

Purification and Characterization of Thermostable α -Galactosidase from *Aspergillus terreus*_{GR}

S. K. Shankar · S. K. Dhananjay · V. H. Mulimani

Received: 25 December 2007 / Accepted: 1 May 2008 /
Published online: 10 June 2008
© Humana Press 2008

Abstract An extracellular thermostable α -galactosidase producing *Aspergillus terreus*_{GR} strain was isolated from soil sample using guar gum as sole source of carbon. It was purified to apparent homogeneity by acetone precipitation, gel filtration followed by DEAE-Sephacel chromatographic step. The purified enzyme showed a single band after sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The molecular weight of the purified enzyme after SDS-PAGE was 108 kDa. The enzyme showed optimum pH and temperature of 5.0 and 65 °C, respectively, for artificial substrate pNP α Gal. α -Galactosidase from *A. terreus*_{GR} is found to be thermostable, as it was not inactivated after heating at 65 °C for 40 min. The K_m for pNP α Gal, oNP α Gal, raffinose, and stachyose are 0.1, 0.28, 0.42, and 0.33 mM, respectively. Inhibitors such as 1,10-phenanthroline, phenylmethylsulfonyl fluoride, ethylenediaminetetraacetic acid, mercaptoethanol, and urea have no effect, whereas *N*-bromosuccinamide inhibited enzyme activity by 100%. Among metal ions tested, Mg²⁺, Ni²⁺, Ca²⁺, Co²⁺, and Mn²⁺ had no effect on enzyme activity, but Ag⁺, Hg²⁺, and Cu²⁺ have inhibited complete activity.

Keywords *Aspergillus terreus*_{GR} · Thermostable · α -Galactosidase · Purification and characterization

Introduction

α -Galactosidase (α -D-galactoside galactohydrolase EC 3.2.1.22) is an exo-acting enzyme that mainly cleaves terminal α -1-6-linked D-galactosyl residues from a wide range of substrates including oligosaccharides of raffinose family sugars such as raffinose, stachyose, verbascose, and polysaccharides of galactomannans like locust bean gum and guar gum [1]. It widely occurs in microorganisms, plants, and animals [2–4]. α -Galactosidase has several biotechnological applications such as processing of beet sugar; the enzyme aided raffinose degradation can be utilized to avoid raffinose inhibition of

S. K. Shankar · S. K. Dhananjay · V. H. Mulimani (✉)
Department of Biochemistry, Gulbarga University, Gulbarga 585106 Karnataka, India
e-mail: v_h_mulimani@rediffmail.com

normal crystallization [5]. Hydrolysis of raffinose and stachyose present in leguminous food and feed is another application area, as these oligosaccharides cause intestinal discomfort, flatulence, and low-feed utilization in monogastrites [6]. In pulp and paper industry, biobleaching can be improved by adding α -galactosidase in combination with xylanase [7]. Furthermore, conversion of cheap gelling agent guar gum to a more attractive locust bean gum like polysaccharide can be achieved by partial removal of some galactoside units from guar polysaccharide [8]. Apart from this, some of the α -galactosidase preparations are known to remove the terminal α -1,3 linked galactose residues from glycans and have potential medical application in transfusion therapy for the conversion of erythrocyte of blood group 'B' specificity to more universally transferable type 'O' [9, 10]. In humans, mutations in the X-chromosomal α -galactosidase gene can lead to an accumulation of α -linked galactosides in tissues, which results in a recessive disorder called Fabry disease [11]. Fabrazym is a α -galactosidase preparation used for enzyme replacement therapy for Fabry's patients.

Stability of enzymes and activity at high temperatures are important and desirable properties for various biotechnological processes. Increasing enzyme thermostability would allow enzymatic reaction to be carried out at higher temperature; this would help to increase conversion rates and substrates solubility and to reduce the possibility of microbial growth and the viscosity of the reaction medium [12, 13]. Most thermostable enzymes in the market have been derived from mesophiles. Thermostable α -galactosidases have been purified and characterized from thermophilic bacteria and fungi [14–19]. However, reports on thermostable α -galactosidase from mesophilic fungi are scanty. Therefore, the present study describes purification and characterization of thermostable α -galactosidase from mesophilic fungus *Aspergillus terreus*_{GR}.

Materials and Methods

Chemicals

p-Nitrophenyl- α -D-galactoside (pNP α Gal), *o*-nitrophenyl- α -D-galactoside (oNP α Gal), 5-bromo-4-chloro-3-indolyl- α -D-galactopyranoside (X- α -D-Gal), 4-methyl-umbelliferyl- α -D-galactoside (MU- α -gal), raffinose, stachyose, melibiose, and sephadex-G 75 were purchased from Sigma Chemicals USA. DEAE-Sephacel was obtained from Pharmacia. All other chemicals used were of analytical grade.

Screening of α -Galactosidase Producing Microorganism

Different soil samples collected locally from gardens, cultivated fields, and composts were used in the present study to isolate α -galactosidase-producing fungi. The screening was done by serial dilution method by taking sterile water containing soil sample and inoculated onto the surface of X- α -Gal selective medium containing (g l⁻¹): X- α -Gal, 0.008; glucose, 10.0; (NH₄)₂SO₄, 3.0, KCL, 0.5, KH₂PO₄, 1; MgSO₄·7H₂O, 0.5; FeSO₄·7H₂O, 0.01; agar, 15; pH 5.5–6. After 5 days of incubation at 37 °C, those colonies displaying deep blue color were selected for determination of α -galactosidase activity. The cultivation medium contained (g l⁻¹): K₂HPO₄, 5; KH₂PO₄, 3.0; (NH₄)₂SO₄, 0.5; MgSO₄·7H₂O, 0.01 and guar gum, 10.0 with pH 6.0. One of the isolates having good amount of α -galactosidase activity was sent to Agharkar Research Institute (ARI), Pune, India for identification. The strain was maintained on potato dextrose agar (PDA) slants at 4 °C.

Media and Culture Condition for α -Galactosidase Production

Cultivation was performed in Erlenmeyer flask (250 ml) containing 100 ml of basal medium shaken. Basal medium consists of (g l^{-1}) peptone, 6; $(\text{NH}_4)_2\text{SO}_4$, 1.5; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.5; urea, 0.3; and KH_2PO_4 , 6. The above constituents were mixed in distilled water, 1% (w/v) of guar gum was added, and pH was adjusted to 6. After sterilization at 121 °C for 15 min, the medium was cooled down for further inoculation. Inoculation was performed by adding 1 ml spore suspension (2×10^5 spores/ml) of *A. terreus*_{GR} prepared by adding sterile distilled water containing 0.08% Tween-80 to the extensively sporulated organism on PDA slant (7-day old). After inoculation, the flasks were incubated at 37 °C for 6 days, on the seventh day, the mycelium was filtered to obtain a clear supernatant containing extracellular α -galactosidase.

Enzyme Activity Assay

α -Galactosidase activity was quantitatively assayed according to the method described by Dey and Pridham [1] using a final concentration of 1 mM pNP α Gal as substrate. Aliquots of 0.1 ml of pNP α Gal (10 mM) and 0.8 ml of acetate buffer (0.1 M and at pH 5) were preincubated at a 65 °C temperature for 2 min before adding 0.1 ml of suitably diluted enzyme to initiate the reaction. This was terminated after 15 min by adding 3 ml of 0.1 M Na_2CO_3 , and the released *p*-nitrophenol was determined spectrophotometrically at 405 nm (Elico Ltd., India). One unit of α -galactosidase activity was defined as the amount of enzyme liberating 1 μmol *p*-nitrophenol in 1 min under the assay conditions.

Determination of Protein Concentration

Protein concentration was estimated according to the method of Lowry et al. [20]. Fractions obtained during the purification procedure were screened for protein content by UV absorbance under spectrophotometer (Elico Ltd) at 280 nm.

Enzyme Purification

After 6 days of incubation period, mycelia were separated by filtration through Whatman filter paper no 1. Filtrate was subjected to precipitation by adding chilled acetone 1:1.5% v/v (culture broth to acetone) at 20 °C, stirred well and the mixture was kept at 4 °C for 12 h. The precipitate was recovered by centrifugation at $8,850 \times g$ for 30 min and dissolved in a minimal volume of 50 mM acetate buffer, pH 5.0. The centrifugate was dialyzed overnight against 5 mM acetate buffer of pH 5.0. This enzyme solution was loaded onto a pre-equilibrated Sephadex-G-75 column (2×100 cm) and was eluted with the same buffer at a flow rate of 0.5 ml/min. The fractions showing α -galactosidase activity were collected and pooled and loaded onto a DEAE-Sephacel column (1.5×10 cm). The column equilibrated with 50 mM Tris-HCl buffer, pH 7.4, was eluted at 0.25 ml/min with a salt gradient concentration (0.0 M to 0.5 M NaCl in 50 mM Tris-HCl buffer of pH 7.4). Five-milliliter fractions were collected, and those showing α -galactosidase activity were pooled and collected fractions were analyzed for protein content (A280) and α -galactosidase activity using pNP α Gal.

Electrophoretic Analysis

Protein homogeneity of purified α -galactosidase from *A. terreus*_{GR} strain was assessed by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) [21]. The proteins

were stained with 0.25% (*w/v*) Coomassie brilliant blue R-250, and protein molecular mass was determined by comparing the relative mobilities to appropriate proteins standards. Medium molecular weight (Sigma Chemicals Ltd, USA) protein markers were used.

Zymogram Analysis

Protein activity was assessed by zymogram analysis of non-denaturing or native PAGE proteins. After electrophoresis, half of the gel was treated with 4-methyl-umbelliferyl- α -D-galactoside (MU- α -gal, 2.0 mM), and activity bands were visualized as fluorescent band under UV transilluminator. Photograph of fluorescing gel was obtained by gel-doc and corresponding band in other half of the gel was stained with Coomassie brilliant blue R-250 in the same way as described above.

pH and Temperature Optima

The effect of pH on enzyme activity was investigated at 65 °C. The pH optimum was determined at pH values ranging from 3.6 to 8. Buffers were either 50 mM acetate buffer (pH 3.6–5.6), 50 mM sodium citrate buffer (pH 5.8–6.5), or 50 mM Tris–HCl buffer (pH 7.2–8.0). α -Galactosidase activity was measured using pNP α Gal as described above. The temperature optimum was determined by measuring the enzyme activity in the 30–75 °C range using 50 mM acetate buffers (pH 5.0).

Stability of the Purified Enzyme

The effect of pH and temperature stability of purified α -galactosidase was determined. For pH stability, the enzyme was incubated in buffers of various pH values for 2 h at 37 °C, and the residual activity was assayed by the standard method as described above. To measure the enzyme temperature stability, preparations were preincubated in the acetate buffer (pH 5.0) in the absence of the substrate and at various temperatures ranging from 65 to 75°C. Samples were taken at different time intervals, and residual activity was measured under standard conditions.

Determination of Kinetic Parameters

Kinetic parameters were determined at 65 °C using various concentrations of pNP α Gal, oNP α Gal, raffinose, and stachyose in 50 mM acetate buffer (pH 5). Hydrolysis of pNP α Gal or oNP α Gal was quantitatively determined by the amount of released *p*-nitrophenol, similarly to the standard activity assay. Hydrolysis of raffinose and stachyose was quantified by determination of released reducing sugars (galactose) by Somogyi-Nelson method [22]. The kinetic parameters were calculated from Linweaver–Burk plot.

Effect of Inhibitors and Metal Ions on α -Galactosidase Activity

The sensitivity of the enzyme to metals was examined by running the standard α -galactosidase assay at the optimum temperature and pH. Metal ions (AgNO₃, CaCl₂, CoCl₂·6H₂O, CuSO₄, HgCl₂, KCl, MgCl₂·6H₂O, MnSO₄, NiCl₂, and ZnSO₄) were dissolved to a final concentration of 10 mM in 50 mM acetate buffer of pH 5.0. To determine the effect of inhibitors on α -galactosidase, enzyme was preincubated with final

concentration of 10 mM 1,10-phenanthroline, *N*-bromosuccinamide, phenylmethylsulfonyl fluoride (PMSF), mercaptoethanol, ethylenediaminetetraacetic acid (EDTA), and urea. After 30 min of preincubation, the reaction was initiated by adding pNP α Gal, and assay was performed as described previously.

Results and Discussion

Screening of α -Galactosidase-Producing Microorganism

To screen active α -galactosidase-producing strains, those colonies that displayed deep blue color after hydrolysis of X- α -Gal on PDA plate were picked and selected for further study. This was further confirmed by growing the isolates in basal medium containing guar gum as the sole carbon source. Of 15 isolates, one showing extracellular α -galactosidase activity was selected for detailed taxonomical and optimization studies. The isolate was identified as *A. terreus*_{GR}.

Purification of α -Galactosidase from *Aspergillus terreus*_{GR}

The fungus cultivated in guar-gum-based medium for 6 days producing extracellular α -galactosidase activity was subjected to acetone precipitation at 1:1.5% v/v (culture filtrate/acetone) saturation. After solvent precipitation, approximately 70.66% yield was obtained. Sephadex-G 75 chromatography resulted to 54.54% yield with increase in 10-fold of purification. After the third step of purification (DEAE-Sephacel chromatography), 19-fold of purification and overall yield of approximately 38% were observed (Table 1). Zymogram analysis with MU-Gal after native PAGE indicated single activity band under transillumination (Fig. 1). Three simple chromatographical steps were required to purify α -galactosidase to apparent homogeneity.

Properties of α -Galactosidase

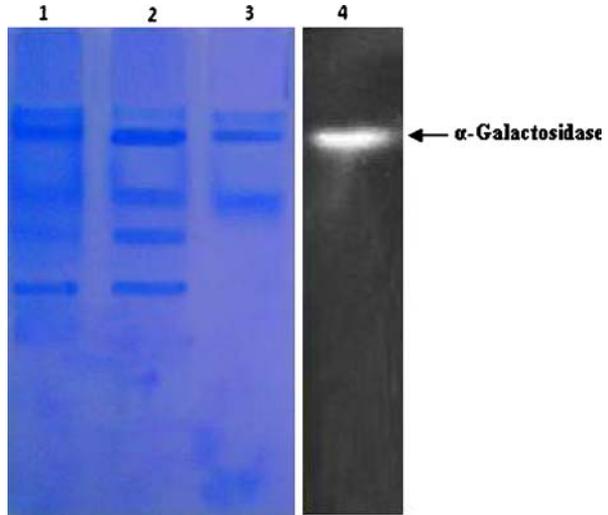
Determination of Molecular Weight by SDS-PAGE

After anion exchange chromatography (DEAE-Sephacel), a single band was observed in both native (data not shown) and SDS-PAGE (Fig. 2). After SDS-PAGE, molecular mass of purified enzyme is 108 kDa, which is comparable to α -galactosidase from white rot fungus *Pleurotus florida* having a monomeric protein of molecular mass approximately 99 kDa [23]. *Thermomyces lanuginosus*-BS39562/b α -galactosidase had molecular mass of 93 kDa [18], whereas some of the α -galactosidases reported from various fungi have comparatively low molecular mass (Table 2).

Table 1 Summary of purification of *Aspergillus terreus*_{GR} α -galactosidase.

Purification step	Total volume (ml)	Total enzyme (U)	Total protein (mg)	Specific activity (U/mg)	Yield (%)	Fold of purification
Crude extract	1,000	242	125	1.936	100	1
Acetone precipitation	30	171	28.5	6	70.66	3
Sephadex-G75	12	132	6.8	19.4	54.54	10
DEAE-Sephacel	5	92	2.5	36.8	38	19

Fig. 1 Native PAGE of the purified α -galactosidase from *Aspergillus terreus*_{GR}. Lane 1 Crude enzyme; lane 2 acetone precipitated enzyme; lane 3 fractions from Gel filtration (Sephadex G-75) chromatography; lane 4 activity staining of α -galactosidase using MU- α -gal



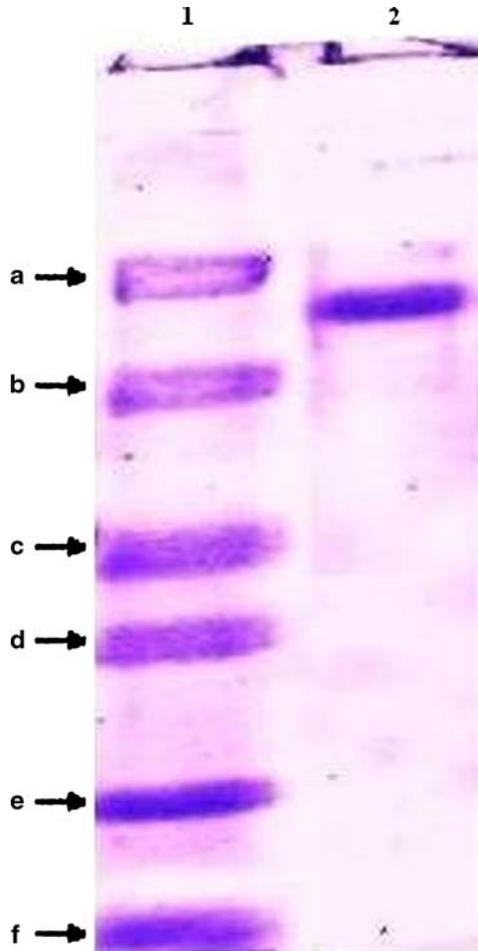
Effect of pH on α -Galactosidase Activity and Stability

From Fig. 3, it is clear that purified α -galactosidase from *A. terreus*_{GR} is optimally active at pH 5; thereafter, activity of the enzyme gradually decreased. There is almost 100% retention of its original activity after incubating the enzyme at various pH (pH range of 3.6–8) at 37 °C up to 2 h (Fig. 3). From the literature, it is evident that α -galactosidase from *Aspergillus* species have optimum activity at acidic pH range. α -Galactosidase from *Trichoderma cutaneus* JCM 2947 was optimally active between pH 5.0 and 6.0 [24]. Multiple α -galactosidase from *Aspergillus niger* had maximal catalytic activity at pH 4.5; α -galactosidase I was the most stable at pH 5–6 for 17 h at 50 °C [25]. Similarly, α -galactosidase from *Debaryomyces hansenii* UFV-1 had maximal substrate hydrolysis (pNP α Gal) at pH 5 [26]. Kotwal et al. [17] reported thermostable α -galactosidase from thermophilic fungus *Humicola* sp., which was optimally active at pH 5 and was 100% stable in the pH range 4–5. On the other hand, optimum pH for α -galactosidase activity from *Trichoderma reesii* was at pH 4, and also the enzyme demonstrated moderated pH stability in buffers having pH values ranging from 4.5 to 6.5 [27]. Li et al. [28] have reported that thermostable α -galactosidase from thermotolerant *Absidia* sp. WL 511 was active at pH 7.5, and the enzyme was stable at pH values ranging from 5.0 to 9.0.

Effect of Temperature on Activity and Stability of α -Galactosidase

Figure 4 depicts temperature optima for *A. terreus*_{GR} α -galactosidase. Enzyme maximally hydrolyzed pNP α Gal at 65 °C (pH 5). Figure 5 shows thermostability profile of α -galactosidase. It is clear from Fig. 5 that enzyme is 100% stable at 65 °C for 40 min. Approximately 55% of activity was retained at 70 °C for 20 min. Half-life of the enzyme was 25 min at 70 °C, but at 75 °C, only 22% of activity was retained after 10 min. Similar observation was made by Rezessy-Szebo et al. [18], who reported that the temperature optimum for *T. lanuginosus* α -galactosidase was 65 °C (at pH range 5 and 5.5). The enzyme was stable for 24 h at 55 °C, but a rapid decrease in enzyme activity occurred at

Fig. 2 SDS-PAGE of *Aspergillus terreus*_{GR} α -galactosidase after DEAE-Sephacel chromatography. *Lane 1* Molecular weight marks **A** β -galactosidase (116); **B** bovine serum albumin (66); **C** ov-albumin (45); **D** lactate dehydrogenase (35); **E** REase Bsp 981 (25); **F** 4- β -lactoglobulin (18.4). *Lane 2* Purified α -galactosidase



temperatures higher than 65 °C having less than 10% of residual activity after 24 h incubation. The α -galactosidase from *Pycnoporus cinnabrium* was thermostable, and no loss in activity was found below 75 °C, but it was inactivated at 80 °C or more and completely lost its activity at 90 °C [29]. On the other hand, α -galactosidase from *Aspergillus fumigatus* had optimum activity at 55 °C and retained about 80% of the original activity after incubation for 90 min at 50 °C [30]. Similarly, Falkoski et al. [31] reported that α -galactosidase from *A. terreus* was optimally active at 55 °C and found to be more thermostable than the α -galactosidase from *Penicillium griseoroseum* and soybean. Table 2 summarizes some of the thermostable α -galactosidase from different fungi. Thermostable α -galactosidase from *Ganoderma lucidum* was maximally active at 70 °C, and the enzyme was fully stable to heating at 70 °C for 30 min [16]. The optimum temperature on activity of thermostable α -galactosidase from thermophilic fungus *Humicola* sp. was at 60 °C and was stable at 60 °C for 1 h, but this enzyme also rapidly inactivated above 60 °C [17]. The α -galactosidase from another thermophilic fungus *T. lanuginosus* was stable for 6 h at temperature up to 60 °C, but at temperature above 70 °C, it was rapidly inactivated [19]. α -

Table 2 Thermostable α -galactosidases purified from different fungi.

Organism	Optimum Temperature and pH on activity		Molecular Weight (kDa)	References
	Temperature (°C)	pH		
<i>Pycnoporus cinnabarinus</i>	75	5–5.5	52	Ohtakara et al. [29]
<i>Daberyomyces hansenii UFV-1</i>	60	5	60	Viana et al. [26]
<i>Gonoderma lucidum</i>	70	6	56	Sripuan et al. [16]
<i>Humicola</i> sp	65	5	87.1	Kotwal et al. [17]
<i>Thermomyces lanuginosus</i> -BS39562/b	65	5–5.5	93	Rezessy-Szabo et al. [18]
<i>Thermomyces lanuginosus</i>	65–70	4.5–5	57	Puchart et al. [19]
<i>Absidia</i> sp WL511	73	7.5	–	Li et al. [28]
<i>Aspergillus terreus</i> _{GR}	65	5	108	This study

Galactosidase from thermotolerant *Absidia* sp. WL511 had optimum activity at temperature of 73 °C. The enzyme was stable at temperature up to 60 °C and had 87% of its full activity at 65 °C after 2 h of incubation [28].

Substrate Specificity and Determination of Kinetic Parameters

Kinetic parameters like K_m and V_{max} of *A. terreus*_{GR} α -galactosidase is summarized in Table 3; the results revealed that α -galactosidase had higher affinity toward pNP α Gal (K_m , 0.11 mM) than that of oNP α Gal. Other natural substrates also had higher K_m value. The relative substrate specificity of α -galactosidase toward the various synthetic and natural galactosides is in the order of pNP α Gal>oNP α Gal>stachyose>raffinose>locust bean gum>guar gum (Table 3). Kinetic study revealed that the enzyme prefers stachyose rather than raffinose. Stachyose in legume-based foods is known to cause more flatulence than raffinose. Therefore, the enzyme is applicable in food-processing industry for the elimination of flatulence-inducing factors present in legume-based foods. In literature α -galactosidases from different organisms have different K_m and V_{max} values. According to Sripuan et al. [16], thermostable α -galactosidase from *G. lucidum* showed higher hydrolytic

Fig. 3 Effect of pH on activity (diamond) and stability (square) of *Aspergillus terreus*_{GR} α -galactosidase

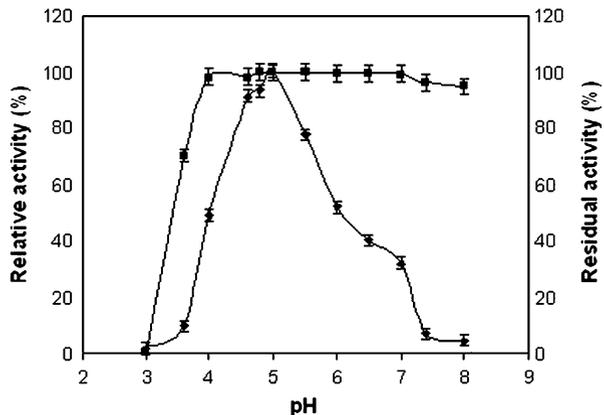
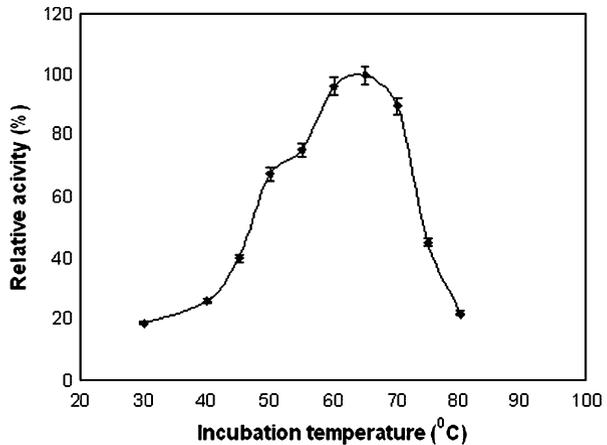


Fig. 4 Effect of temperature on activity of *Aspergillus terreus*_{GR} α -galactosidase



activity toward pNP α Gal compared to oNP α Gal. The K_m for pNP α Gal was 0.3 mM, whereas 16.0 and 9.66 K_m , respectively, was observed for raffinose and stachyose in *D. hansenii* α -galactosidase [26]. Based on the above results as the enzyme hydrolyses, both synthetic and natural substrates, such as pNP α Gal, oNP α Gal, oligosaccharides of raffinose family, and polysaccharides of galactomannans were the keen interest of the present investigation.

Effect of Inhibitors and Metal Ions on α -Galactosidase Activity

The effect of different inhibitors and metal ions on α -galactosidase activity is depicted in the Table 4. Among the inhibitors tested, EDTA and 1,10-phenanthroline did not inhibit α -galactosidase activity, and this indicates that α -galactosidase is not a metalloenzyme. Inhibition of α -galactosidase from *A. terreus*_{GR} by *N*-bromosuccinamide indicates the role of tryptophan at or near the active site. Inhibition of α -galactosidase in *Humicola* sp. by *N*-bromosuccinamide was also noticed [32]. From Table 4, it is also evident that PMSF did not inhibit α -galactosidase, and this indicates the absence of serine group at or near active site of the enzyme. Among the metal ions tested on α -galactosidase activity, mercuric (Hg^{2+}) ions

Fig. 5 Effect of temperature on stability of *Aspergillus terreus*_{GR} α -galactosidase at 65 °C (diamond), 70 °C (square), and 75 °C (triangle)

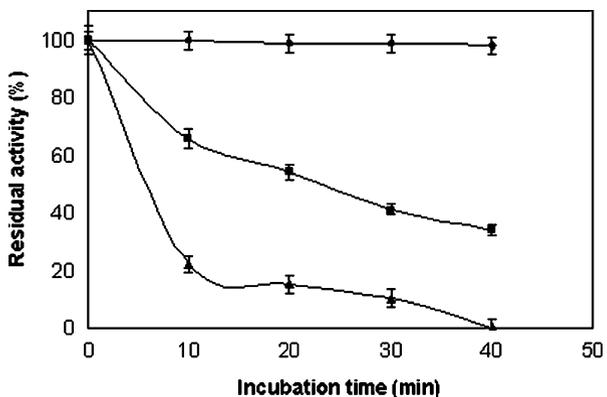


Table 3 K_m and V_{max} values of *Aspergillus terreus*_{GR} α -galactosidase determined by Michael–Menten plot.

Substrates	Relative activity (%)	K_m (mM)	V_{max} ($\mu\text{mol ml}^{-1} \text{min}^{-1}$)
pNP α Gal	100	0.11	7.2
oNP α Gal	93	0.28	11.23
Raffinose	39	0.42	16.33
Stachyose	65	0.33	14.13
Guar gum	18	–	–
Locust bean gum	29	–	–

inhibited complete enzyme activity. Inhibition by Hg^{2+} suggests that the enzyme contains an essential sulphhydryl group. Significant inhibitory effect was also observed in the presence of Zn^{2+} and Ag^+ . Similar with our results, α -galactosidase from *P. cinnabarium* was strongly inhibited by Ag^+ and Hg^{2+} [29]. α -Galactosidase from *Monascus pilous* was strongly inhibited by Hg^{2+} and Cu^{2+} [5]. Metals such as Mg^{2+} , Ni^{2+} , Co^{2+} , Mn^{2+} , and Ca^{2+} had no effect on enzyme action. Kotwal et al. [17] reported that Mg^{2+} had no effect, while Hg^{2+} completely inhibited α -galactosidase from thermophilic fungus *Humicola* sp.

Conclusion

Extracellular α -galactosidase purified from *A. terreus*_{GR} to apparent homogeneity and purified enzyme showed molecular mass weight of 108 kDa as revealed by SDS-PAGE analysis. α -Galactosidase from *A. terreus*_{GR} is thermostable, as it was not inactivated by heating at 65 °C for 40 min and also it has higher temperature optima and pH stabilities. The enzyme shows low K_m for synthetic substrates, and affinity toward stachyose is high compared to raffinose. The enzyme is also active on large molecular weight substrates like guar gum and locust bean gum. α -Galactosidase from *A. terreus*_{GR}, having acidic pH optima and heat stability, may prove useful in industrial application for degradation of

Table 4 Effect of inhibitors and metal ions on *Aspergillus terreus*_{GR} α -galactosidase activity.

Inhibitors/metal ions	Residual activity (%)
None	100
1,10-Phenanthroline	100
<i>N</i> -bromosuccinamide	0
PMSF	100
EDTA	100
Mercaptoethanol	100
Urea	100
KCl	100
Mg^{2+}	100
Hg^{2+}	0
Zn^{2+}	68
Cu^{2+}	0
Ag^+	0
Ni^{2+}	100
Co^{2+}	100
Mn^{2+}	100
Ca^{2+}	100

raffinose oligosaccharide in legumes and conversion of guar gum to a more attractive polysaccharide like locust bean gum by selectively cleaving α -galactoside linkages.

Acknowledgment One of the authors, Shankar S. K., thanks Council of Scientific and Industrial Research (CSIR) New Delhi, India for providing financial assistance in the form of Senior Research Fellowship (SRF) during this work.

References

- Dey, P. M., & Pridham, J. B. (1972). *Advances in Enzymology*, 36, 911–930.
- Ulezlo, V., & Zaprometova, O. M. (1982). *Applied Biochemistry and Microbiology*, 18, 3–15.
- Soh, C. P., Ali, M. Z., & Lazan, H. (2006). *Phytochemistry*, 67, 242–254.
- Sismerska, P., Monti, D., Cechova, I., Pelantova, H., Mackova, M., Bezouska, K., et al. (2007). *Journal of Biotechnology*, 128, 61–71.
- Shibuya, H., Kabayashi, H., Sato, T. W. S., Kims Yoshida, S., Kaneko, S., & Kusukabe, I. (1997). *Bioscience, Biotechnology, & Biochemistry*, 61, 592–598.
- Cruz, R., & Park, Y. K. (1982). *Journal of Food Science*, 47, 1973–1975.
- Clarke, J. H., Davidson, K., Rixon, J. E., Halstead, J. R., Franssen, M. P., Gilbert, H. J., & Harzlewood, G. P. (2000). *Applied Microbiology and Biotechnology*, 53, 661–667.
- Bulpin, P. V., Gildley, M. J., Jeffcoat, R., & Underwood, D. R. (1990). *Carbohydrate Polymers*, 12, 155–168.
- Harpaz, N., Flowers, H. M., & Sharon, N. (1975). *Archives of Biochemistry and Biophysics*, 1720, 676–683.
- Goldstein, J., Siviglia, G., & Hurst, R. (1982). *Science*, 215, 168–170.
- Brouns, S. J. J., Smits, N., Wu, H., Sniijders, A. P. L., Wright, P. C., de Vos, W. M., & van der Oost, J. (2006). *Journal of Bacteriology*, 188, 2392–2399.
- Vielle, C., Burdette, D., & Zeikus, J. (1996). Thermozyms. In M. El-Gewely (Ed.), *Biotechnology Annual Review*, vol. 2 (pp. 1–83). New York: Elsevier Science.
- Bruce, L. Z., Henrik, K. N., & Robert, L. S. (1999). *Journal of Industrial Microbiology and Biotechnology*, 8, 71–81.
- Gote, M., Umalkar, H., Khan, I., & Khire, J. (2004). *Process Biochemistry*, 39, 1723–1729.
- King, M. R., Yernool, D. A., Eveleigh, D. E., & Chassy, B. M. (1998). *FEMS Microbiology Letters*, 163, 37–42.
- Sripuan, T., Aoki, K., Yamamoto, K., Tongkao, D., & Kumagai, H. (2003). *Bioscience, Biotechnology, and Biochemistry*, 67, 1485–1491.
- Kotwal, S. M., Khan, M. I., & Khire, J. M. (1995). *Journal of Industrial Microbiology*, 15, 116–120.
- Rezessy-Szabo, J. M., Ngyue, Q. D., Christophe, A. H., Gyongyi, B., & Marc, H. C. (2007). *Biochimica et Biophysica Acta*, 1777, 55–62.
- Puchart, V., Vranska, M., Bhat, M. K., & Diely, P. (2000). *Biochimica et Biophysica Acta*, 1524, 27–37.
- Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). *Journal of Biological Chemistry*, 193, 265–275.
- Laemmli, U. K. (1970). *Nature*, 227, 680–685.
- Somogyi, M. (1952). *Journal of Biological Chemistry*, 195, 19–25.
- Ramalingam, , Saraswathy, N., Sadasivam, S., Subha, K., & Poorani, N. (2007). *Indian Journal of Biochemistry and Biophysics*, 2, 76–81.
- Yuji, O. D. A., & Kenzo, T. (1997). *Memor Facu Eng Fukuyama University*, 21, 67–71.
- Adamark, P., Larsson, M., Tjerneld, E., & Stalbrand, H. (2001). *Enzyme and Microbial Technology*, 29, 441–448.
- Viana, P. A., De Rezende, S. T., Marques, V. M., Trevizano, L. M., Passos, F. M. L., Oliveira, M. G. A., et al. (2006). *Journal of Agricultural and Food Chemistry*, 54, 2385–2391.
- Zeilinger, S., Kristufek, D., Arisan-Atacinci, H. R., & Kubecek, C. (1993). *Applied and Environmental Microbiology*, 59, 1347–1353.
- Li, H., Liang, W. Q., Wang, N., Luao, X. Y., Wu, J. M., Hu, J. Q., et al. (2006). *World Journal of Microbiology and Biotechnology*, 22, 1–7.
- Ohtakara, A., Mitsutomi, M., & Uchida, Y. (1984). *Journal of Biological Chemistry*, 48, 1319–1327.
- De Rezende, S. T., & Felix, C. R. (1999). *Folia Microbiology*, 44, 191–195.
- Falkoski, D. L., Guimaraes, V. M., Callegari, R. A. P., De Barros, E. G., & De Rezende, S. T. (2006). *Journal of Agricultural and Food Chemistry*, 54, 10184–10190.
- Sabir, M. K., Jayanth, M. K., & Islam, M. K. (2000). *Journal of Biochemistry and Molecular Biology*, 4, 65–72.