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Biochemical and *In Silico* Characterization of a Recombinant, Highly Thermostable α-Glucosidase from *Thermococcus radiotolerans* DSM-15228

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HA and MSN conceived the research idea and prepared the study plan. JAK and MAAG supervised the studies. HA, SI and MSN carried out experimental work.

Key words
Thermococcus radiotolerans*, Recombinant, α-glucosidase, Kinetics, *In silico

ABSTRACT

α-glucosidase (EC 3.2.1.20) is the enzyme catalyzing α-1,4-glycosidic linkage between the glucose moleculecules in disaccharides like maltose. The enzyme is ubiquitous in nature and has applications in the glucose and ethanol production processes on industrial scale. α-glucosidase found in the human intestine is considered as am important target in the management of diabetes type II. We have produced a recombinant of α-glucosidase from *Thermococcus radiotolerans* in *E. coli* strain BL21 (DE3) codon plus RIPL in the presence of 1 mM IPTG. The enzyme was purified by selective precipitation and cation exchange chromatography, it displayed a 30 kDa band on SDS-PAGE. The purified recombinant of enzyme exhibited an optimal activity at pH 6, and 85°C, it retained more than 50% activity after incubation at 100°C for 5 min. A Km value of 28.1 mM of maltose and 26.3 µmol per min Vmax was determined by Lineweaver-burk plot. In silico studies have suggested Glu^{109}, Ala^{107}, Tyr^{104}, Glu^{41}, Arg^{44}, Asn^{45}, Glu^{48} as the main active site residues of the enzyme. Molecular docking studies have shown that the enzyme investigated in the present study and human α-glucosidase have similar active site composition. Affinity studies with ten well established inhibitors have shown similar free energy change (ΔG) values for human and microbial enzymes indicating the evolutionary conserved structural and functional domains in the enzyme from phylogenetically different species.

INTRODUCTION

Alpha-Glucosidase is an exoenzyme catalyzing the hydrolysis of di and oligo-saccharides and aryl glucosides in a manner analogous to that of glucoamylase (Ramchandran and Shah, 2008). The enzyme can hydrolyze the non-reducing terminal of (α1→4) linked residues of glucose to release a lone molecule of α-glucose. α - Glucosidase is considered as carbohydrate-hydrolase which releases single molecule of α-glucose. The enzyme is found in seeds of plants (Mohiuddin *et al*., 2016; Tan *et al*., 2018), fungi (Kaur *et al*., 2019), bacteria (Mawlankar *et al*., 2020), and animals (Kotońska-Feiga *et al*., 2015). The enzyme has several important applications, many medications targeting type II diabetes mellitus comprise of α-glucosidase inhibitors (Akmal and Wadhwa, 2022). Deficiency of lysosomal α-glucosidase deficiency is associated with a rare multisystem genetic

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condition known as Pompe disease. α-glucosidase from plant sources has applications in the food, pharmaceutical, and biotechnology industries (Kashtoh *et al*., 2022). α-glucosidases employed in the preparation of isomalto and maltooligosaccharides (IMOs) such isomaltose, maltotriose which are used in brewing, syrup and biofuel production on industrial scales (Soumya *et al*., 2015). The enzyme also plays a critical role in the human digestive process. Starch digested primarily by amylases into simpler sugars (Kim *et al*., 2011), the last step in glucose utilization is governed by α-glucosidases located at the enterocyte brush boundary (Sukumaran *et al*., 2016). All of the above applications of α-glucosidase advocate for the bulk requirements of enzyme to fulfil its ever-increasing demands worldwide. The native sources produce limited amounts of such enzymes that is sufficient for their physiological requirements. Genetic engineering provides useful tools and procedures for the large-scale production of recombinants of useful enzymes in the selected host systems (Demain and Vaishnav, 2009; Proença *et al*., 2016; Druzhinina and Kubicek, 2017). *E. coli* cells are considered as the best host cells for the production of heterologous proteins using different plasmids under the control of specific operon systems (Pontrelli *et al*., 2018; Kaur *et al*., 2018). For practical applications in the relevant industries, the enzymes capable to operate under stringent conditions like high temperature, high salt or sugar content, and unusual pH. Such novel enzymes are often produced by the bacterial of archaeal species isolated from rigorous conditions (Liu and Kokare, 2023). It leads to a continuous investigation of enzymes from microbial species reported from the harsh environmental conditions. *Thermococcus radiotoerans* is a hyperthermophilic archaeon that has been isolated Mid-Atlantic Ridge (23°22′N, 44°57′W) at a depth of 3.5 km. The species grows at high salt concentration with an optimum growth as pH 6 and 88°C (Jolivet *et al*., 2004; Polymenakou *et al*., 2021). Exploring microbial genomes at NCBI revealed that the *Thermococcus radiotolerans* genome sequence contains genes for α-glucosidase. It was anticipated that the enzyme would have certain unique features that would be easily exploited in tough industrial conditions. For the first time, the current study aimed to clone, heterologously produce, biochemically describe, and *in silico* analyse α-glucosidase from *Thermococcus radiotolerans*.

MATERIALS AND METHODS

Materials

DSMZ, Germany, provided the genomic DNA of *Thermococcus radiotolerans* DSM-15228, which was employed as a template for amplification of α-glucosidase gene. TransformAid bacterial transformation kit (catalog no. k2710), 1 kb DNA marker (catalog no. SM0311), prestained protein marker PAGERulerTM- 10-180 kDa (26616). quick digest DNA restriction enzymes (NdeI and BamHI), CloneJET PCR cloning kit (K1231), and 2x PCR master mix (catalogue no. K0171) were provided by thermofisher scientific. GeneEluteTM PCR clean-up kit (Merck catalog number NA1020), GenEluteTM gel extraction kit (Merck catalogue No. NA1111), T7 promoter based expression vector pET21a (+) was obtained from Novagen, substrate maltose was obtained from local suppliers at KSA. Genetically modified *E. coli* strain BL21 (DE3) codon plus RIPL (catalogue no. 230280) were obtained from Agilent Technologies.

PCR amplification β-glucosidase and gene cloning

Thermococcus radiotolerans DSM-15228 possesses a 729 bp gene that codes for -glucosidase (accession no: NZ CP015106). The target gene was amplified by PCR using the forward and reverse primer sequences 5'-CATATGAGGAGGTTCAGAGATTCTGCGAGAT-3′ and 5'- GGATCCTAGAGGAAAGCGTGCCTG-3′, respectively. 2x master mix (Thermofisher catalogue no. K0171), 40 picomole primers, and 15-20 ng template DNA were employed in the reaction mixture. The PCR thermocycler was adjusted at 94°C for 40 sec of denaturation, 58°C for 30 sec of annealing, and 72°C for 30 sec of extension; these conditions were repeated for 35 cycles, followed by an additional 10 min of extension time. Agarose gel electrophoresis was used to examine the PCR amplified α-glucosidase gene. To purify the PCR product that was not loaded onto the agarose gel, the Gene EluteTM PCR Clean-Up Kit (Merck catalog No. NA1020) was used. The pJET1.2 cloning vector was available with the CloneJET PCR Cloning Kit (Thermo Fisher Scientific Catalogue No. K1231) for the ligation of purified PCR product (Thermofisher catalogue No. K1231). Recombinant plasmids were produced according to manufacturer's instructions and utilized to transform competent *E. coli* (DH5). Transform Aid bacterial transformation kit (Thermofisher catalogue no. k 2710) was used in the transformation procedure. In LB-agar plates with 100 mg of ampicillin per ml of medium, transformed *E. coli* (DH5) cells emerged as independent colonies. The presence of target gene in the recombinant plasmid was confirmed by restriction analysis of the isolated plasmid using NdeI and BamHI enzymes. The restriction reaction contained approximately 100 ng of isolated plasmid DNA, 5 U of each enzyme, 1x green buffer, and nuclease free water in a 50 ul final reaction volume. Recombinant plasmids were created per the manufacturer's instructions and used to transform

competent *E. coli* (DH5). In the transformation method, a TransformAid bacterial transformation kit (Thermofisher catalogue no. k2710) was employed. Transformed *E. coli* (DH5) cells developed as independent colonies in LB-agar plates with 100 mg of ampicillin per ml of medium. The presence of the target gene in the recombinant plasmid was verified by restriction analysis with NdeI and BamHI on the isolated plasmid. In a 50ul reaction mixture, the restriction reaction comprised roughly 100ng of isolated plasmid DNA, 4 U of each enzyme, 1x green buffer, and nuclease free water.

Production of enzyme as a recombinant

A 250 ml conical glass flask containing 30 ml LB broth supplemented with 100 mg/ml ampicillin per ml media was inoculated with a single colony of recombinant BL21 (DE3) RIPL codon plus cells and incubated overnight in an orbital shaker set to 37°C and 200 rpm. Using the same circumstances, 1% of the overnight culture was grown in a new flask to achieve an optical density of 0.5 at 600 nm. To monitor cell development, the OD at 600 nm was measured on a regular basis. Protein synthesis was stimulated by incubating cells for 3 hours at 37°C and 200 rpm with 1 mM IPTG. In a separate experiment, cells transformed with the pET21a (+) minus α-glucosidase gene were treated in parallel. The cells were harvested by centrifugation at 10,000 g for 5 min, and the pellet was dissolved in distilled water and separated on SDS-PAGE (Laemmli, 1970).

Assay for α-glucosidase activity

A modified version of procedure described by (Dietvorst *et al*., 2007) was used for the measurement of enzyme activity. The experimental and control tubes were prepared in parallel. In the experimental tube, 1500 µl acetate buffer (100mM), pH 6 containing 2 mM EDTA (buffer A), and 500 µl of 500 mM maltose prepared in the buffer A were carefully added. Both tubes were incubated at 40°C for 5 min and reaction was stopped by boiling the reaction mixtures in the water bath for 5 min. In the next step, 100 µl pre-treated sample was transferred to fresh cuvettes of 3mL capacity. To each cuvette following reagents were added: 2.5 ml of 0.5M triethanolamine buffer (TEA), pH 7.6, 100 µl of 0.1M $MgCl_2$, 100 µl of 20mM ATP, 100 µl of 10mM NADP, 20 µl of 1mg/ml solution of glucose-6-phosphate dehydrogenase (G6PDH). To the experimental tube 10 µl diluted enzyme sample and to the negative control same volume of distilled water were added. The reaction was started by the addition of 20 µl hexokinase. Change in absorbance per min (ΔdO.D) at 340nm was determined by the following equation:

$$\text{Enzyme activity}\left(\frac{\text{U}}{\text{mL}}\right) = \frac{\frac{\Delta \text{OD340nm}}{\text{min}} \times \text{Total reaction vol. (mL)} \times \text{Enzymedilution factor}}{(6.22) \times \text{Enz. Vol. (mL)}}$$

Total soluble protein content of enzyme sample was determined by Bradford method (Bradford, 1976), and specific activity of enzyme was calculated as units of enzyme per mg of solution.

Purification of recombinant α-glucosidase

Bacterial cells were precipitated by centrifugation at 7000 x g for 10 min after gene induction and suspended in buffer B. (ice-chilled 20 mM phosphate buffer pH 7). After sonicating the suspended cells under optimal conditions (Ferdous *et al*., 2021; Dopp *et al*., 2018), a clear supernatant was produced by centrifugation at 12,000 g. The soluble fraction was purified by selective heat denaturation at 70°C for 10 min to denature the majority of proteins from E. coli origin, followed by centrifugation as described above. The enzyme from the supernatant was then precipitated by ammonium sulphate (0 to 55% ammonium sulphate in the final sample solution) and dialyzed in buffer B. After clarifying the dialysate by centrifugation at 12000 x g for 15 min at 4°C, the clear supernatant was kept in an ice box. Buffer B was used to equilibrate a 2.5 x 36 cm chromatographic column with 30 mL of CM-Sephadex column. The sample solution was loaded onto the column at a rate of 2 ml per min. The unbound proteins were kept in an ice box. To elute the bound proteins, a linear gradient of sodium chloride (0 to 500mM) was utilized. Higher protein concentration fractions are maintained in an ice box and tested for activity and purity. Protein fractions containing 0.5 to 2 mg/ml were pooled and kept at -20°C.

Effect of temperature, pH, and substrate concentration on enzyme activity

The influence of temperature on enzyme activity was studied by running the process at various temperatures ranging from 40°C to 95°C while keeping all other parameters constant. To test the influence of pH on enzyme activity, buffer solutions with pH values ranging from 4 to 7.5 were produced and served as the assay medium. For pH 4, 4.5, 5, and 5.5, sodium acetate buffer was created, whereas sodium phosphate buffer was made for pH 6, 6.5, 7 and 7.5. The pKa values and effective buffering range of these buffer solutions were used to select them. The temperature stability of the enzyme was tested by incubating the enzyme sample for 5 min before the reaction at temperatures ranging from 40°C to 100°C. The effect of increasing the substrate content in the reaction mixture on enzyme activity was studied. Using Microsoft Excel, the values of 1/V and 1/[S] were subjected to a Lineweaver-Burk plot, and Km and Vmax values were calculated.

In silico *studies*

The protein sequences of α-glucosidase from *Thermococcus radiotolerans* and human lysosomal α-glucosidase were submitted to the AlphaFold server (Jumper *et al*., 2021) for the generation of 3D structure. The structures of the compounds (Table I) was obtained from the PubChem database (Kim *et al*., 2016), the co-crystalized ligand with human protein was as a control and for comparison. The protein and ligand preparation, active site prediction, and extra precision docking were performed using the Maestro interface from Schrödinger Suite, LLC, NY (Release, 2020), as described in the work reported (Zamzami, 2021). Due to the high similarity of AlphaFold generated human lysosomal α-glucosidase structure with human lysosomal α-glucosidase, GAA, in complex with cyclosulfamidate 4 (PDB ID:7P2Z), we used the similar active site for docking. The bacterial active site was predicted using the sitemap interface of Maestro. Protein residues interaction was studied using the online server Protein-Ligand Interaction Profiler (PLIP).

RESULTS

Characteristics and kinetics of recombinant α-glucosidase

A 729 bp open reading frame encoding the α-glucosidase gene was PCR amplified, cloned into pJET1.2, then subcloned onto pET21a (+) plasmids. The cells were exposed to gene expression after being transformed with the recombinant pET-AG plasmid, which resulted in the emergence of a significant protein band of around 30 kDa on SDS-PAGE (Fig. 1). All of the recombinant enzyme was determined to be soluble and active after purification, and fractions with protein contents ranging from 0.5 to 2mg/ml were pooled together and tested on SDS-PAGE (Fig. 1).

Recombinant enzyme was purified using CM-sephadex based cation-exchange chromatography. An ice chilled buffer B (phosphate buffer 20 mM, pH 7) was used as the mobile phase, and a linear NaCl gradient from 0 to 0.5 M was used to elute the protein. SDS-PAGE analysis was performed on fractions consisting of purified enzyme (Fig. 1). Purified enzyme had a specific activity of 98.6 U/mg, and a total of 740 units were obtained after 46.2% recovery and a 24.65 fold purification (Table I).

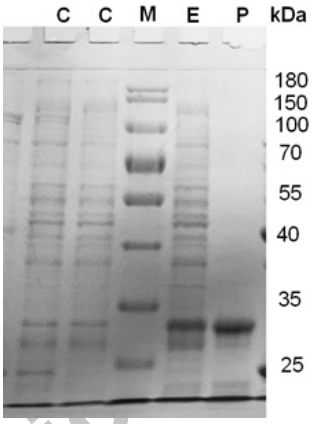


Fig. 1. SDS-PAGE showing the expression and purification of α-glucosidase. Lane C, a control experiment (without the gene), Lane-M, Protein marker (Thermofisher - unstained protein ruler). Lane E, experimental with the expression of the gene. P, purified enzyme, the molecular weight of the purified enzyme was found as 30 kDa.

An optimal enzyme activity was measured at 85°C and pH 6, the enzyme retained up to 50% activity when incubated at 100 °C for 5 min, Km values for maltose was 28.1 mM of maltose and Vmax value for enzyme was 26.3 µmol per min (Fig. 2).

3D structure and molecular dicking of α-glucosidase

Among the screened compounds the molecular docking study revealed a high binding affinity of acarbose (PubChem ID: 41774) to both bacterial and human alpha-glucosidase, both showed similar docking affinity (-9.6 and -9.5 kcal/mol for bacterial and human protein respectively). Four compounds (glyset, miglitol, Precose, and voglibose) showed better binding affinity (more than -6.5) with human alpha-glucosidase than the control compounds (Table II).

Table I. Activity of the enzyme, total protein content, specific activity, percentage yield, and fold purification of recombinant α-glucosidase. Crude extract (50 ml) was processed in three steps. One unit is the amount of enzyme that can liberate one micromole of glucose from maltose per minute under our assay conditions.

Purification step	Activity (U/ml)	Proteins (mg/ml)	Activity (U/mg)	Total units	Percentage yield	Fold purification
Crude extract	32	8	4.0	1,600	100	1
Dialysis of ammonium sulphate precipitate	110	15.5	7.09	1210	75.6	1.8
CM- Sephadex Column	148	1.5	98.6	740	46.2	24.65

Table II. Molecular docking of α-glucosidase with its potential inhibitors a comparison of human and Thermococcus enzyme.

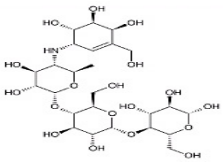
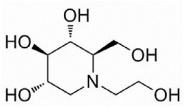
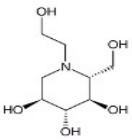
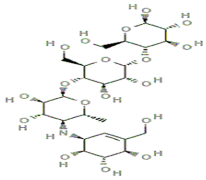
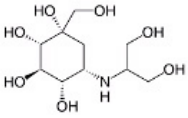
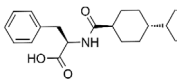
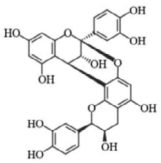
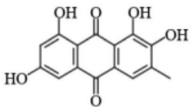
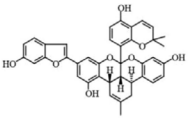
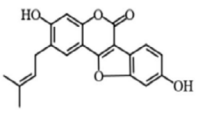
S. No.	Name of alpha glucosidase inhibitor	Pub Chem ID	Chemical structure	Binding energy by molecular docking (Thermococcus) ΔG =	Binding energy by molecular docking (Human) ΔG =
1	Acarbose	41774		-9.6	-9.5
2	Glyset	441314		-6	-8
3	Miglitol	441314		-6	-8
4	Precose	444254		-9.2	-5.8
5	Voglibose	444020		-6.9	-10.7
6	Nateglinide	5311309		-5.6	-4.9
7	Procyanidin A2	124025		-5.7	-7.25
8	Alaternin	5748627		-7.1	-5
9	Mulberrofuran K	495296		-3.2	-3.3
10	Psoralidin	5281806		-3.6	-4.7

Table III. Interacted residues of bacterial alpha-glucosidase and acarbose.

Index	Residue	AA	Distance H-A	Distance D-A	Donor angle	Donor atom	Acceptor atom
1	41A	GLU	2.01	2.96	165.41	3939 [O3]	681 [O.co2]
2	45A	ASN	2.19	3.08	147.43	751 [Nam]	3939 [O3]
3	48A	GLU	1.87	2.82	164.12	3931 [O3]	806 [O-]
4	48A	GLU	1.66	2.62	163.17	3929 [O3]	806 [O-]
5	48A	GLU	2.22	3.13	156.31	3928 [O3]	801 [O2]
6	52A	ASN	3.32	4.01	126.88	860 [Nam]	3928 [O3]
7	104A	TYR	3.26	3.87	122.98	3937 [O3]	1722 [O3]
8	107A	ALA	2.96	3.69	133.39	3932 [O3]	1768 [O2]
9	109A	GLU	1.96	2.92	171.73	3936 [O3]	1790 [O.co2]
10	109A	GLU	2.07	3.02	154.26	3941 [N3]	1789 [O.co2]

Table IV. Interacted residues of human alpha-glucosidase and acarbose.

Index	Residue	AA	Distance H-A	Distance D-A	Donor angle	Donor atom	Acceptor atom
1	404A	ASP	2.25	3.18	159.46	13288 [O3]	4914 [O.co2]
2	404A	ASP	1.87	2.82	165.14	13289 [O3]	4915 [O.co2]
3	411A	ARG	2.02	3.03	172.45	5021 [Ng+]	13291 [O3]
4	411A	ARG	3.13	3.82	127.06	5022 [Ng+]	13291 [O3]
5	518A	ASP	2.93	3.66	134.24	6691 [O3]	13290 [O3]
6	600A	ARG	3.28	4.05	134.28	7919 [Ng+]	13282 [O3]
7	616A	ASP	1.58	2.52	160.94	13290 [O3]	8148 [O3]
8	616A	ASP	1.54	2.5	162.98	13282 [O3]	8149 [O-]

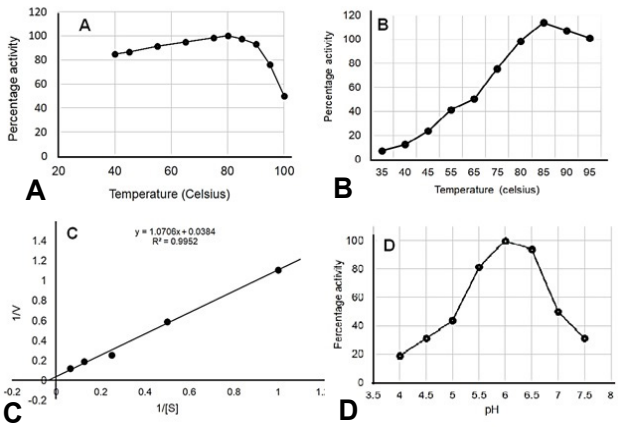


Fig. 2. A study of the kinetics of recombinant α-glucosidase. A, Enzyme stability against temperature. B, Effects of temperature on enzyme activity. C, Lineweaver-Burk plot for Km and Vmax. D, Effects of pH on enzyme activity.

Ten H-bonds were generated from the interaction of bacterial alpha-glucosidase with the following residues GLU41, ASN45, GLU48 (three) ASN52, TYR104, ALA107, and GLU109 (two) (Table III, Fig. 3).

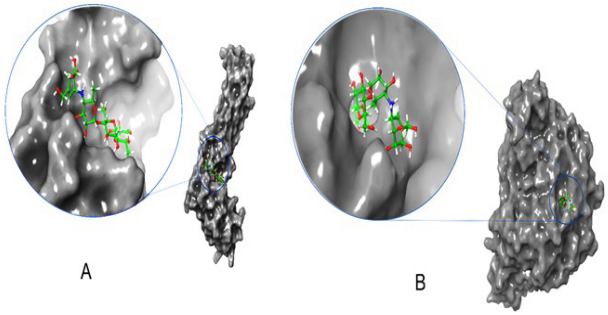


Fig. 3. Depicts a 3D interaction of alpha-glucosidase and acarbose. Panel A shows an enlarged model of the complex, highlighting the ligand in the active site of bacterial alpha-glucosidase. Panel B provides a closer view of the complex, with the ligand displayed in the center of human alpha-glucosidase.

While the interaction of human alpha-glucosidase and acarbose generated eight H-bonds with ASP404 (two), ARG411 (two), ASP518, ARG600, and ARG616 (two) (Table IV, Fig. 4).

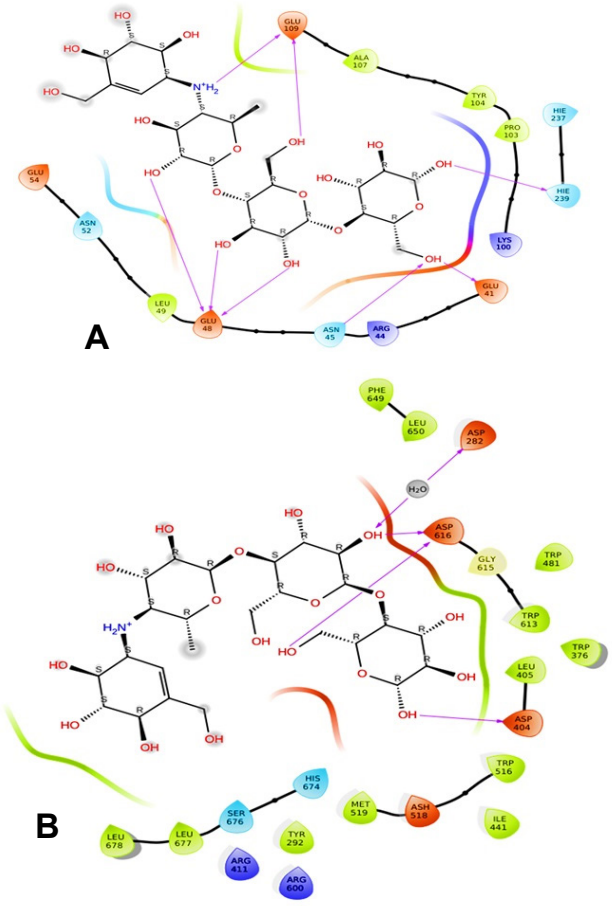


Fig. 4. Depicts a 2D interaction of alpha-glucosidase and acarbose. A, enlarged model of the complex, highlighting the ligand in the active site of bacterial alpha-glucosidase. B, provides a closer view of the complex, with the ligand displayed in the center of human alpha-glucosidase.

DISCUSSION

α-Glucosidase (EC 3.2.1.20) also known as maltase is the enzyme which liberates glucose from maltose by hydrolyzing the α-1, 4-glycosidic linkage. In combination with amylase, α-glucosidase is use in the processing of starch to glucose, the process which has tremendous importance in the glucose and bioethanol production industries (Synowiecki *et al*., 2006; Chen *et al*., 2010). In the human body, the activity of intestinal α-glucosidase has been associated with the development of diabetes type II (Hedrington and Davis, 2019). Therefore, the inhibitors of enzyme provide a useful medicine for the management of disease which affects 537 million adults worldwide (Cisneros-Yupanqui *et al*., 2022). There is big pool of literature about α-glucosidase and continuous research on the enzyme from different unexplored species. Many recent studies have reported the cloning, recombinant production and properties of enzyme from various organisms (Wang *et al*., 2022; Wu *et al*., 2023). In the present study we have cloned, purified, and characterized α-glucosidase from Thermococcus radiotolerans, which is an unexplored species for its proteins and enzymes. A complete open reading frame consisting of 729 bp coding for α-glucosidase was PCR amplified, cloned and subcloned in BL21 (DE3) codon plus RIPL strain of *E. coli* using pJET1.2 and pET21a (+) plasmid vectors. Production of recombinant of enzyme in *E. coli* was induced by addition of 1 mM IPTG (isopropyl β- D-1-thiogalactopyranoside). The IPTG concentration for the induction of gene expression has been reported from 0.2 to 1 mM (Yin *et al*., 2023). We found a 30 kDa protein on SDS-PAGE (Fig. 1), the active and soluble form of enzyme was detected in the sonicated *E. coli* cellular extracts. In the literature, α-glucosidases with a molecular weight of 70 kDa (Ohashi *et al*., 1996), 76 kDa (Benešová *et al*., 2005), and 98 kDa (Dhanawansa and Faridmoayer, 2002), have been reported. Selective heat denaturation, ammonium sulphate precipitation and cation exchange chromatography techniques were used for the purification of recombinant enzyme. It gave 98.6 U/mg activity, 46.2 percentage final recovery and more than 24 folds purification as indicated in the table 1. The purified enzyme was subjected to kinetics and in silico evaluation. It exhibited maximum activity at 85°C and pH 6. An optimum pH of 5 to 6.5 and temperature of 36°C has been reported for α-glucosidase from pistachio green stink bug (Ramzi and Hosseininaveh, 2010). The enzyme was found highly thermostable, it retained more than 50% activity after incubation at 100°C for five min. Such hyperthermophilic activity has been reported by the α-glucosidase reported from *Thermococcus aggregans* (Niehaus *et al*., 2000). Lineweaver-burk plot method was used to determine Km and Vmax values which were found 28.1 mM and 26.3 µmol per min respectively when maltose was used as the substrate (Fig. 2). Enzyme with Km of 4.3 mM and Vmax values of 3.7mmol per min has been reported from coastal medicine plants (Gurudeeban *et al*., 2012). According to in silico studies of bacterial α-glucosidase Glu^{109}, Ala^{107}, Tyr^{104}, Glu^{41}, Arg^{44}, Asn^{45}, Glu^{48} were found as the active site amino acids which interact with the substrate by hydrogen binding. In case of human enzyme Asp^{404}, Arg^{411}, Asp^{518}, Arg^{600}, and Arg^{616} were found as the main active site aresidues (Figs. 3, 4). A comparative analysis of 10 well establish α-glucosidase inhibitors were made by molecular docking to determine the affinity of each inhibitor for both of the human and microbial enzymes (Table II). With a few exceptions the enzymes from entirely different species have shown

similar affinities with the inhibitors. As for example, minimum affinities as indicated by free energy changes of (ΔG -3.2) and (ΔG -3.3) were exhibited by microbial and human enzyme respectively for Mulberrofuran K. The highest affinities [ΔG -9.6 (microbial) and ΔG -9.5 (human)] were found with acarbose indicating acarbose as the strongest available inhibitor for both enzymes. The way the of interaction of our compounds with human alpha-glucosidase is almost exactly the same as what has been seen before in the interaction of this protein with other inhibitors (Kok *et al*., 2022). This similarity is especially evident in the way Arg600, and Asp404 are involved. The blocking of catalytic residue ARG616 (Kok *et al*., 2022; Roig-Zamboni *et al*., 2017) with strong H-bonds may lead to the strong blocking of the protein's active site which may lead to the impairing its function.

CONCLUSION

α-glucosidase produced as recombinant from *Thermococcus radiotolerans* is a uniquely low molecular weight enzyme which has exhibited extremely high temperature stability, and relatively low Km value. The comparative analysis of structures of microbial and human enzyme have shown a huge difference in the primary structure and molecular weight, the active site amino acids found are similar in nature, with the exception that the glutamate in microbial enzyme active site has been replaced by aspartate in human enzyme active site, arginine is mostly conserved. According to our in silico data, both of the enzymes have very similar affinities with the 10 well known inhibitors indicating an evolutionary conservation of some features among the enzymes and proteins from species with extremely different phylogenetics.

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Statement of conflict of interest

The authors have declared no conflict of interest.

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