

Full Length Research Paper

Production and partial characterization of α -galactosidase activity from an Antarctic bacterial isolate, *Bacillus* sp. LX-1

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An Antarctic *Bacillus* sp. isolate was found to exhibit extracellular α -galactosidase activity. On the basis of the results of 16S rRNA sequencing, the strain was named *Bacillus* sp. LX-1. In a one-factor-at-a-time experiment, galactose, peptone and Mn^{2+} were found to be the medium components that facilitated enzyme production. The new strain showed optimal α -galactosidase activity at pH 7.0 and temperature of 40°C. The enzyme exclusively hydrolyzed α -D-galactosides such as *p*-nitrophenyl- α -galactopyranoside, melibiose, raffinose and stachyose, and showed no effect with proteases such as trypsin, pancreatin, and pronase. Enzyme activity was almost completely inhibited by the presence of Ag^+ , Hg^{2+} , Cu^{2+} , and sodium dodecylsulfate (SDS) but was unaffected by β -mercaptoethanol and ethylenediaminetetraacetic acid (EDTA). The LX-1 α -galactosidase may be a promising candidate as a biocatalyst for soybean processing in food and feed industries.

Key words: Antarctic, *Bacillus* sp., α -galactosidase, one-factor-at-a-time, biocatalyst.

INTRODUCTION

α -Galactosidase (EC 3.2.1.22, α -D-galactoside galactohydrolase) is an exo-type glycoside hydrolase that catalyzes the hydrolysis of α -1,6-galactosidic linkages in galactose-containing oligosaccharides such as melibiose (galactose- α -1,6-glucose), raffinose (galactose- α -1,6-sucrose), and stachyose (galactose- α -1,6-raffinose) and in galactomannan, which is commonly found in legumes and seeds (Viana et al., 2006; Comfort et al., 2007). Although α -galactosidase has been extensively screened from various sources such as bacteria, fungi, plants, and animals (Matsuura, 1998; Marraccini et al., 2005; Cao et al., 2007, 2010), its enzymatic properties differ remarkably according to the source (Viana et al., 2006).

Furthermore, there is ongoing interest in screening and characterizing novel α -galactosidase sources that yield enzymes with high levels of activity.

α -Galactosidase has been usefully exploited in many fields such as the feed, food, chemical, pulp, and medicinal industries. These applications include enhancing the nutritive quality in animal feed by hydrolyzing non-metabolizable sugars (Ghazi et al., 2003), improving crystallization and yield of sucrose by eliminating raffinose from sugar beet molasses (Ganter et al., 1988), alleviating the flatulence-causing property of soybean products (Viana et al., 2006), improving oil and gas recovery by hydrolysis of proppant matrix (McCutchen et al., 1996), enzymatic bleaching of softwood pulps (Talbot and Sygusch, 1990), and converting erythrocyte antigens from type B to type O (Kruskall et al., 2000). To date, α -galactosidases with different levels of enzyme activities have been extracted from a variety of bacterial species, including *Bacillus stearothermophilus* (Talbot and Sygusch, 1990), *Thermus brockianus* (Fridjonsson et al., 1999), *Bifidobacterium bifidum* (Goulas et al., 2009), *Streptomyces griseoalbus* (Anisha et al., 2009), *Pedobacter nyackensis*

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Abbreviations: PCR, Polymerase chain reaction; BLAST, Basic local alignment search tool; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; CMC, carboxymethylcellulose; DNS, dinitrosalicylic acid.

(Liu et al., 2009), *Pseudoalteromonas* sp. KMM701 (Balabanova et al., 2010) and *Lactobacillus acidophilus* (Farzadi et al., 2011). However, no studies have reported the activity of α -galactosidase from cryophilic bacterial species, such as those inhabiting the polar regions or deep sea. The main objectives of the present study are to analyze the optimal medium components for extracellular production of α -galactosidase by an Antarctic bacterial isolate, *Bacillus* sp. LX-1, and to partially characterize the enzyme.

MATERIALS AND METHODS

Bacterial strain and culture conditions

The bacterial isolate, LX-1 derived from the Antarctic soil samples was supplied by the KOPRI (Korea Polar Research Institute) operating the King Sejong station (South Korea) in Antarctica. α -Galactosidase activity was identified by the presence of blue colonies on selective Luria-Bertani (LB) agar (1% tryptone, 0.5% yeast extract, 1% NaCl, and 1.5% bacto agar (Difco); pH 7.2), supplemented with 0.2% lactose and 32 μ g/mL of 5-bromo-4-chloro-3-indolyl- α -D-galactopyranoside (X- α -Gal) at 28°C, as described in a previous study (Goulas et al., 2009).

Taxonomic identification of strain LX-1

Genomic DNA of LX-1 was extracted using the FastDNA Kit (Qbiogene) according to the manufacturer's protocol. The 16S rRNA from the genomic DNA was amplified by polymerase chain reaction (PCR) using the universal primers, 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCT-GTTACGACTT-3') (William et al., 1991). The amplified 1446 bp sequences were analyzed using an automated ABI PRISM 3730 XL DNA analyzer (Applied Biosystems) and then compared with sequences in the Genbank database [National Center for Biotechnology Information (NCBI)] using Basic local alignment search tool (BLAST) (Altschul et al., 1990). Sequences showing a relevant degree of similarity were aligned using CLUSTAL W software (Thompson et al., 1994). The evolutionary distances with other strains of *Bacillus* strains were computed using the maximum composite likelihood method (Tamura et al., 2004), and the phylogenetic relationships were determined using the Molecular evolutionary genetics analysis software (MEGA, version 4.0) (Tamura et al., 2007).

Nucleotide sequence accession numbers

The 16S rRNA nucleotide sequence has been deposited in the Genbank database (Accession No. HQ660811).

Optimization of media components

To screen the media components for α -galactosidase production, carbon sources (1% galactose, 2% galactose, 1% wheat bran and 2% wheat bran), nitrogen sources (1% yeast extract, 1% tryptone, 1% soybean meal, 1% peptone and 1% ammonium sulfate) and mineral sources (1% NaCl, 0.01% CaCl₂, 0.01% MgSO₄, 0.07% KH₂PO₄, 0.001% MnSO₄ and 0.001% FeSO₄) were tested. The preferable nutrient for α -galactosidase production was determined by varying one factor at a time while keeping the others constant (Liu et al., 2007). The data were presented as mean \pm standard

errors from three experiments, and their significance was analyzed using Student's *t*-test.

Partial purification of the enzyme

Strain LX-1 was cultured in 1 L of LB medium (pH 7.2) containing 0.2% galactose for 96 h at 28°C. The culture medium containing secreted α -galactosidase was centrifuged (10000 \times *g*; 30 min; 4°C) to remove cells, and the protein in the supernatant was precipitated with ammonium sulfate (50% saturation) and centrifuged. The obtained pellet was dissolved in 50 mM Tris-HCl (pH 8.0) and dialyzed overnight against 50 mM Tris-HCl (pH 7.4) at 4°C. This dialyzed solution was used as the α -galactosidase source to study the catalytic properties throughout this work.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and zymogram analysis

SDS-PAGE of partially purified enzyme was carried out using a Xcell II Mini-Cell and NuPAGE Novex 4 to 12% Bis-Tris Gels (Invitrogen) according to the manufacturer's recommendations. The separated proteins were visualized by staining with Simply Blue Safe Stain (Invitrogen). The enzyme was also subjected to non-denaturing 6.5% polyacrylamide gel electrophoresis (PAGE) using a modular Mini-protean II electrophoresis system (Bio-Rad) according to the manufacturer's instructions. The gel was then placed on a 1.5% (w/v) bacto agar plate containing 4 mg/mL X- α -Gal and incubated at 40°C for 12 h. α -Galactosidase activity was observed as blue bands on the gel.

Enzyme assay and substrate specificity

Unless otherwise stated, α -galactosidase activity was measured at 40°C for 15 min by assaying the release of *p*-nitrophenol from *p*-nitrophenyl- α -D-galactopyranoside, with final concentration of 1 mM in 1 mL of 50 mM sodium phosphate (pH 7.0). Enzyme activity on other *p*-nitrophenyl (*p*NP) or *o*-nitrophenyl (*o*NP) conjugated substrates, such as *p*NP- α -L-arabinofuranoside, *p*NP- β -D-xylopyranoside, *p*NP- β -D-cellobioside, *p*NP- α -D-glucopyranoside, *o*NP- β -D-galactopyranoside, and *o*NP- β -D-glucopyranoside, was determined with a final substrate concentration of 1 mM in 1 mL of 50 mM sodium phosphate (pH 7.0) at 40°C. The reactions were then stopped by adding 1 mL of 1 M Na₂CO₃, and color development was measured at a wavelength of 405 nm (OD_{405 nm}). One unit (U) of enzyme activity was defined as the amount of enzyme required to produce 1 μ mol of *p*-nitrophenol or *o*-nitrophenol per minute, under the assay conditions described above. When carboxymethylcellulose (CMC), xylan, galactomannan, maltose, and starch were used as substrates with final concentrations of 0.4%, the amount of reducing sugar produced was determined by the dinitrosalicylic acid (DNS) method under standard assay conditions (Miller, 1959). Enzyme activity on substrates like lactose, raffinose, melibiose, and stachyose was evaluated by assaying the release of D-galactose using a galactose test kit (Boehringer Mannheim GmbH). One enzyme unit (U) was defined as the amount of enzyme required to produce 1 μ mol of reducing sugar equivalent, or galactose per minute under standard assay conditions.

Effect of pH and temperature on enzyme activity

α -Galactosidase activities were evaluated in pH range of 3 to 8.5 [50 mM glycine-HCl (pH 3); 50 mM sodium acetate (pH 4 to 5.5); 50

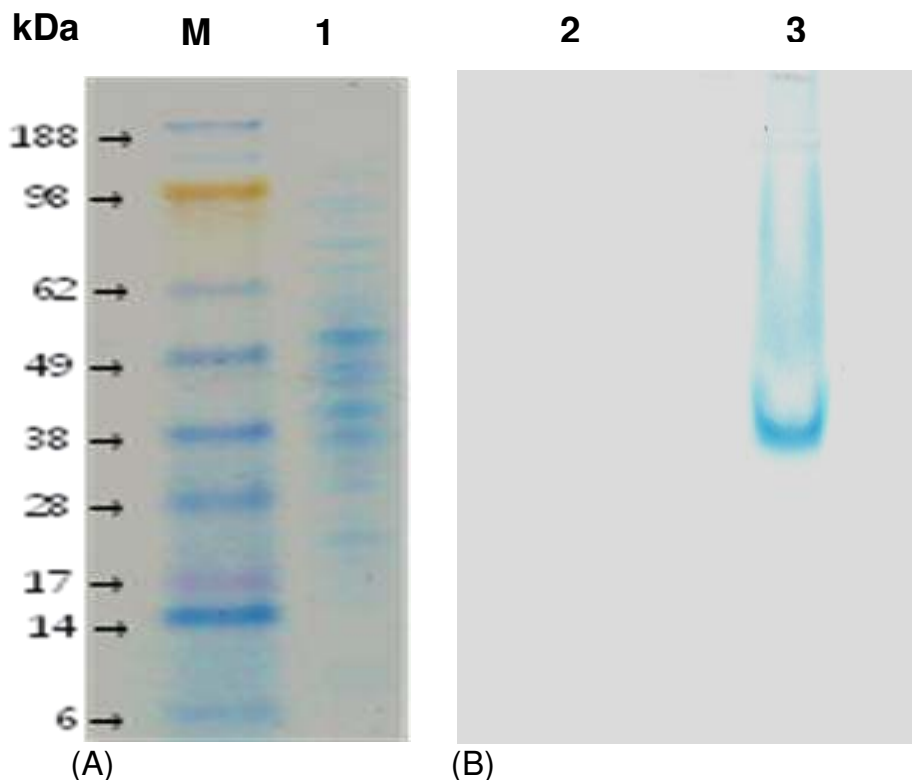


Figure 1. SDS-PAGE (A) of partially purified enzyme and zymogram analysis, (B) showing α -galactosidase activity on a non-denaturing electrophoretic gel. Lane M, Seeblue plus2 pre-stained standard (Invitrogen); Lane 1, partially purified enzyme; lane 2, bovine serum albumin (negative control); lane 3, LX-1 α -galactosidase.

mM sodium phosphate (pH 6 to 7); 50 mM Tris-HCl (pH 7.4 to 8.5)] at 30°C and at temperatures between 0 and 70°C, at the optimum pH identified.

Thermal stability

The thermal stability was measured by assessing the residual activity after pre-incubating the enzyme in 50 mM sodium phosphate (pH 7.0) at various temperatures ranging from 0 to 70°C for 30 min.

Effect of metal ions and chemicals on enzyme activity

The effect of different metal ions and chemicals on α -galactosidase activity was determined under standard assay conditions after pre-incubating the enzyme with 2 mM Mg^{2+} , Ca^{2+} , Co^{2+} , Zn^{2+} , Ni^{2+} , Cu^{2+} , Mn^{2+} , Fe^{2+} , Hg^{2+} , Ba^{2+} , Ag^+ , Na^+ , K^+ , β -mercaptoethanol, ethylenediaminetetraacetic acid (EDTA), sodium dodecyl sulfate (SDS), or phenylmethylsulfonyl fluoride (PMSF) in 50 mM sodium phosphate (pH 7.0) for 20 min at 25°C.

Determination of protease resistance

The resistance of LX-1 α -galactosidase to proteolysis was investigated by using a slightly modified version of previously described method (Cao et al., 2010). The partially purified enzyme (9 μ g) was incubated with 0.9 μ g of trypsin (Sigma), proteinase K (Sigma),

subtilisin Carlsberg (Sigma), pancreatin (Sigma) or pronase (Sigma) at 37°C for 30 min in 0.1 M sodium phosphate (pH 7.0), and a combination of protease and α -galactosidase in a ratio of 1:10 (w/w). Then, the enzyme activity was assayed under standard conditions.

RESULTS AND DISCUSSION

Identification and naming of the isolated strain

To identify the isolated strain (LX-1) showing α -galactosidase activity (Figure 1), the 16S rRNA gene was cloned and its sequence was compared with those available in the National Center for Biotechnology Information (NCBI) database. A phylogenetic tree modeled using 16S rRNA sequences from the isolated strain and eight other *Bacillus* strains showed that the isolated strain shared 99.4% sequence identity with the type strain, *Bacillus megaterium* DSM 32^T (Figure 2). Therefore, it was named *Bacillus* sp. LX-1.

Effect of different carbon sources on α -galactosidase production

As shown in Figure 3, the highest α -galactosidase

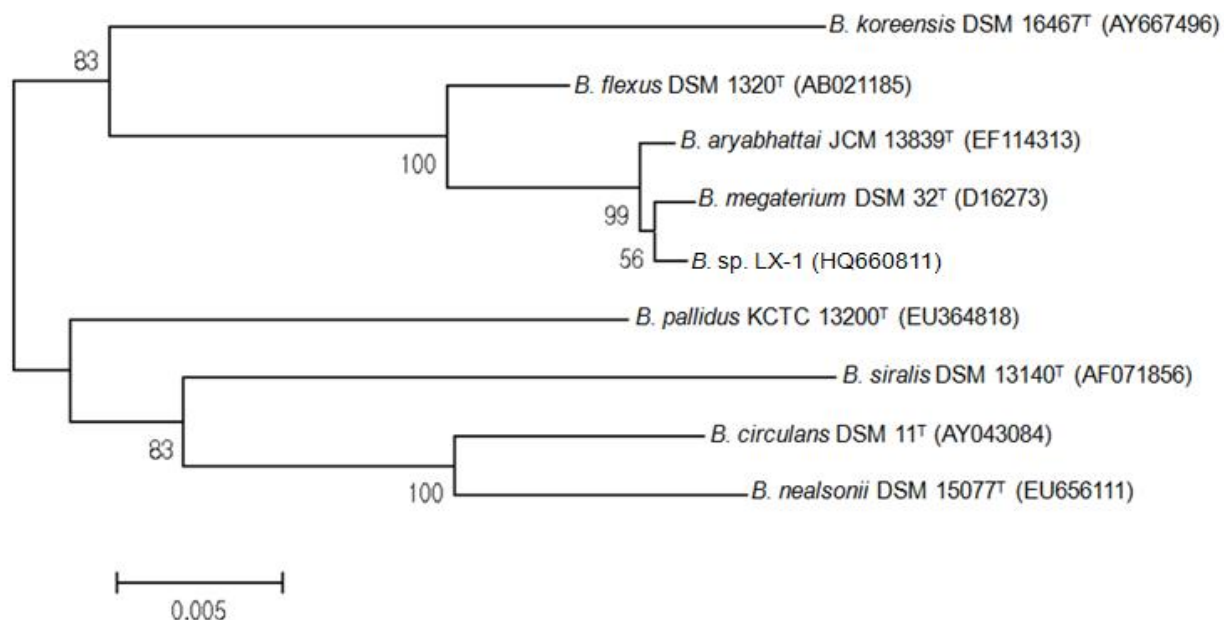


Figure 2. Phylogenetic tree constructed using 16S rRNA sequences of *Bacillus* sp. LX-1 and eight other *Bacillus* strains. Bootstrap values (based on 1,000 trials and only values more than 50%) are shown at the nodes. The GenBank accession numbers are indicated with parentheses. Bar, 5-base substitutions per 1,000 nucleotide positions.

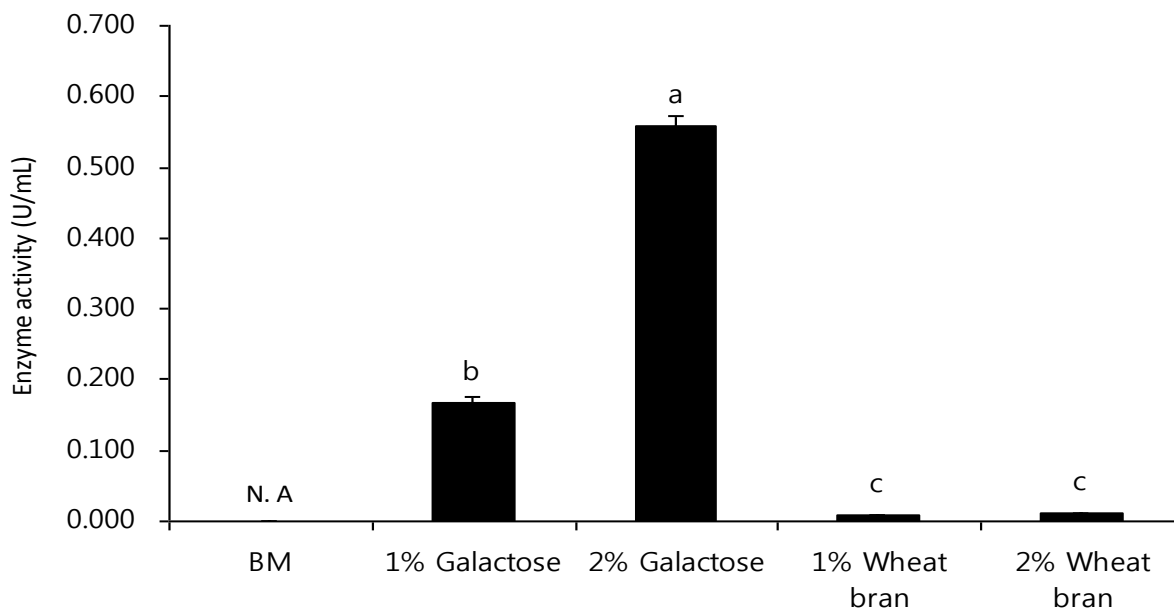


Figure 3. Effect of carbon sources on α -galactosidase production by *Bacillus* sp. LX-1. The basal medium (BM) is composed of 0.5% yeast extract, 1% tryptone, 1% NaCl, 0.01% $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.01% $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.07% KH_2PO_4 , 0.001% $\text{MnSO}_4 \cdot \text{H}_2\text{O}$, and 0.001% $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (initial pH, 7.0; culture time, 24 h). N.A.: no activity. Data were expressed as mean \pm standard errors from three experiments. Values with unlike lower case letter differ ($P < 0.05$).

production (0.558 ± 0.014 U/mL) was observed when 2% galactose was used as the carbon source. A previous study showed that galactose was the best carbon source for enzyme production by *Aspergillus parasiticus* MTCC-2796 (Shivam et al., 2009). The LX-1 strain displayed

poor enzyme production when wheat bran was used as the substrate. However, the same substrate elicited maximum enzyme activity from *Aspergillus foetidus* ZU-G1 (Liu et al., 2007) when used as the carbon source. Thus, it was assumed that α -galactosidase production by

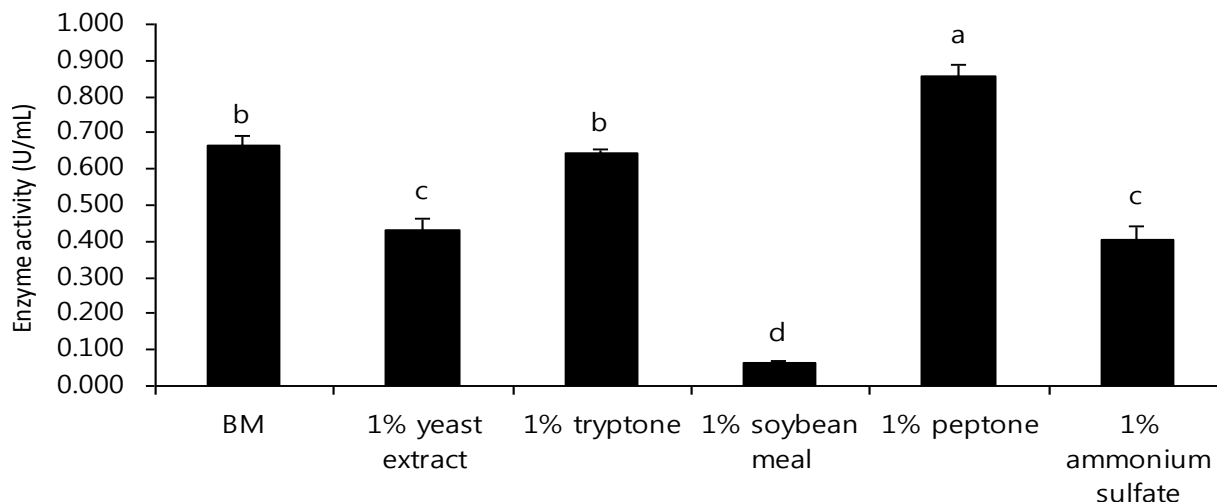


Figure 4. Effect of additional nitrogen sources on α -galactosidase production by *Bacillus* sp. LX-1. The basal medium (BM) is composed of 2% galactose, 0.5% yeast extract, 1% tryptone, 1% NaCl, 0.01% $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.01% $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.07% KH_2PO_4 , 0.001% $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$, and 0.001% $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (initial pH, 7.0; culture time, 24 h). Data were expressed as mean \pm standard errors from three experiments. Values with unlike lower case letter differ ($P < 0.05$).

different microbial strains was substrate specific.

Effect of different nitrogen sources on α -galactosidase production

Although 0.5% yeast extract and 1% tryptone were present in the basal medium, an additional 1% tryptone, yeast extract, ammonium sulfate [$(\text{NH}_4)_2\text{SO}_4$] or soybean meal had little positive effect or a negative effect on α -galactosidase production (Figure 4). Soybean meal, in particular, significantly inhibited enzyme production (Figure 4), whereas it supported α -galactosidase production by *A. foetidus* ZU-G1 in a dose-dependent manner (Liu et al., 2007). The additional supply of peptone supported enzyme production (0.856 ± 0.033 U/mL) (Figure 4).

Effect of essential elements on α -galactosidase production

Among the essential elements tested, MnSO_4 induced a marked increase in α -galactosidase activity (0.901 ± 0.044 U/mL) (Figure 5). Only MgSO_4 slightly inhibited enzyme production, whereas NaCl, CaCl_2 , KH_2PO_4 or FeSO_4 had no marked effect on enzyme production (Figure 5).

Effect of pH on enzyme activity

As shown in Figure 6A, maximum enzyme activity was observed at pH 7.0, and over 50% of the peak activity

was exhibited at pH 6.0 to 7.0. However, no activity was detected in the acidic pH range (pH 3 to 5). Generally, the optimum pH for bacterial α -galactosidases is slightly alkaline (pH 6.0 to 7.5), whereas that for fungal and yeast enzymes is acidic (pH 3 to 5) (Patil et al., 2010). Nevertheless, the α -galactosidases from the lactic acid bacteria strain, *Leuconostoc mesenteroides* JK55 and *Streptomyces griseoloalbus* exhibited 90 and 70% of maximal enzyme activity at an acidic pH of 4.0, respectively (Yoon and Hwang, 2008; Anisha et al., 2009). Furthermore, the acidic α -galactosidase was unfavorable for treating galacto-oligosaccharides present in soymilk, because lowering the natural pH (around 6.2 to 6.4) of soymilk leads to precipitation of soy proteins and a sour taste (Patil et al., 2010).

Effect of temperature on enzyme activity and thermal stability

LX-1 α -galactosidase exhibited more than 50% of maximal enzyme activity at 25 to 45°C. The optimum temperature for enzyme activity was 40°C, and the enzyme exhibited relatively high activity (22% of the maximal activity) even at 5°C (Figure 6B). However, only negligible activity was observed above 50°C. Thermal stability of the LX-1 α -galactosidase was tested after pre-incubation of the enzyme at 0 to 70°C for 30 min. The enzyme maintained about 88% of the initial activity at 45°C, but no activity was detected at temperatures of 50°C and above (Figure 7). Although a thermostable α -galactosidase can be considerably advantageous in the food processing industry because of the increased reaction rates at higher temperatures and lower risk of

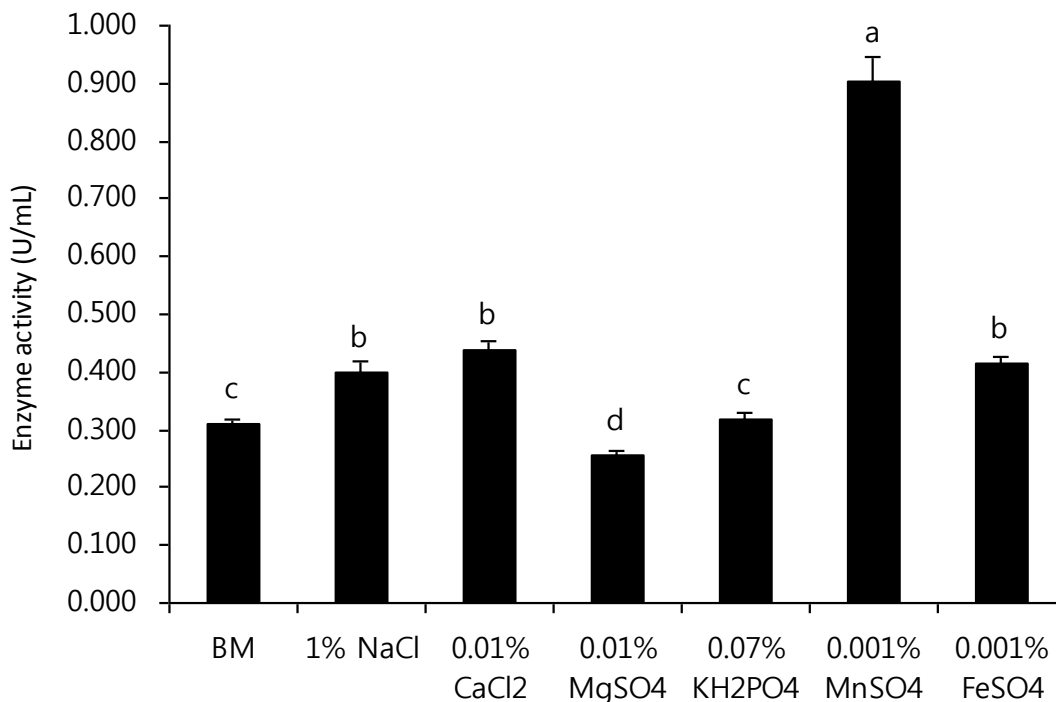


Figure 5. Effect of essential elements on α -galactosidase production by *Bacillus* sp. LX-1. The basal medium (BM) is composed of 2% galactose, 0.5% yeast extract, 1% tryptone, and 1% peptone (initial pH, 7.0; culture time, 24 h). Data were expressed as mean \pm standard errors from three experiments. Values with unlike lower case letter differ ($P < 0.05$).

contamination by mesophilic organisms (King et al., 2002; Patil et al., 2010), it seems that the optimum temperature for thermostable enzymes is not always desirable for industrial applications (King et al., 2002). For example, to improve the nutritive and sensory qualities of soymilk, it is often fermented using lactic acid bacteria with α -galactosidase activity at 25 to 45°C (Yoon and Hwang, 2008). Moreover, in the case of enzymes used in the feed industries, even if the physiological temperature range of monogastric animals, including poultry and swine, is 37 to 40°C, enzyme supplementation to cold feed and the initial stages of digestion of the feed occurs at low temperatures (Boyce and Walsh, 2006). Thus, LX-1 α -galactosidase may be a suitable enzyme supplement for soybean processing in the food and feed industries.

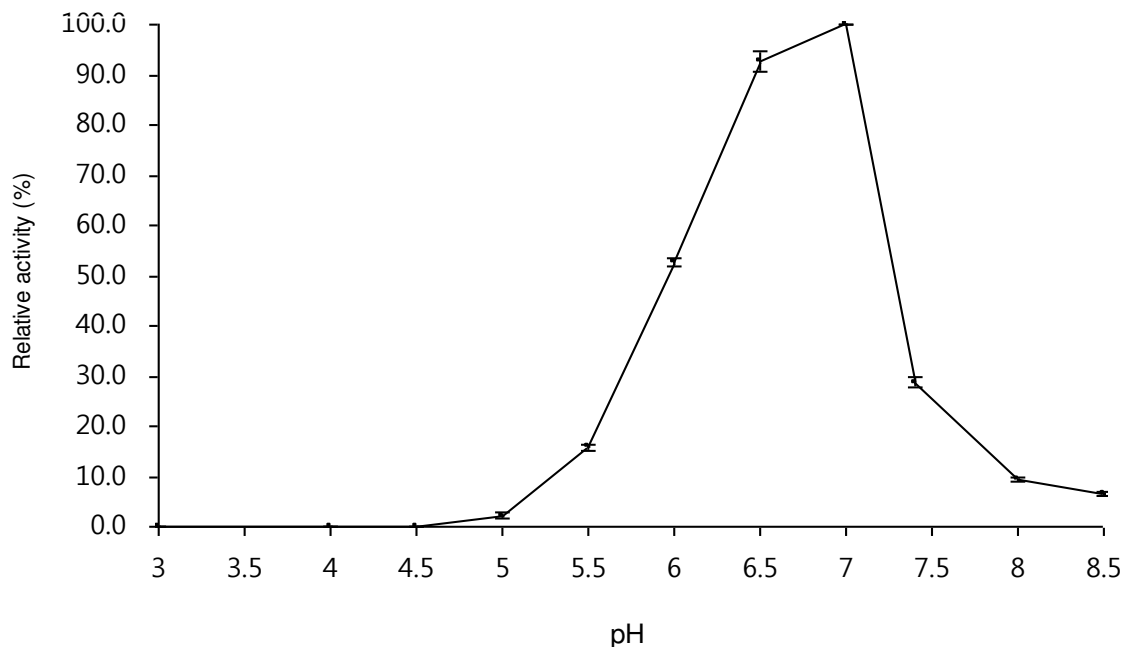
Effect of metal ions and inhibitors on enzyme activity

LX-1 α -galactosidase activity in the presence of various metal ions and chemicals is shown in Table 1. The enzyme was almost completely inhibited by Ag^+ , Hg^{2+} , and Cu^{2+} , similar to the results shown in previous studies on α -galactosidases produced by *B. megaterium* (Patil et al., 2010), *Aspergillus terreus* (Falkoski et al., 2006) and *Penicillium griseoroseum* (Falkoski et al., 2006). In α -galactosidases from several organisms, sulfhydryl-reactive metal ions such as Ag^+ and Hg^{2+} interfere with

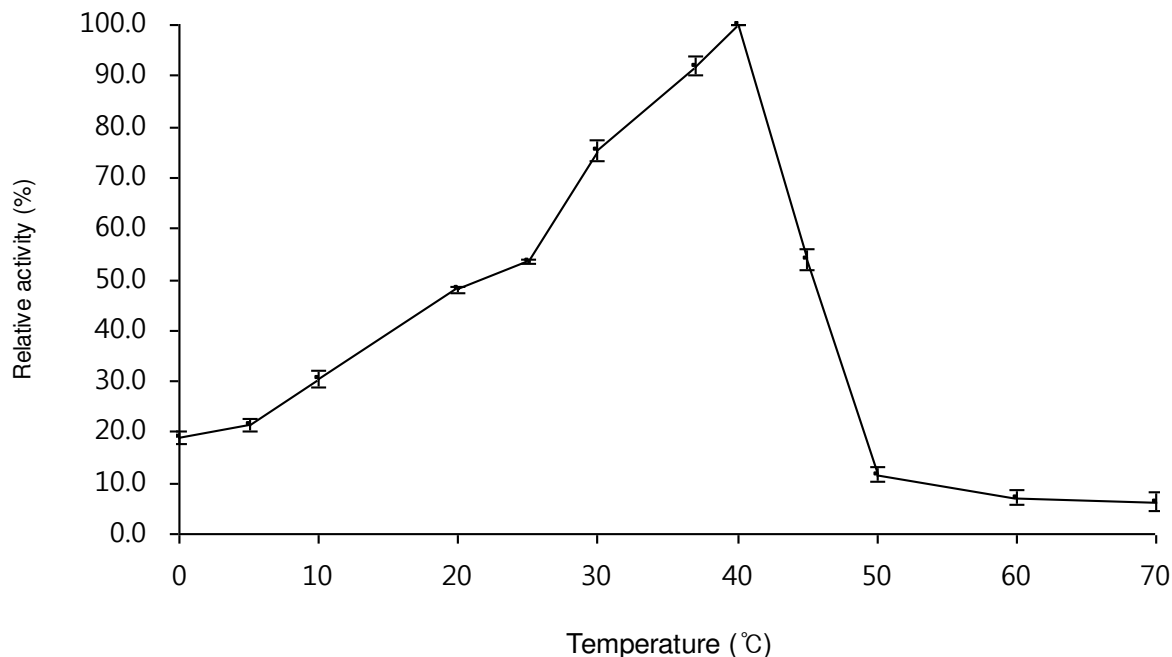
the substrate-enzyme interaction by binding to the cysteine residues in the active site (Wang et al., 2010). The enzyme activity was decreased by 48 to 63% in the presence of Zn^{2+} and Ni^{2+} . In contrast, the activity of α -galactosidase from fungal *Bispora* sp. was slightly stimulated by Zn^{2+} , Ni^{2+} and Cu^{2+} (Wang et al., 2010). However, Ca^{2+} , Co^{2+} , Mn^{2+} , Fe^{2+} , Ba^{2+} , Mg^{2+} , Na^+ , and K^+ had minimal or no inhibitory effects on the enzyme activity. LX-1 α -galactosidase was very sensitive to the common anionic detergent SDS, implying complete loss of enzyme function with the disruption of enzyme spatial structure (Falkoski et al., 2006). The enzyme was almost unaffected by β -mercaptoethanol and EDTA, suggesting that it is not a metalloenzyme and SH groups are not involved in enzyme function (Viana et al., 2006). Additionally, the enzyme was moderately inhibited by the well known serine protease inhibitor, PMSF (Hutadilok-Towatana et al., 1999).

Substrate specificity

As shown in Table 2, the enzyme exhibited strict specificity towards the synthetic aryl- α -galactosidic substrate, *p*-nitrophenyl- α -galactopyranoside. However, little or no activity was detected when it was used with substrates such as maltose, lactose, and other synthetic substrates containing β -linkages or arabinose and



(A)



(B)

Figure 6. Optimal pH (A) and temperature (B) activity profiles. (A) Relative activity at 40°C and various pH levels, where 100% equates to 1.55 ± 0.037 U/mL; (B) relative activity at pH 7 and various temperatures, where 100% equates to 1.57 ± 0.012 U/mL. The assays were performed at a final concentration of 1 mM *p*-NP- α -galactopyranoside. Data were expressed as mean \pm standard errors from three experiments.

glucose residues. The enzyme could not hydrolyze polysaccharides such as CMC, xylan, galactomannan (locust bean gum), and starch. Among galacto-oligosaccharides, such as melibiose, raffinose, and

stachyose, melibiose was the most effectively hydrolyzed; a similar effect was observed for α -galactosidases from *Penicillium* sp. 23 (Varbanets et al., 2001) and *B. stearothersophilus* (Talbot and Sygusch, 1990).

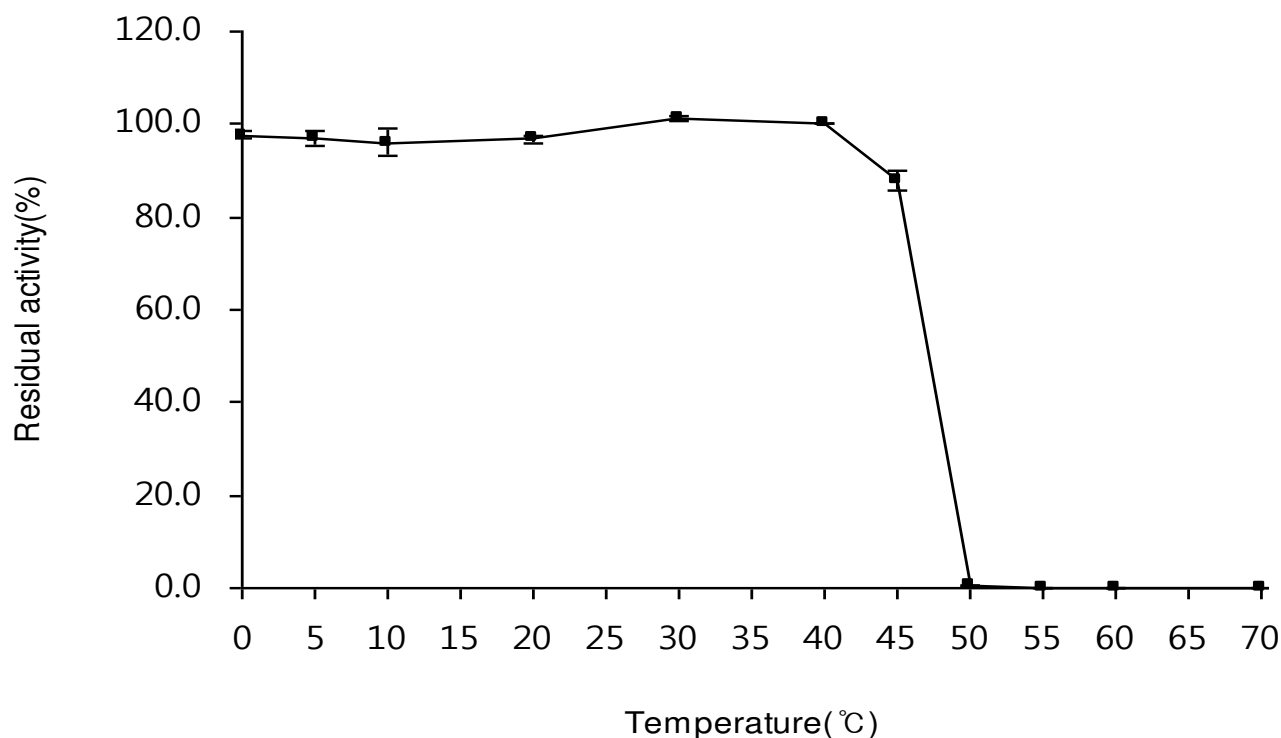


Figure 7. Thermal stability of LX-1 α -galactosidase activity. One hundred percent of the residual activity equates to 1.544 ± 0.053 U/mL. Data were expressed as mean \pm standard errors from three experiments.

Table 1. Effect of metal ions and chemicals on LX-1 α -galactosidase activity.

Reagent ^a	Relative activity (%) ^b
No addition	100
Ca ²⁺	92.3 \pm 1.8
Co ²⁺	79.2 \pm 3.1
Fe ²⁺	81.1 \pm 4.3
Mg ²⁺	98.4 \pm 4.0
Mn ²⁺	95.8 \pm 4.1
Ba ²⁺	100.0 \pm 0.3
Cu ²⁺	3.6 \pm 0.6
Zn ²⁺	52.1 \pm 2.1
Ni ²⁺	37.1 \pm 1.7
Hg ²⁺	0.7 \pm 0.7
K ⁺	103.8 \pm 2.7
Na ⁺	101.0 \pm 1.9
Ag ⁺	0
β -mercaptoethanol	98.5 \pm 2.1
EDTA	103.5 \pm 0.7
SDS	2.9 \pm 0.7
PMSF	82.3 \pm 0.9

^aThe final concentration of each reagent was 2 mM in the assay buffer. ^bThe activity in the absence of all reagents was considered 100%, which equates to 1.626 ± 0.029 U/mL. Data were expressed as mean \pm standard errors from three experiments.

Generally, most α -galactosidases degrade raffinose rapidly and stachyose slowly (Ishiguro et al., 2001). Furthermore, many of the so called family 27 α -galactosidases catalyze the release of galactose from intact galactomannan polymers, whereas the substrate specificity of family 36 enzymes is restricted to small oligosaccharides, including raffinose and stachyose (Wang et al., 2010). Thus, it is presumed that the LX-1 α -galactosidase belongs to family 36.

Effect of proteolysis on enzyme activity

LX-1 α -galactosidase showed different levels of resistance to the proteases tested (Figure 8). The enzyme activity was completely inhibited after 30 min incubation with proteinase K and subtilisin Carlsberg, whereas trypsin, pancreatin and pronase had almost no effect. This is important because susceptibility of feed enzymes to proteolytic attack can help determine the rate and site of inactivation of an enzyme (Wang et al., 2007). Additionally, the α -galactosidases, RmGal36 and Aga-F78, from *Rhizomucor miehei* and *Rhizopus* sp. F78 ACCC30795, respectively, were found to be resistant to trypsin (Cao et al., 2009; Katrolia et al., 2012). Meanwhile, approximately 50% of the initial enzyme activity shown by α -galactosidase from *Streptomyces* sp. S27

Table 2. Substrate specificity of LX-1 α -galactosidase.

Substrate	Concentration (mM)	Enzyme activity (U/mL)
<i>p</i> -nitrophenyl- α -D-galactopyranoside	1	1.622 \pm 0.063
<i>o</i> -nitrophenyl- β -D-galactopyranoside	1	0.036 \pm 0.010
Raffinose	1	1.384 \pm 0.073
Melibiose	1	14.563 \pm 0.146
Stachyose	1	0.777 \pm 0.064

Data were expressed as mean \pm standard errors from three experiments. No activity was detected on the substrates such as *p*-nitrophenyl- α -D-glucopyranoside, *p*-nitrophenyl- β -D-xylopyranoside, *p*-nitrophenyl- β -D-cellobioside, *o*-nitrophenyl- α -D-glucopyranoside, *p*-nitrophenyl- α -L-arabinofuranoside, lactose, CMC, xylan (birchwood), galactomannan (locust bean gum), starch and maltose.

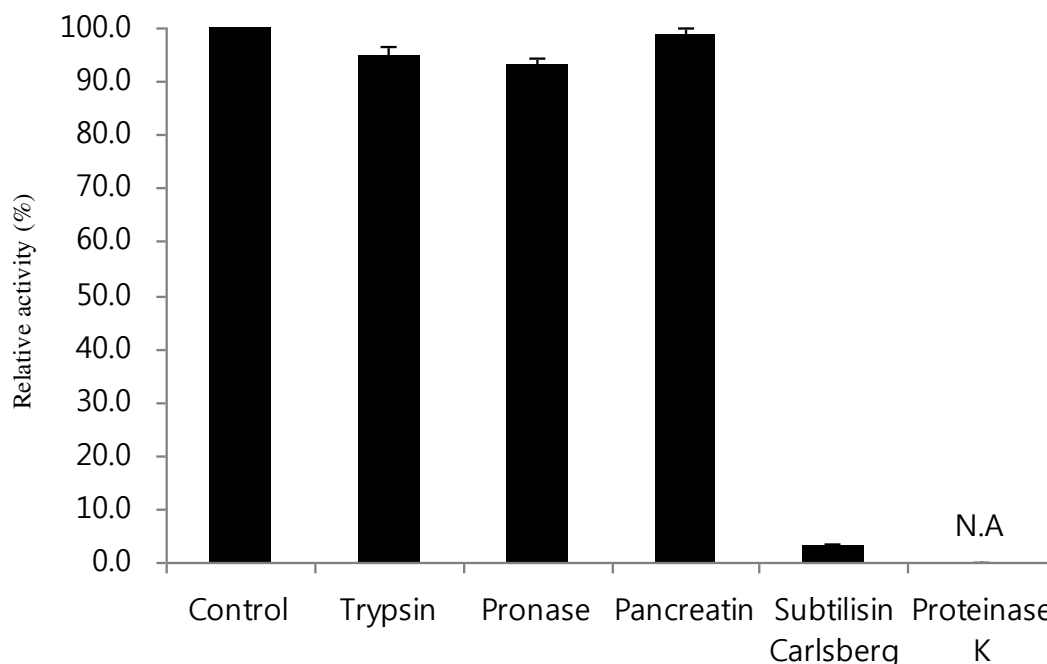


Figure 8. Effect of proteases on LX-1 α -galactosidase activity. The activity of protease-untreated control was defined as 100%, which equates to 1.678 \pm 0.008 U/mL. N.A.: no activity. Data were expressed as mean \pm standard errors from three experiments.

was lost after a 30 min trypsin digestion (Cao et al., 2010).

Conclusion

The LX-1 α -galactosidase may be a promising biocatalyst for soybean processing in the food and feed industry, because it showed optimal enzyme activity at physiologically-relevant and operationally-suitable temperature and pH ranges, and good stability against intestinal proteolytic enzymes such as trypsin and pancreatin. A more detailed characterization of the enzyme, including gene cloning, protein engineering, and fermentation technology assessments is warranted to achieve maximum catalytic efficiency and productive yield

of the enzyme.

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