzyme was obtained from 4 L of high cell density culture (Table 1).

In order to obtain purified enzyme homogeneity, we choose only the most active fractions from the individual purification steps. The final enzyme preparation displayed only about 263.5 fold increase in specific activity compared with the culture, and 0.5 mg of homogeneously purified β-galactosidase was obtained from 4 L of crude filtrate.

Crude cell extract was obtained 230 mL with 20.16 unit/mg specific activity. After purification of Mono-Q HR 5/5 chromatography of FPLC specific activity of β-galactosidase was increased until 5,312.0 unit/mg and fold purification was 263.5. The purified β-galactosidase was obtained 0.5 mg from 1,100 mg crude enzyme powder.

Electrophoresis of Purified Enzyme

Molecular weight of other β-galactosidase has been investigate in the range from 86 to 540 kDa. β-Galactosidases were analyzed from different resources: *Escherichia coli*: 540 kDa, *Aspergillus oryzae*: 108 kDa, *Saccharomyces lactis*: 135 kDa (Leila and Maria, 2001), *Lactobacillus reuteri*: two 105 kDa subunits (Nguyen *et al.*, 2006), *Lactobacillus delbruckii* subsp. *bulgaricus*: two 110 kDa subunits (Adams *et al.*, 1994), *Sterigmatomyces elviae* CBS8119: two 86 kDa subunits (Onishi and Tanaka, 1995), *Bifidobacterium longum* KCTC3215: 180 kDa (Kang *et al.*, 1991) and *Streptococcus thermophillus*: four 130 kDa subunits (Kang and Park, 1989). After these purification steps, the β-galactosidase was considered pure as judged from SDS-PAGE electrophoresis (Fig. 4). The enzyme eluted from DEAE-Sepharose chromatography showed singe peak at a NaCl concentration of 0.25 M and Mono-Q HR 5/5 column showed two peaks at a NaCl of 0.25 M. Molecular weight of β-galactosidase of *L. salivarius* subsp. *salivarius* Nam27 was about 30 kDa and we obtained one band of pure enzyme.

Table 1. Effect of different steps in the purification of β-galactosidase of *L. salivarius* subsp. *salivarius* Nam27

Purification step	Volume (mL)	Protein concentration (mg/mL)	Total protein (mg)	Activity* (unit/mL)	Specific activity (unit/mg)	Total enzyme activity (unit)	Fold purification	Yield (%)
Cell extract	230	4.78	1.100	2.57	20.16	22,184.0	1	100
DEAE chromatography	26	2.36	11	2.51	565.56	6,221.17	28.05	28.04
Mono-Q chromatography	3	0.16	0.5	9.165	5,312.0	2,656.0	263.5	11.97

*All activity tests were done in triplicate. The maximum variation from the mean values (shown) was less than 5%.

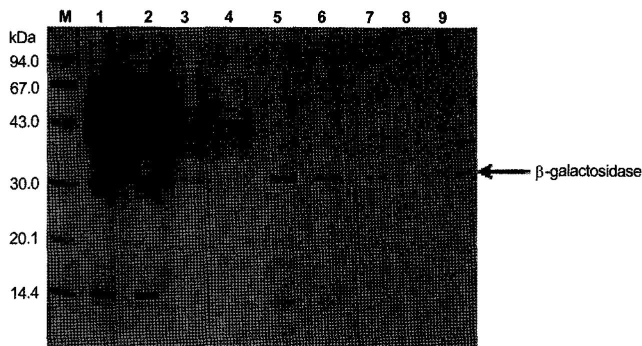

Fig. 3. SDS-PAGE of the different steps in the purification of β-galactosidase from *L. salivarius* subsp. *salivarius* Nam27. M: molecular mass of standard proteins in range from 14,400 to 94,000 Da. Lane 1 to 4: supernatant after breakage of cells with glass bead mill. Lane 5 to 8: Fraction of DEAE-Sepharose chromatography (Lanes (1-8) are different by loading volume). Lane 9: is Mono-Q HR 5/5 column chromatography fraction.

Optimum pH and Stability on the Enzyme Activity

Knowledge of the effects that different environmental factors have on enzymatic activity and molecular structure should be highly useful to industrial applications (Jurado *et al.,* 2004). Mostly optimum pH of β-galactosidase of other lactic acid bacteria were pH in range from 5.0 to 7.0 (Kang *et al.,* 1991; Park *et al.,* 2001).

The β-galactosidase was characterized by measuring its activity in buffer of varying pH using 5 mM ONPG as substrate. The optimum pH for the activity was estimated to be around 7.0 at 40°C. Enzyme maximal activity was at pH 5.0. The β-galactosidase exhibited a narrow pH range between 4.0 and 7.0 of maximal activity with approximately 30% of maximal activity at pH 5.0 (Fig. 4). Fig. 4 shows that the enzyme demonstrated moderate pH stability with more than 80% of the maximum activity remained following 60 min incubation in buffers having pH values ranging from 4 to 7. The enzyme was relatively stable at acidic conditions.

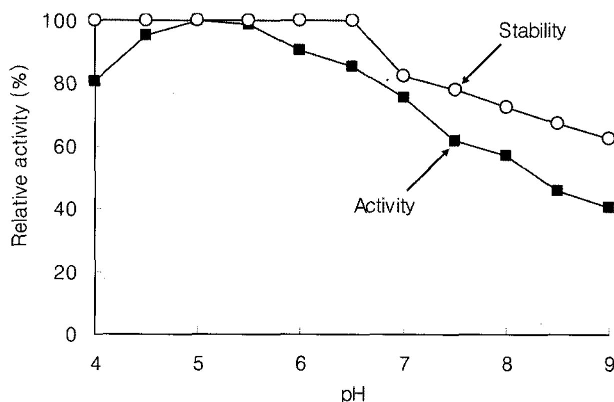

Fig. 4. Effect of pH on β-galactosidase activity and stability from *L. salivarius* subsp. *salivarius* Nam27. The optimum pH for activity was determined by measuring enzyme activity over the pH range from 4 to 9 by using 50 mM acetate buffer (pH 4.0-6.0), 50 mM K-phosphate buffer (pH 6.0-8.0) and 50 mM Tris-HCl (8.0-9.0). The pH effect was examined using ONPG as substrate. The pH stability was determined by holding the enzyme for 1 hr at room temperature over the pH range from 4 to 9 in 0.5 pH unit increments. All activity tests were done in triplicate. The maximum variation from the mean values was less than 5%.

Optimum Temperature and Stability on the Enzyme Activity

Mostly temperature of β-galactosidase of other lactic acid bacteria was in the range from 40 to 65°C (Kang *et al.,* 1991; Park *et al.,* 2001). Optimum temperature was analyzed by holding the enzyme for 10 minutes at different temperatures from 5 to 100°C at 5°C intervals and returning to standard condition for activity assay. The β-galactosidase exhibited the highest activity in the temperature range of 35 to 55°C and enzyme activity was significantly decreased above 60°C (Fig. 5). Temperature stability of β-galactosidase was examined at 45, 50, 55, 60 and 65°C (Fig. 6). The temperature stability of β-galactosidase of *L. salivarius* subsp. *salivarius* Nam27 is higher then that of *Arthrobacter* sp. and *E. coli* (Petra *et al.,* 2003; Hoyoux *et al.,* 2001). While this mesophilic β-galactosidase from *E. coli* has lost only 20% of its activity during the 80 min incubation in 45°C (Hoyux *et al.,* 2001), the β-galactosidase of *L. salivarius* subsp. *salivarius* Nam27 has lost only 40% of its activity during the 60 min incubation in 60°C. At 50 and 55°C the enzyme was more stable and the enzyme retained 100% activity after 60 min incubation. The enzyme rapidly lost activity at 65°C after 20 min. The enzyme was relatively stable at high temperature.

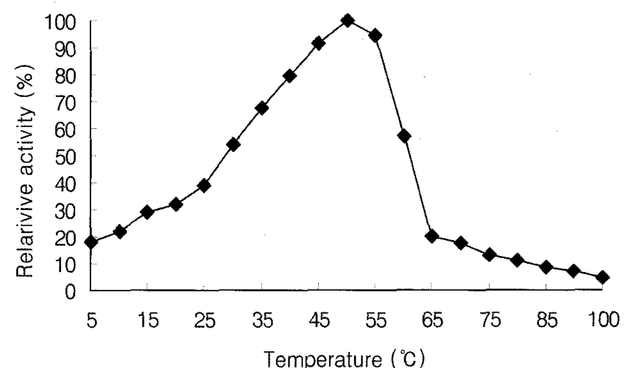

Fig. 5. Effect of temperature on β-galactosidase activity from *L. salivarius* subsp. *salivarius* Nam27. Activity was determined by assaying the enzyme at various incubation temperatures using ONPG as a substrate. All activity tests were done in triplicate. The maximum variation from the mean values (shown) was less than 5%.

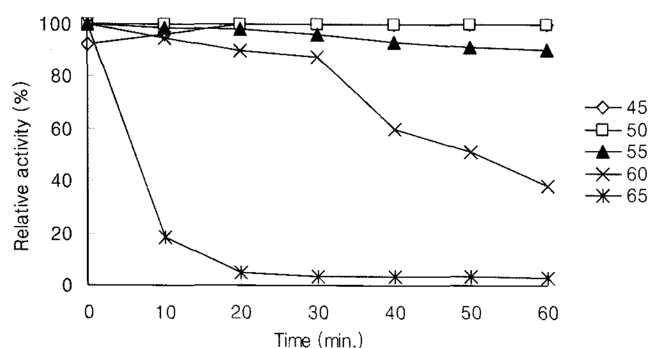

Fig. 6. Effect of temperature on stability of β-galactosidase was incubated at 45, 50, 55, 60 and 65°C, and residual enzyme activity was determined from 0 to 60 min at 10 min intervals. All activity tests were done in triplicate. The maximum variation from the mean values (shown) was less than 5%.

Effect of Metal Ions for β-Galactosidase Activity

Of the most studied factors affecting the activity and stability of β-galactosidase is the influence of ions such as Ca^{2+}, Mg^{2+}, Na^{+}, NH_4^{+} and K^{+}. Regardless of the origin of the enzyme, in all the works consulted, it is indicated that Ca^{2+} ions increase the functioning of the enzyme, Mg^{2+} ions inhibit the their activity (Jurado *et al.,* 2004; Kang *et al.,* 1991; Kang and Park, 1989).

Metal ions were present different effect on β-galactosidase of *L. salivarius* subsp. *salivarius* Nam27. Cu^{2+} and Fe^{3+} ions cause the decreas in the activity of β-galactosidase from other resources (Jurado *et al.,* 2004; Kang *et al.,* 1991). β-galactosidase, which we obtained, was similar to β-galactosidase of other bacteria and other resources by effect of calcium, copper, iron, potassium and lithium. The requirement of metal cations for the β-galactosidase activity was examined using Ca^{2+}, Cu^{2+}, Fe^{3+}, K^{+}, Li^{+}, Mg^{2+}, $Mn^{2+}$and Zn^{2+}as a counter part ion. K^{+}, Li^{+}, Mg^{2+} metal ions at 1 mM concentration not affect β-galactosidase and Ca^{2+}, Zn^{2+} increased β-galactosidase activity by 164.09%, and 127.37%. But Cu^{2+}, Fe^{3+} and Mn^{2+} decreased β-galactosidase activity by 58.29%, 85.10% and 77.66%, respectively (Table 2).

Table 2. Effect of metal ion on β-galactosidase activity from *L. salivarius* subsp. *salivarius* Nam27

Metal	Relative activity (%) (Means ± SE*)
Control[e]	100 [bc]
Ca^{2+}	164.09 [a] ± 6.94
Cu^{2+}	58.29 [d] ± 8.10
Fe^{3+}	85.10 [cd] ± 6.42
K^{+}	100.97 [bc] ± 6.55
Li^{+}	95.81 [bcd] ± 7.84
Mg^{2+}	98.35 [bc] ± 6.51
Mn^{2+}	77.66 [cd] ± 12.70
Zn^{2+}	127.37 [ab] ± 20.45

[a,b,c,d] In a column, means followed by a common letter are not significantly different at the 1% level by DMRT.
* SE : Standard Error.
[e] Activity of the control, in which no metal ion was added, was taken as 100.

CONCLUSION

β-Galactosidase are known to occur widely in plants, animals, fungi and bacteria and have been studied most widely in plants and fungi. This report describes the purification and characterization of the β-galactosidase from *L. salivarius* subsp. *salivarius* Nam27 that is a novel lactic acid bacteria isolated from human. Many researchers have purified and characterized the β-galactosidase from several bacteria, fungi, and yeast. Innumerable microorganisms produce β-galactosidase of different properties.

L. salivarius subsp. *salivarius* Nam27 possesses a high level of β-galactosidase activity. Purified β-galactosidase was obtained after sonication of harvested cell pellet followed by DEAE-Sepharose and Mono-Q chromatography. The crude enzyme extract was found to contain 4.78 mg/mL protein and had a β-galactosidase activity of 2,218.4 unit/mg. The final enzyme preparation displayed only about 263.5 fold increases in specific activity compared with the culture.

Optimum pH of β-galactosidase was pH 5.0. β-Galactosidase was 70% activity at pH range from 4.0 to 7.0. β-Galactosidase was more stable at pH 4.0 or β-galactosidase was stable at acidic conditions.

Metal ions were present different effect on β-galactosidase of *L. salivarius* subsp. *salivarius* Nam27. Cu^{2+} and Fe^{3+} metal ions decreased activity of β-galactosidase from other resources, other metal ions were similar to that of other β-galactosidase.

Molecular weight of other β-galactosidase has been investigated in range from 86 to 540 kDa. Dimeric structure is a common feature found in many β-galactosidase described in the literature (Nguyen *et al.,* 2006). However, β-galactosidase of *L. salivarius* subsp. *salivarius* Nam27 is assumed to be a monomer with a molecular weight of about 30 kDa, which is smaller than that of other microorganisms.

The temperature stability of β-galactosidase of *L. salivarius* subsp. *salivarius* Nam27 is higher than that of *Arthrobacter* subsp. and *E. coli* (Petra *et al.,* 2003; Hoyux *et al.,* 2001).

These results show that studied enzyme could be used for the production of galactooligosaccharides, either directly in milk or for the preparation of galactooligosaccharides from

whey as an additive in dairy products.

REFERENCES

1. Adams, R. M., Yoast, S., Mainzer, S. E., Moon, K., Palombella, A. L., Estell, D. A., Power, S. D., and Schmidt, B. F. (1994) Characterization of two cold-sensitive mutants of the β-galactosidase from *Lactobacillus delbruckii* subsp. *bulgaricus*. *J. Biol. Chem.* **269**, 5666-5672.
2. Bae, H. C., Nam, M. S., and Lee, J. Y. (2002) Probiotic characterization of acid- and bile-tolerant *lactobacillus salivarius* subsp. *salivarius* from korean faeces. *Asian-Aust. J. Anim. Sci.* **15**, 1798-1807.
3. Bae, H. C., Choi, S. H., and Nam, M. S. 2001. Isolation and identification of acid- and bile-tole rant *Lactobacillus salivarius* subsp. *salivarius* from human faeces. *Asian-Aust. J. Anim. Sci.* **14**, 1170-1178.
4. Balotescu, M. G., and Petrache, L. M. (2004) Adherence of lactobacilli to intestinal mucosa and their antagonistic activity against pathogens. *Rom. Biotechnol. Letters.* **9**, 1737-1749.
5. Boon, M. A., Janssen, A. E., and Van't Riet, K. (1999) Effect of temperature and enzyme origin on the enzymatic synthesis of oligosaccharides. *Enzyme Microbial Technol.* **26**, 271-281.
6. Bradford, M. M. (1976) A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**, 248-254.
7. Garman, J., Coolbear, T., and Smart, J. (1996) The effect of cations on the hydrolysis of lactose and transferase reactions catalyzed by β-galactosidase from six strains of lactic acid bacteria. *Appl. Microbiol. Biotechnol.* **46**, 22-27.
8. Hoyoux, A., Jennes, I., Dubois, P., Genicot, S., Dubail, F., Francois, J. M., Baise, E., Feller, G., and Gerday, C. (2001) Cold-Adapted β-Galactosidase from the Antarctic Psychrophile *Pseudoaltermonas haloplanktis*. *Appl. Environ. Microbiol.* **67**, 1529-1535.
9. Ibrahim, S. A. and O'Sullivan, D. J. (2000) Use of Chemical Mutagenesis for the Isolation of Food grade β-Galactosidase Overproducing Mutants of *Bifidobacteria, Lactobacilli* and *Streptococcus thermophillus*. *J. Dairy Sci.* **83**, 923-930.
10. Jurado, E., Camacho, F., Luzón, G., and Vicaria, J. M. (2004) Kinetic models of activity for β-galactosidases: influence of pH, ionic concentration and temperature. *Enzyme Microbial Technol.* **34**, 33-40.
11. Kang, K. H. and Park, S. I. (1989) Production, purification and characterization of β-galactosidase from *Streptococcus thermophillus* 510. *Kor. J. Appl. Microbiol. Biotechnol.* **17**, 35-45.
12. Kang, K. H., Min, H. K., Yi, H. K., and Jang, Y. H. (1991) Production, purification and characterization of β-galactosidase from *Bifidobacterium longum KCTC 3215*. *Kor. J. Appl. Microbiol. Biotechnol.* **19**, 456-463.
13. Leila, B. and Maria, D. P. B. (2002) β-galactosidase activity as affected by apparent pH and physical properties of reduced moisture systems. *Enzyme Microbial technol.* **30**, 367-373.
14. Meller, P. L., Jorgansen, F., Hansen, O. C., Madsen, S. M., and Stougaard, P. (2001) Intra- and extracellular β-galactosidases from *Bifidobacterium bifidium* and *Bifidobacterium infantis* molecular cloning, heterologous expression, and comparative characterization. *Appl. Environ. Microbiol.* **67**. 2276-2283.
15. Nguyen, T. H., Splechtna, B., Steinbock, M., Kneifel, W., Lettner, H. P., Kulbe, K. D., and Haltrich, D. (2006) Purification and characterization of two novel β-galactosidase from *Lactobacillus reuteri*. *J. Agri. Food Chem.* **54**. 4989-4998.
16. Onishi, N. and 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.
17. Park, M. S., Yoon, H. J., Rhim, S. L., and Ji, G. E. (2001) Molecular cloning and characterization of the β-galactosidase gene from *Bifidobacterium adolescentis Int57*. *Microbiol. Biotechnol.* **11**, 106-111.
18. Petra, K. L., Strnad, H., Spiwok, V., Mala, S., Kralova, B., and Nicholas, J. R. (2003) The cloning, purification and characterization of a cold-active β-galactosidase from the psychrotolerant antarctic bacterium *arthrobacter* sp. C2-2. *Enzyme Mcrobial Technol.* **33**, 836-844.
19. Woo, P. C. Y., Fung, A. M. Y., Lau, S. K. P., and Yuen, K. Y. (2002) Identification by 16S rRNA gene sequencing of *Lactobacillus salivarius* bacteremic cholecystitis. *J. Clin. Microbiol.* **40**, 265-267.

(2007. 2. 7. 접수/2007. 3. 21. 채택)