한국축산식품학회지
Korean J. Food Sci. Ani. Resour.
Vol. 27, No. 1, pp. 102~109(2007)

Purification and Characterization of α-Galactosidase from *Lactobacillus salivarius* subsp. *salivarius* Nam27

Hyoung Cchurl Bae, Jong Woo Choi[1], and Myoung Soo Nam*
Division of Animal Science & Resources, Chungnam National University, Daejeon 305-764, Korea
[1]Department of Food Science, University of Wisconsin, Madison Wisconsin 53706, U.S.A.

ABSTRACT

Lactobacillus salivarius subsp. *salivarius* CNU27 possessed a high level of α-galactosidase activity. Purified α-galactosidase was obtained after sonication of harvested cell pellet followed by DEAE-Sephadex A-50 and Mono Q anion exchange chromatography. The specific activity of the purified enzyme was 8,994 units/mg protein which is 17.09 times higher than that in crude extract. The native enzyme was a monomer with a molecular mass of 56,397.1 dalton. The optimum temperature and pH for the enzyme were 40°C and 6.0, respectively. The enzyme was stable between 25 and 50°C. However, α-galactosidase activity was lost rapidly below pH 4.5 and above pH 8.5. The enzyme activity decreased to 6.73% and 4.30% of the original activity by addition of Cu^{2+} and Hg^{2+}, respectively. Other metal compounds did not affect the enzyme activity significantly. The enzyme liberated galactose from melibiose, raffinose, and stachyose. The rate of substrates hydrolysis was measured by HPLC. Raffinose, stachyose and melibiose were completely decomposed after 24 hr at 40°C.

Key Words : *Lactobacillus salivarius,* α-galactosidase, purification, enzyme

INTRODUCTION

Lactobacillus salivarius subsp. *salivarius* Nam27 is a novel subspecies of lactic acid bacteria isolated from human feces, It is a gram-positive, non-spore forming rod with ability to grow in both anaerobic and aerobic conditions (Bae *et al.,* 2001). The probiotic properties of *L. salivarius* subsp. *salivarius* Nam27 include antimicrobial activity and cholesterol assimilation (Bae *et al.,* 2002). *L. salivarius* subsp. *salivarius* Nam27 possesed high α-galactosidase activity (Bae *et al.,* 2001). α-Galactosidases (EC 3.2.1.22, α-D-galactoside galactohydrolase) are produced by animals (Ohshima *et al.,* 1997), fungi (Zeilinger *et al.,* 1993) and bacteria (Tzortzis *et al.,* 2003). They have been studied most widely in plant seeds (Gao and Schaffer, 1999). Many researchers have purified and characterized the α-galactosidase from several bacteria, such as *Bacteroides ovatus* (Gherardini *et al.,* 1985), *Bifidobacterium adolescentis* (Leder *et al.,* 1999), *Pycnoporus cinnabarinus* (Mitsutomi and Ohtakara, 1988), *Bifidobacterium infantis* (Roy *et al.,* 1992), *Trichoderma reesei* (Savel'ev *et al.,* 1996), *Lactobacillus reuteri* (Tzortzis *et al.,* 2003), *Lactobacillus plantarum* (Silvestroni *et al.,* 2002), *Lactobacillus fermentum* (Garro *et al.,* 1993), *Bacillus* sp. (Jin *et al.,* 2001), and *Streptococcus mutans* (Aduse-Opoku *et al.,* 1991).

It is known that α-galactosidase hydrolyzes prebiotics such as non-digestible oligosaccharides, which cannot be digested in the upper part of the human gastrointestinal tract. All α-galactosides, such as raffinose, stachyose, and melibiose, are found in human foods. α-Galactosidase hydrolyses α-D-galactosidic bonds present in raffinose and stachyose, and releases galactoside. Animals and humans lack the enzymes to degrade such α-galactosides (Shabalin *et al.,* 2002). Raffinose and stachyose are known as flatulence factors. Thus, they may serve as natural sources of carbohydrate for *Lactobacillus*, *Bifidobacterium* and *Bacillus* in the colon (Gherardini *et al.,* 1985). It is likely that α-galactosidase, as the initial enzyme in the metabolic pathway of stachyose and raffinose catabolism (Keller and Pharr, 1996), plays an important role in the carbohydrate partitioning in the cucurbits (Gao and Schaffer, 1999).

α-Galactosidase is of interest because of its many potential biotechnological applications, including utilization of molasses by baker's yeast, hydrolysis of raffinose in sugar beet and soy processing, serological conversion of blood group type

*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

and, most importantly, its use as a dietary additive for humans and animals (Murphy and Power, 2002). If the enzyme is used for hydrolysis of the raffinose into galactose and sucrose in the sugar beet industry, the sugar beet yield could be increased (Jin *et al.*, 2001). The α-galactoside oligosaccharides that constitute 6% of soybean meal are neither digested nor utilized by poultry and young pigs. Consequently, they contribute little nutritional value and may cause intestinal distress. Processing of feeds with α-galactosidase could reduce the levels of undesirable side effects and increase the available energy content of feeds. Since humans and other monogastric animals do not possess the enzyme α-galactosidase that is required to metabolize α-galactosides, consumption of soybeans and other legumes may cause intense gastrointestinal disturbance (flatulence). This lowers the consumer acceptability of soy foods. Soybean-derived foods containing lower amounts of α-galactosidase would reduce the problem, and potentially increase the economic value of soybean.

Although a number of studies on α-galactosidases have been carried out, there is a lack of physiological information on the enzymes that originate from *Lactobacillus*, especially those from *L. salivarius* subsp. *salivarius*. We selected *L. salivarius* subsp. *salivarius* with probiotics characteristics from Korean feces (Bae *et al.*, 2001; Bae *et al.*, 2002). This study describes the purification and characterization of the α-galactosidase from a *L. salivarius* subsp. *salivarius* Nam27.

MATERIALS AND METHODS

Microorganisms and Media

L. salivarius subsp. *salivarius* Nam27 was identified by 16s rDNA (Bae *et al.*, 2001) and was maintained at -70°C in 20% glycerol (Sigma Chemical Co., St. Louis, Mo, USA). Before use, the bacteria were propagated twice in lacto bacilli MRS broth (Difco Laboratories, Detroit, MI, USA) at 37°C.

Preparation of Cell Extract from *L. salivarius* subsp. *salivarius* CNU27

Two liters of MRS broth were inoculated with *L. salivarius* subsp. *salivarius* Nam27 and incubated 24 hr at 37°C. The cells were harvested by centrifugation at 10,800 g for 15 min at 4°C. Cells were mixed 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) and disrupted in a Bead Beater (Biospec Products, Barlesville, OK, USA) operated in 6 cycles of 3 min followed by 2 min chilling on ice. The glass beads were allowed to settle and the supernatant was 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 α-Galactosidase

Centrifugation was used to collect 200 mL of crude supernatant. The first step in the purification procedure used DEAE-Sephadex α-50 anion-exchange chromatography (6× 12 cm, 130 mL volume; Sigma Chemical Co.). Samples were eluted with a segmented gradient of 0.2, 0.3, and 0.5 M NaCl in 5 mM sodium phosphate buffer (pH 6.0) with a flow rate of 1 mL per min at 4°C. The active fractions were pooled and dialyzed (cellulose membrane cutoff M.W. 12,400 Dalton; Sigma Chemical Co.) against distilled water for 2 days at 4°C. Samples were freeze-dried, and dispersed in 25 mM Bis-Tris propane buffer (pH 6.9). Samples were further purified by loading onto an anion-exchange Mono Q HR 5/5 column (Amersham Pharmacia Biotech, Uppsala, Sweden) attached to a FPLC (Amersham Pharmacia Biotech). The sample was eluted with a segmented gradient of 0.2, 0.3, and 0.5 M NaCl over 120 min with a flow rate of 0.5 mL per min. Fractions of 0.5 mL were collected and assayed for activity. Active fractions were pooled for further analysis. Purified enzyme was stored at -20°C until use.

Prior to use, all buffers were filtered through a 0.22 μm pore size filter. All columns and column media used were obtained from Pharmacia unless otherwise stated. Samples were filtered through a 0.2 μm membrane filter, and were used directly for HPLC (Waters Co., Milford, MA, USA) analysis. The HPLC system consisted of a Waters model 600E Multisolvent Delivery System, a Rheodyne model 7725i injector with a 20 μL sample loop, and a Waters model 2487 Dual Absorbance Detector fitted with 280 nm to which C4 column (Waters Co.), which was heated to 40°C by a Waters Column Heater Module, was attached. Eluent A solution was 0.1% (w/v) trifluoroacetic acid (TFA) in water and eluent B solution was 0.1% (w/v) TFA in acetonitrile (AcN). Sample (20 μL) was loaded onto the column and eluted with a linear gradient from 20% to 50% B solution for 20 min. The flow rate was 1.5 mL /min. The separation time was 20 min. Detector output was recorded using an Autochro-WIN 2.0 plus software package (Young Lin Instrument Co., Ltd., Seoul, Korea).

Enzyme Activity

α-Galactosidase activity was determined by the rate of hydrolysis of 10 mM *p*-nitrophenyl-α-D-galactopyranoside (pNPG) 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 μL of 5% (w/v) Na_2CO_3. The increase in absorbance at 400 nm was measured spectrophotometrically. One unit of α-galactosidase was defined as the amount of enzyme which released 1 μmol of *p*-nitrophenol from pNPG per min under the standard assay conditions. The specific activity was expressed as units per mg protein.

Optimum pH and Stability

The effect of pH on α-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 pNPG as substrate. The effect of pH on α-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 hr, the pH was adjusted to 6.5 with 50 mM K-phosphate buffer (pH 6.5) containing 10 mM (final concentration) pNPG.

Optimum Temperature and Stability

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

Effect of Metal Ions on α-Galactosidase

The effect of metal ions on α-galactosidase activity was determined by adding 1 mM of metal salts, and enzyme activity was determined after incubation of the reaction mixture at 40°C for 1 hr.

Protein Concentration

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

Determination of Molecular Mass

The native molecular mass of the α-galactosidase was determined by matrix-assisted laser desorption ionization time-of-flight (MALDI TOF) mass spectrometry (Shimadzu Co., Kyoto, Japan) at the Center for Research Facilities of the Chungnam National University (Daejeon, Korea). Before the determination of the molecular weight, α-galactosidase was completely denatured at 100°C for 10 min.

Electrophoresis of Enzyme Obtained from Purification Procedure

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). Lyophilized samples were dissolved in the sample buffer. The electrode chamber buffer consisted of 0.025 M Tris-base and 0.192 M glycine at pH 8.3. After polymerization of the stacking gel for 1 hr, 10 μL of sample were loaded into each well. Molecular weight standards in the range of 14,400 to 97,400 dalton (Bio-Rad Laboratories, Inc., Hercules, CA, USA) were used for identification.

Substrate Specificity

A reaction mixture containing 3% (w/v) natural oligosaccharides (melibiose, raffinose, and stachyose) was used to determine the ability of α-galactosidase to release D-galactose. The enzyme solution was incubated at 40°C for 24 hr. The products of the hydrolysis were determined by a high-performance liquid chromatography (HPLC) analysis, which was performed by using a SUPELCOGEL C-610H column (30 cm × 7.8 mm i.d., Sigma-Aldrich Co. USA), operated at 40°C at a flow rate of 1 mL/min and a refractive index detector (Waters Co. Milford, MA, USA). The sample was filtered through a 0.2 μL membrane filter and used directly for HPLC (Waters Co. Milford, MA, USA) analysis. The HPLC system contained a Waters model Waters 600E Multisolvent Delivery System and a Rheodyne model 7725i injector with a 20 μL sample loop. Detector output was recorded with a Autochro-WIN 2.0 plus of software package (Young Lin Instrument Co., Ltd., Seoul, Korea).

RESULTS AND DISCUSSION

Purification of α-Galactosidase from *L. salivarius* subsp. *salivarius* CNU27

Cells of *L. salivarius* subsp. *salivarius* Nam27 grown in MRS broth were harvested by centrifugation at the beginning of the stationary phase. After washing the cells and sonification, the enzyme extract (200 mL) was found to contain 740 mg protein and had a α-galactosidase activity of 1,947 U/mL using pNPG as a substrate (Table 1).

The α-galactosidase was bound to a DEAE-Sepharose

chromatography column, which was equilibrated with 5 mM sodium phosphate buffer pH 6.0 and stepwise eluted with 0.2, 0.3, and 0.5 M NaCl (Fig. 1). The fractions having the highest activity were collected and further purified on an anion-exchange Mono Q HR 5/5 column of FPLC (Fig. 2). The highest activity of α-galactosidase was observed with

Table 1. Impact of the different steps of purification on activity of α-galactosidase

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	200	3.70	740.0	1,947	526	389,472	1.00	100
DEAE chromatography	60	0.38	23.0	700	1,825	41,975	3.47	10.8
Mono Q chromatography	4.5	0.20	0.9	1,799	8,994	8,095	17.09	2.1

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

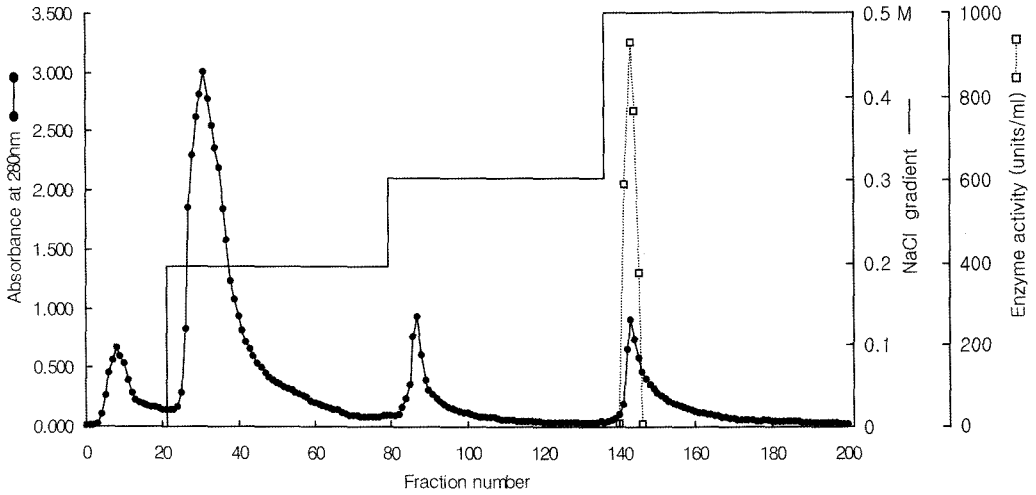

Fig. 1. DEAE-sepharose anion exchange chromatogram of α-galactosidase of *L. salivarius* subsp. *salivarius* Nam27. The samples were eluted with a segmented gradient of 0.2, 0.3, and 0.5 M NaCl in 5 mM sodium phosphate buffer (pH 6.0) with a flow rate of 1 mL per min at 4°C. Proteins were eluted using the same buffer along with measurement of absorbance at 280 nm (-●-). The α-galactosidase activity (unit/mL, -□-) was monitored using pNPG as a substrate. One fraction volume was 20 mL. Zero activity points are not shown.

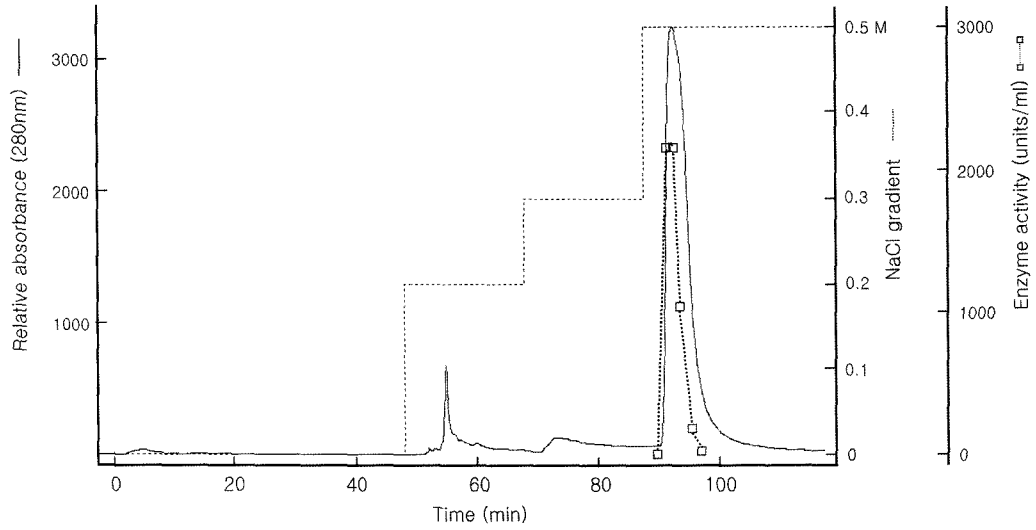

Fig. 2. Mono Q anion exchange chromatography of α-galactosidase from *L. salivarius* subsp. *salivarius* Nam27. The sample was eluted with a segmented gradient of 0.2, 0.3, and 0.5 M NaCl in 25 mM Bis-Tris propane buffer (pH 6.9) over 120 min with a flow rate of 0.5 mL per min. Proteins were eluted using the same buffer along with measurement of absorbance at 280 nm (—). The α-galactosidase activity (unit/mL, -□-) was monitored using pNPG as a substrate. One fraction volume was 0.5 mL. Zero activity points are not shown.

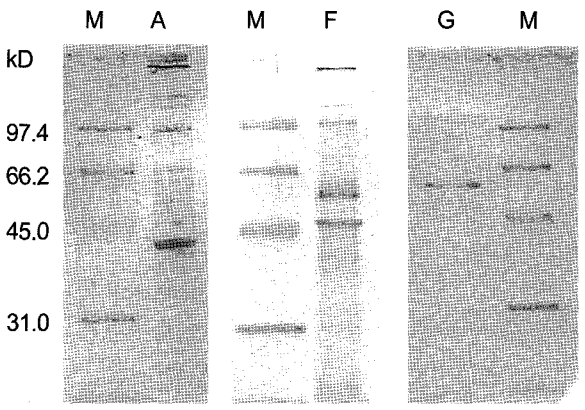

Fig. 3. SDS-PAGE of different steps in the purification of α-galactosidase from *L. salivarius* subsp. *salivarius* Nam27. M is low molecular mass standard proteins, A is a crude enzyme obtained by disturbing cells with glass bead mill, F is a DEAE-chromatography fraction, G is a Mono Q chromatography fraction.

the 0.5 M NaCl gradient on the DEAE-Sepharose chromatography and anion-exchange Mono Q HR 5/5 columns of FPLC. After these purification steps, the purity of α-galactosidase was confirmed by SDS-PAGE electrophoresis (Fig. 3).

In order to obtain a highly purified enzyme we chose only the most active fractions from the individual purification steps. The final enzyme preparation showed only about 17.09 fold increase in specific activity compared with the cell extract, and 0.9 mg of homogeneously purified α-galactosidase were obtained from 2 L of cell culture.

Molecular Mass Determination of α-Galactosidase

The molecular mass of the native α-galactosidase was determined by MALDI-TOF was 56,397.1 dalton (Fig. 4). Other studies reported molecular masses of 50 kDa of dimer composition from *Escherichia coli* (Nagao *et al.*, 1988), 50 kDa of monomer composition from *Trichoderma reesei* RUT-30 (Zeilinger *et al.*, 1993), 300 kDa of tetramer composition from *L. salivarius* (Aduse-Opoku *et al.*, 1991) and 60 kDa of *L. reuteri* (Tzortzis *et al.*, 2003).

Effect of pH and Temperature on the Enzyme Activity

The α-galactosidase enzyme was characterized by measuring its activity in buffers at various pH values with 10 mM pNPG as a substrate. The optimum pH for the activity was estimated to be around 6.0. The α-galactosidase showed 67% of its maximum activity at pH 7 and only 56% at pH 5 (Fig. 5).

The enzyme had moderate pH stability with more than 90% of the maximal activity remained after 60 min incubation in buffers at pH values ranging from 4.5 to 8.5. The enzyme was relatively unstable under acidic conditions (Fig. 5).

Optimum temperature was analyzed by holding the enzyme for 15 min at temperatures ranging from 5 to 100°C (5°C intervals) and returning to standard conditions for activity assay. The α-galactosidase exhibited the highest activity in the temperature range between 25 to 50°C and the activity significantly decreased above 55°C (Fig. 6).

Temperature stability of α-galactosidase was examined at 40, 45, 50, and 55°C (Fig. 7). At 40 and 45°C the enzyme retained 100% activity after a 60 min incubation. The enzyme retained more than 50% of its original activity during a 60

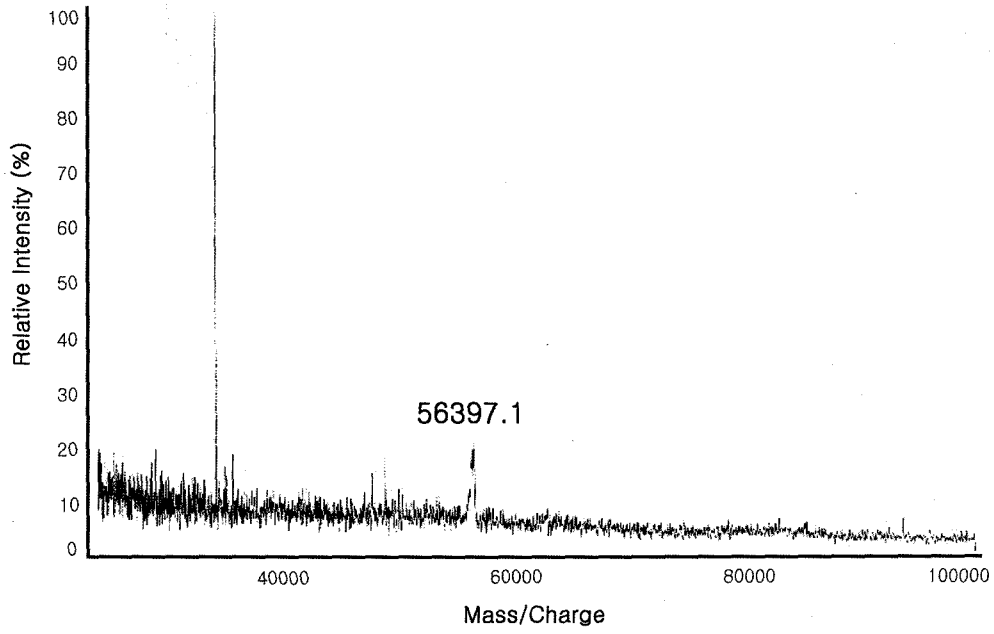

Fig. 4. Molecular mass spectrum of native α-galactosidase from *L. salivarius* subsp. *salivarius* Nam27.

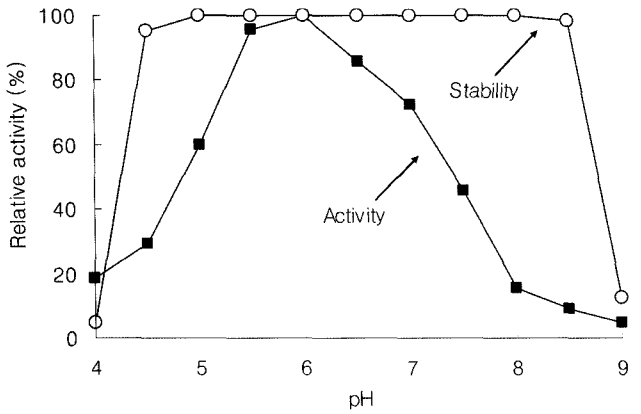

Fig. 5. Effect of pH on α-galactosidase activity and stability from *L. salivarius* subsp. *salivarius* Nam27. The optimum pH for activity was determined by measuring enzyme activity over the pH range from 4 to 9 by using 50 mM acetate buffer (pH 4.0-6.0), 50 mM K-phosphate buffer (pH 6.0-8.0) and 50 mM Tris-HCl (pH 8.0-9.0). The pH effect was examined using pNPG as a 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%.

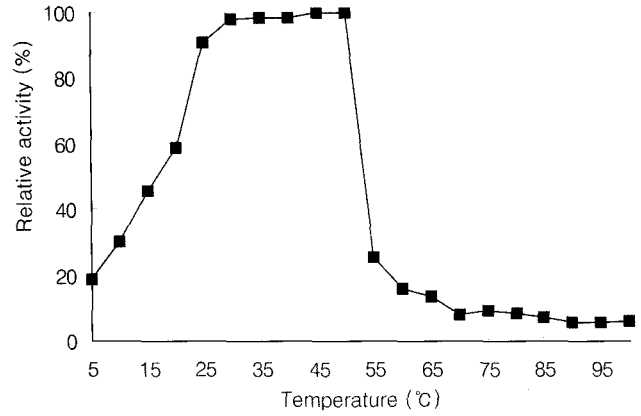

Fig. 6. Effect of temperature on α-galactosidase activity from *L. salivarius* subsp. *salivarius* Nam27. Activity was determined by assaying the enzyme at various incubation temperatures using pNPG as a substrate. All activity tests were done in triplicate. The maximum variation from the mean values (shown) was less than 5%.

min incubation period at temperatures up to 50°C. The enzyme had no residual activity after a 20 min incubation period at 55°C.

Effect of Metal Ions on α-Galactosidase Activity

The requirement of metal cations for the α-galactosidase activity was examined using Ca^{2+}, Co^{2+}, Cu^{2+}, Fe^{2+}, Hg^{2+}, K^{+}, Li^{+}, Mg^{2+}, Mn^{2+}, and Zn^{2+} as a counter ion. Some metal ions at 1 mM concentration did not affect α-galactosidase, but Cu^{2+} and Hg^{2+} significantly decreased α-galactosidase

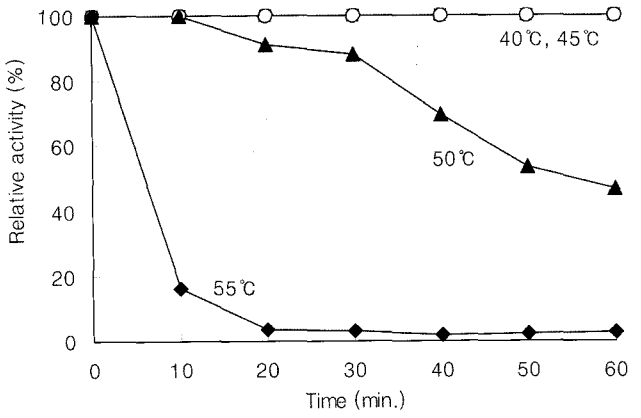

Fig. 7. Effect of incubation time on stability of α-galactosidase at 40, 45, 50 and 55°C. Residual enzyme activity was determined from 0 to 60 min at 10 min intervals. All activity tests were done in triplicate. The maximum variation from the mean values (shown) was less than 5%.

activity to 6.73% and 4.30%, respectively (Table 2). Regarding the effects of metal ions on the enzymatic activity, some differences were observed between our enzyme and others previously studied. Mercury and cupric ions inhibited most well-known α-galactosidases, including those from *Humicola* sp. (Kotwal *et al.*, 1999), *Aspergillus tamarii* (Civas *et al.*, 1984), *Bifidobacterium* sp. Int-57 (Yeo *et al.*, 1993), *Diplococcus pneumoniae* (Li *et al.*, 1963), *L. fermentum* (Garro *et al.*, 1993) as well as the enzyme produced by *L. salivarius* subsp. *salivarius* Nam27.

Substrate Specificity

Raffinose, stachyose and melibiose are the galactooli-

Table 2. Effect of metal ions (1 mM) on the activity of the purified α-galactosidase from *L. salivarius* subsp. *salivarius* Nam27

Metal	Relative activity (%) (Means ± SE*)
Control[c]	100.00[a]
$CaCl_2$	109.69[a] ± 20.2
$CoCl_2$	90.21[a] ± 6.1
$CuSO_4$	6.73[b] ± 4.3
$FeCl_2$	112.09[a] ± 1.0
$HgCl_2$	4.30[b] ± 2.2
KCl	92.81[a] ± 11.5
LiCl	92.71[a] ± 10.6
$MgCl_2$	94.94[a] ± 5.9
$MnCl_2$	125.34[a] ± 9.3
$ZnSO_4$	120.36[a] ± 5.4

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

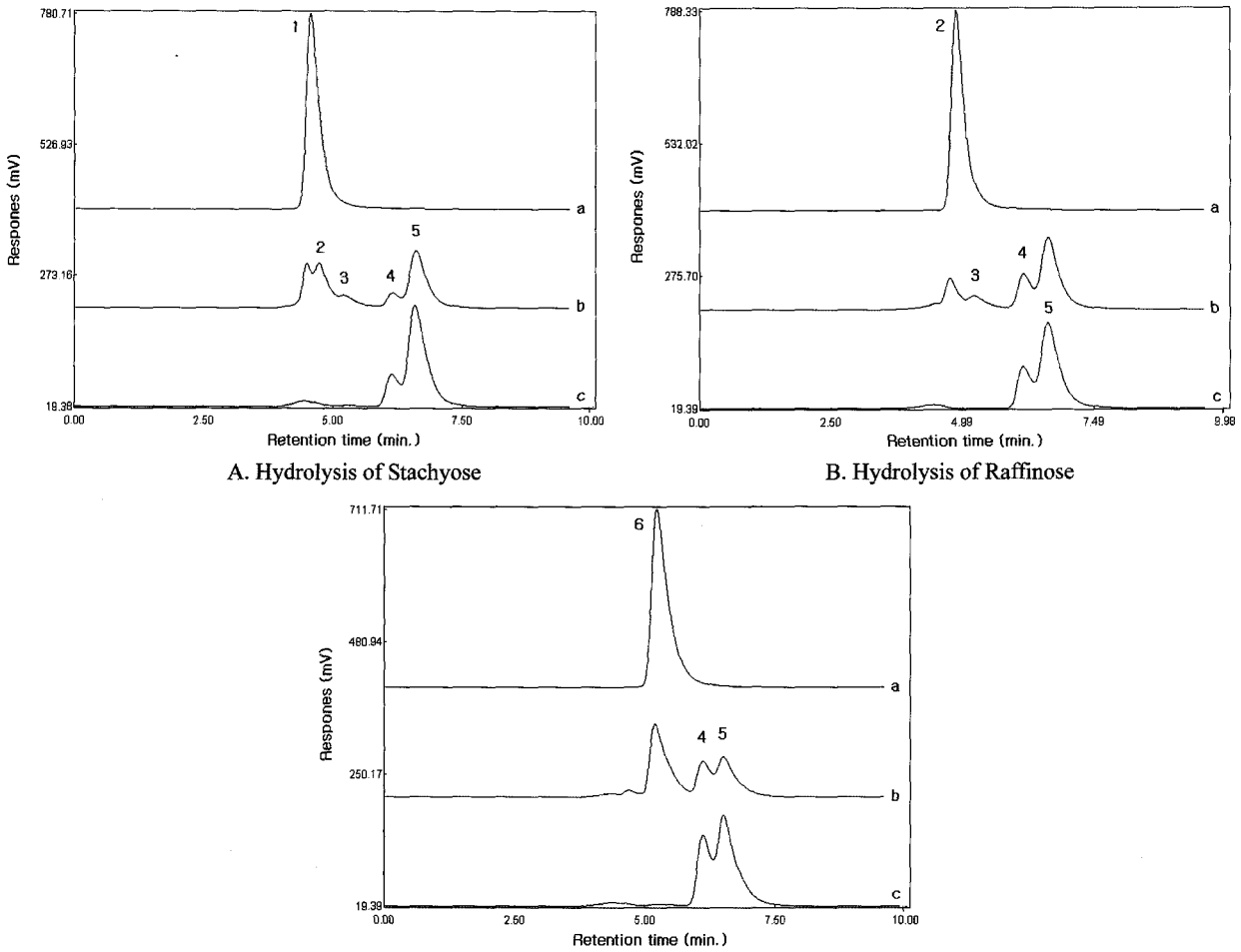

Fig. 8. Hydrolysis patterns of stachyose (A), raffinose (B) and melibiose (C) by α-galactosidase from *L. salivarius* subsp. *salivarius* Nam27. A is after 0 hr hydrolysis, B is after 6 hr hydrolysis, C is after 24 hr hydrolysis. 1 is stachyose, 2 is raffinose, 3 is sucrose, 4 is glucose, 5 is galactose, 6 is melibiose.

gosaccharides most commonly found in feedstuffs with relatively high levels in leguminous seeds. These oligosaccharides are not digested in the small intestine of monogastric animals but are degraded to some degree by the microflora of the hindgut (Murphy and Power, 2002).

The enzyme liberated galactose from melibiose, raffinose, and stachyose, and the hydrolysis rate of the substrate was studied by HPLC. Raffinose, stachyose and melibiose were completely decomposed after 24 h of incubation (Fig. 8).

CONCLUSION

α-Galactosidases are found in plants, animals, fungi and bacteria. They have been studied most widely in plant seeds. This study describes the purification and characterization of the α-galactosidase from a feces isolate, *L. salivarius* subsp. *salivarius* Nam27.

Comparison of published data with those obtained in this study indicates many similarities, including a temperature optimum of 40-50°C, broad pH stability (5-7) and inhibition profiles in the presence of metal ions. The molecular mass of α-galactosidase in the *L. salivarius* subsp. *salivarius* Nam27 (56,397.1 Da) differed from that previously found in other microorganisms (50-300 kDa).

α-Galacto-oligosaccharides, particularly raffinose and stachyose, are prebiotic which can stimulate the growth and activity of probiotic bacteria in the colon. The α-galactosidase of several intestinal bacteria, mainly bifidobacteria and lactobacilli, encouraged us to look for new prebiotic substrates that are not digested by intestinal enzymes and might be fermented by several intestinal bacteria. Raffinose and stachyose are probably the best known α-galactosides and the fermentation of raffinose by different bacteria has been extensively studied. α-Galactosidase could be used for the production of α-galactosides, which might be used as a prebiotic to improve growth of desirable intestinal bacteria.

ACKNOWLEDGEMENT

The authors are very grateful to Dr. John Lucey (Dept. of Food Science, University of Wisconsin, Madison) and Dr. Ashraf Nasr Eldin Hassan (Dept. of Dairy Science, South Dakota State University, Brookings) for proofreading this manuscript.

REFERENCES

1. Aduse-Opoku, J., Tao, L., Ferretti, J. J., and Russell, R. R. B. (1991) Biochemical and genetic analysis of *Streptococcus mutans* α-galactosidase. *J. Gen. Microbiol.* **137**, 2271-2272.
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. 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.
5. Civas, A., Eberhard, R., Dizet, P. L., and Petek, F. (1984) Glycosidases induced in *Aspergillus tamarii* mycelial α-D-galactosidases. *J. Biochem.* **219**, 849-855.
6. Gao, Z., and Schaffer, A. A. (1999) A novel alkaline α-galactosidase from melon fruit with a substrate preference for raffinose. *Plant Physiol.* **119**, 979-987.
7. Garro, M. S., de Giori, G. S., de Valdez, G. F., and Oliver, G.. (1993) Characterization of alpha-galactosidase from *Lactobacillus fermentum*. *J. Appl. Bacteriol.* **75**, 485-488.
8. Gherardini, F., Babcock, M., and Salyers, A. A. (1985) Purification and characterization of two α-galactosidases associated with catabolism of guar gum and other α-galactosides by *Bacteroides ovatus*. *J. Bacteriol.* **161**, 500-506.
9. Jin, F., Li, Y., Zhang, C., and Yu, H. (2001) Thermostable α-amylase and α-galactosidase production from the thermophilic and aerobic *Bacillus* sp. JF strain. *Process Biochem.* **36**, 559-564.
10. Keller, F., and Pharr, D. M. (1996) Metabolism of carbohydrates in sink and sources: galactosyl-sucrose oligosaccharides. In: Photoassimilate Distribution in Plants and Crops. Marcel Dekker, New York. pp. 157-183.
11. Kotwal, S. M., Gote, M. M., Khan, M. I., and Khire, J. M. (1999) Production, purification and characterization of a constitutive intracellular α-galactosidase from the thermophilic fungus *Humicola* sp. *J. Ind. Microbiol. & Biotechnol.* **23**, 661-667.
12. Leder, S., Hartmeier, W., and Marx, S. P. (1999) Alpha-galactosidase of *Bifidobacterium adolescentis* DSM 20083. *Curr. Microbiol.* **38**, 101-106.
13. Li, Y. T., Li, S. C. C., and Shetlar, M. R. (1963) α-Galactosidase from *Diplococcus pneumoniae*. *Arch. Biochem. Biophys.* **103**, 436-442.
14. Mitsutomi, M., and Ohtakara, A. (1988) Isolation and identification of oligosaccharides produced from raffinose by transgalactosylation reaction of thermostable α-galactosidase from *Pycnoporus cinnabarinus*. *Agric. Biol. Chem.* **52**, 2305-2311.
15. Murphy, R. A., and Power, R. F. G. (2002) Expression of an α-galactosidase from *Saccharomyces cerevisiae* in *Aspergillus awamori* and *Aspergillus oryzae*. *J. Industrial Microbiol. & Biotechnol.* **28**, 97-102.
16. Nagao, Y., Nakada, T., Imoto, T., Shimamoto, M., Sakai, T., Tsuda, S., and Tsuchiya, T. (1988) Purification and analysis of the structure of α-galactosidase from *Escherichia coli*. *Biochem. Biophys. Res. Commun.* **151**, 236-241.
17. Ohshima, T., Murray, G. J., Swaim, W. D., Longenecker, G., Quirk, J. M., Cardarelli, C. O., Sugimoto, Y., Pastan, I., Gottesman, M. M., Brady, R. O., and Kulkarni, A. B. (1997) α-Galactosidase A deficient mice: a model of Fabry disease. In: Proceedings of the National Academy of Sciences of the United States of America, pp. 2540-2544.
18. Roy, D., Blanchette, L., Savoie, L., Ward, P., and Chevalier, P. (1992) Alpha- and beta-galactosidase properties of *Bifidobacterium infantis*. *Milchwissenschaft.* **47**, 18-21.
19. Savel'ev, A. N., Ibatyllin, F. M., Eneyskaya, E. V., Kachurin, A. M., and Neustroev, K. N. (1996). Enzymatic properties of α-galactosidase from *Trichoderma reesei*. *Carbohydrate Research,* **296**, 261-273.
20. Shabalin, K. A., Kulminskaya, A. A., Savel'ev, A. N., Shishlyannikov, S. M., and Neustroev, K. N. (2002) Enzymatic properties of α-galactosidase from *Trichoderma reesei* in the hydrolysis of galactooligosaccharides. *Enzyme Microbial Technol.* **30**, 231-239.
21. Silvestroni, A., Connes, C., Sesma, F., De-Giori, G. S., and Piard, J. C. (2002) Characterization of the melA Locus for alpha-Galactosidase in *Lactobacillus plantarum*. *Appl. Environ. Microbiol.* **68**, 5464-5471.
22. Tzortzis, G., Jay, A. J., Baillon, M. L. A., Gibson, G. R., and Rastall, R. A. (2003) Synthesis of α-galacto-oligosaccharides with α-galactosidase from *Lactobacillus reuteri* of canine origin. *Appl. Microbiol. Biotechnol.* **63**, 286-292.
23. Yeo, N. I., Lee, S. K., and Ji, G. E. (1993) Characterization of α-galactosidase from *Bifidobacterium* sp. Int-57. *Kor. J. Food Sci. Technol.* **25**, 689-693.
24. Zeilinger, S., Kristufek, D., Inci, A. A., Hodits, R., and Kubicek, C. P. (1993) Conditions of formation, purification and characterization of an α-galactosidase of *Trichoderma reesei* RUT-30. *Appl. Environ. Microbiol.* **59**, 1347-1353.

(2007. 2. 7. 접수/2007. 3. 21. 채택)