

Heterologous expression of a novel β -1, 4-glucosidase originated from *Aspergillus fresenii* and its enzymatic characters

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Research

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Abstract

Background: β -1, 4-glucosidases play important roles in the degradation of lignocellulosic biomass. It helps generate glucose from cellobiose and oligosaccharides, which could enhance the productivity in biorefinery and bioconversion process for energy and chemicals. Discovering novel β -1, 4-glucosidases can provide broader possibilities and understanding. The purpose of this study was to find a new β -1, 4-glucosidase in *Aspergillus fresenii* by an efficient method based on the high throughput sequencing technique.

Results: With the high throughput sequencing technique, a novel β -1, 4-glucosidase, named bgl T2, was cloned from *Aspergillus fresenii*, which was 2586 bp encoding 862 amino acid residues based on the sequencing analysis. Its amino acid sequence shared 91%, 80%, 80%, and 78% identity with the β -glucosidases of *Aspergillus steynii* IBT 23096 (XP_024702113.1), *Aspergillus oryzae* (5FJJ_A), *Aspergillus aculeatus* (P48825.1), and *Aspergillus fumigatus* A1163 (BOXPE1.1), respectively. The β -glucosidase bgl T2 gene was optimized according to the codon bias of *Komagataella phaffii* (\equiv *Pichia pastoris* (nom. illeg.)) and synthesized. The optimized bgl T2 gene was inserted into plasmid pPICZ α A, and transformed into *K.phaffii* X33 for its heterologous expression and enzymatic characters determination. The heterologous expressed β -glucosidase bgl T2 presented the highest activity at 55 °C and pH 5.5. When bgl T2 treated in citric acid- disodium hydrogen phosphate buffer (from pH 2.5 to pH 8.0) for one hour, the enzymatic activity was stable for pH 3.0 to pH 8.0 treatment, while the enzymatic activity dropped down to 22% with the pH 2.5 treatment. The thermostability half-life of bgl T2 was 9 min 36 s, 4 min 22 s, 117 s, and 68 s under 50 °C, 55 °C, 60 °C, 65 °C incubation, respectively. The Michaelis constant (K_m) of bgl T2 was 0.0007 mol/L. The maximum rate of bgl T2 theoretical enzymatic reaction (V_{max}) was 9×10^{-8} mol/L/s. In a 5 L fermentation vessel, the recombinant *K.phaffii* X33 could yield a β -1, 4-glucosidase activity of 4.45 U/mL after 96 h methanol inducement.

Conclusions: As the important enzyme to release glucose in the hydrolysis processes of cellulose, the novel bgl T2 provide a possibility to apply in bioenergy engineering, food processing, feed industry, and nutritional study, etc. This study also developed a path to obtain new enzymes depending on high throughput sequencing technique.

Background

β -1, 4-glucosidase (bgl; EC 3.2.1.21) catalyzes the cellobiose and oligosaccharides hydrolysis into glucose. It could remit the inhibition action of cellobiose against endoglucanases and cellobiohydrolases during the enzymatic catalysing cellulose hydrolysis [1, 2]. Cellulase enzymes, as the most important and costly part of the generating glucose from lignocellulose biomass [3, 4], are always interested to be understood, especially a novel one.

β -1, 4-glucosidase exists in plenty of organisms, which gives it a variety properties from each other. The study of β -1, 4-glucosidase started as early as in 1837, found by Wöhler and Liebig from almond

emulsion [5]. Through years of studying, plentiful β -1, 4-glucosidase were discovered [6]. Acquiring a new β -1, 4-glucosidase might help with the treatments of renewable agricultural, industrial and municipal cellulosic wastes for biofuels, chemicals, or animal feed. The conflict between humans and animals for consuming food is becoming critical with the growing population. Alternative feed material is required to subdue this problem. Glucose obtained from lignocellulosic biomass might be an energy source for animals if the lignocellulose biomass wastes digest properly [7, 8]. Since monogastric animals lack the capacity to convert cellobiose into glucose, β -1, 4-glucosidase must play important role in the lignocellulose biomass digestion process.

This study focused on a novel β -1, 4-glucosidase (bgl T2) that predicted by the result of the high throughput sequencing for mRNA. To understand the potential of bgl T2 for further utilization, it was heterologous expressed by *K.phaffii*. The bgl T2 was identified as a member of glycoside hydrolase family 3 (GH3) and its properties were determined. This study broadened the knowledge of β -1, 4-glucosidase from *A.fresenii* and provided a candidate for applications related to cellulose degradation.

Results

Identification of the β -1, 4-glucosidase bgl T2

According to the result of mRNA high throughput sequencing, assembled bgl T2 mRNA (GenBank accession number: MK986475) contain 2586 nucleotides including initiation codon and termination codon. Thus, the heterologous expressed bgl T2 contains 861 amino acids with a predicted molecular mass of 93.55 kDa, and had a theoretical pI of pH 5.11 disregarding the 6 \times His-tag [9]. The *A. fresenii* up-regulated mRNA of bgl T2 on the Avicel induction plate, while no reads was detected on the control plate. Comparing the sequence of PCR amplification product from *A. fresenii* genomic DNA with the assembled bgl T2 mRNA, 5 introns were found in the bgl T2 open reading frame containing 2901 bp (MK986476). The mRNA of bgl T2, the bgl T2 open reading frame, and amino acids sequences of bgl T2 is presented in Supplementary file 1.

Conserved domain of glycosyl hydrolase family 3 N terminal domain, BglIX, glycosyl hydrolase family 3 C-terminal domain, and fibronectin type III-like domain were found in the bgl T2. Due to the conserved domain found, the bgl T2 should be considered as a member of the glycosyl hydrolase family 3 (GH3). The amino acid sequence of bgl T2 shared 91%, 80%, 80%, and 78% identity with the β -glucosidases of *Aspergillus steynii* IBT 23096 (XP_024702113.1), *Aspergillus oryzae* (5FJJ_A), *Aspergillus aculeatus* (P48825.1), and *Aspergillus fumigatus* A1163 (BOXPE1.1), respectively. The alignment of amino acid sequences among these β -glucosidases was presented in Fig. 1.

Construction of the expression plasmid and strain

The optimized bgl T2 coding sequence for *K.phaffii* (MK965547) and original bgl T2 sequence were presented in Supplementary file 2. The codon adaptation index (CAI) value was increased from 0.63 to

0.93 by the optimization for *K.phaffii* expression, while the CG content decreased from 57.12–42.09% in order to avoid rare codons in *K.phaffii*.

The optimized *bgl* T2 coding sequence was successfully inserted into pPICZαA plasmid and preserved in *E. coli* TOP 10 confirmed by sequencing and double digestion. Eight zeocin-resistant clones of recombinant *K.phaffii* X-33 were picked up and preserved on YPD plates (1% yeast extract, 2% peptone, 2% dextrose and 2% agar). They were induced in flask by methanol under 28 °C with 250 rpm. The recombinant *K.phaffii* labeled *bgl* T2-7 gave the highest β-1, 4-glucosidase activity. Thus, the optimized *bgl* T2 coding sequence were successfully recombined with the *K.phaffii* X-33 genome.

Expression *bgl* T2 by recombinant *K.phaffii* X-33

After 96 h inducement in 500 mL flask, the supernatant of *bgl* T2-7 transformant were collected and determined for its β-1, 4-glucosidase activity. The *bgl* T2-7 transformant gave 0.23 U/mL toward p-nitrophenyl-β-D-glucopyranoside (pNPG) as substrate. While the *bgl* T2-7 transformant was induced in 5 L fermentation vessel for 96 h, it yielded 4.45 U/mL. The specific activity of *bgl* T2 was calculated as 3.6 U/mg.

Although the predicted theoretical molecular mass of *bgl* T2 was 93.55 kDa, the heterologous expressed protein appeared a band of approximately 130 kDa (Fig. 2).

Character study of *bgl* T2

bgl T2 showed highest activity at pH 5.5 and 55 °C. At pH 5.0, the *bgl* T2 activity was very close to its activity at pH5.5. There was a mere 2% difference between pH 5.0 and pH 5.5 for the *bgl* T2 relative activity. At the range of pH 4.5 to pH 6.5, *bgl* T2 stood more than 50% of its activity, while its activity was almost inhibited completely below pH 3.5 or above pH 8.0. From 25 °C to 55 °C, the *bgl* T2 activity increased gradually, while it dropped down dramatically started from 55 °C to a higher temperature. The dynamic graph between pH and *bgl* T2 activity was presented in Fig. 3, while the relationship between temperature and its activity was presented in Fig. 4.

When the *bgl* T2 treated in a range of pH 3.0 to pH 8.0 at 4 °C for one hour, *bgl* T2 kept stable. Its activity became the same or even more than the controls (Fig. 5). The treatment of pH 5.0 gave 60% more relative activity than the control. Treating *bgl* T2 in pH 2.5 for one hour resulted in residue activity drop down to 22%.

The half-life of *bgl* T2 were 9 min 36 s, 4 min 22 s, 117 s, and 68 s under 50 °C, 55 °C, 60 °C, 65 °C, respectively. The *bgl* T2 was slightly affected by the chemicals of sodium sulphate, copper sulphate, calcium chloride, ammonium sulphate, potassium chloride, sodium chloride, magnesium chloride, sodium nitrate, manganese sulphate, while it lost 7% activity with zinc sulphate and cobalt sulphate (Table 1). The K_m and V_{max} of *bgl* T2 against pNPG were 0.0007 mol/L and 9×10^{-8} mol/L/s, respectively.

Table 1
Effects of eleven chemicals on the activity of bgl T2.

Chemicals	Relative activity (%)
Control	100 ± 0.31
sodium sulphate	99.36 ± 0.36
copper sulphate	102.25 ± 0.10
ammonium sulphate	100.16 ± 0.38
manganese sulphate	94.53 ± 0.58
zinc sulphate	93.57 ± 0.25
cobalt sulphate	92.93 ± 0.75
calcium chloride	94.21 ± 0.29
potassium chloride	96.95 ± 0.48
sodium chloride	94.37 ± 0.20
magnesium chloride	94.69 ± 0.22
sodium nitrate	98.71 ± 0.37
Control was set as 100% relative activity. These results are the mean of three replicates with standard deviation.	

Discussion

This study discovered a novel β -1, 4-glucosidase, bgl T2, and its gene from *Aspergillus fresenii* depending on the results of high throughput sequencing of mRNA. When the full length of bgl T2 mRNA was assembled, its ORF was amplified from the genomic DNA of *A.fresenii* to confirm the accuracy of the assembled bgl T2 mRNA. It turned out that the bgl T2 mRNA sequence obtained by high throughput sequencing was solid. Furthermore, the bgl T2 was heterologous expressed by *K.phaffii* X33 and its characters were determined. Mastering this method to acquire a new functional enzyme was pretty reliable based on the success in this study. Comparing the traditional method to obtain a new enzyme and its gene, the route that this study took appears to show more of guarantee. Traditionally, having a new enzyme and its gene sequence requires the enzyme purification, isolation, and identification to initialize the enzyme discovery [10, 11], which seems difficult to achieve. Without knowing the nature of the new enzyme, purification and isolation of the new enzyme normally requires several attempts through very complicated steps using combinations of various chromatographic columns [12, 13], which is a time consuming, costly, and risky process. Identification of a new enzyme through LC-MS/MS may present partial peptide sequences of the enzyme, which would be the basic knowledge for the enzyme gene cloning. As an amino acid could share multiple codon, the primers design to amplify the full length gene

of the new enzyme would sometimes be problematic. On the other hand, the case of this study got the mRNA sequence directly by the high throughput sequencing, which made the gene cloning much more convenient. Normally, a partial mRNA sequence of the new enzyme would be found by the high throughput sequencing instead of the full length mRNA. Using the information of partial mRNA sequence to cloning the new enzyme gene is easier than having it by a partial peptide sequence. To uncover new enzyme under the help of high throughput sequencing is not like the transcriptome resequencing study that require at least 3 replicates, but a sufficient clean reads quantity. For the case of this study, 6 GB clean reads were enough.

Since the genome of *A.fresenii* is not yet reported, this study shall be the first one to uncover the characters and the encoding sequence of bgl T2. Although bgl T2 having 91% identity to the β -glucosidases of *Aspergillus steynii* IBT 23096 (XP_024702113.1), it still should be considered as a novel β -glucosidases, because the β -glucosidases of *Aspergillus steynii* is merely a putative one that is not confirmed yet. bgl T2 stands in the same line of many other β -1, 4-glucosidases for its optimal catalytic conditions. β -1, 4-glucosidases are commonly seen the optimal catalytic pH range from 4.0 to 6.0 [14, 15]. Most members of β -1, 4-glucosidases in GH3 originated from fungi have the optimal temperature between 50 °C to 65 °C, which fit the case of bgl T2 who has the optimal temperature at 55 °C. For the kinetic properties, the K_m of bgl T2 is lower, which means it has higher affinity to substrates. Table 2 present the comparison of some main properties of bgl T2 with other β -1, 4-glucosidases.

Table 2
Properties of β -glucosidase from various fungi

	Mr (kDa)	K_m (mM)	Opt. pH	Opt. Temp. (°C)	V_{max} (U mg^{-1})	Reference
<i>Aspergillus niger</i>	105	21.70	5	55	124.4	[16]
<i>Aspergillus oryzae</i>	110	NR	5.5	50	NR	[17]
<i>Aspergillus oryzae</i>	77	0.74	5.0	60	19.4	[18]
<i>Aspergillus oryzae</i> ^a	90	2.91	4.5	55	0.138 ^b	[19]
protoplast fusant of <i>Aspergillus oryzae</i> and <i>Aspergillus niger</i>	125	0.04	5.4	65	215.2	[20]
<i>Ceriporiopsis subvermispora</i>	110	3.29	3.5	60	0.113 ^b	[21]
<i>Phanerochaete chrysosporium</i>	114	0.10	4.0– 5.2	NR	NR	[22]
<i>Daldinia eschscholzii</i>	64	1.52	5.0	50	3.2	[23]
<i>Penicillium purpurogenum</i>	110	5.10	5.0	65	934	[11]
<i>Aspergillus fresenii</i>	130	0.70	5.5	55	5.4 ^b	This study

NR: Not reported. a: the properties were determined against cellobiose as the substrate. b: the unit of V_{max} was reported as $\mu\text{mol}/\text{min}$

bgl T2 is tolerant a wide range of the pH value, from pH 3.0 to pH 8.0, which means that bgl T2 might adapt to some application that have varied pH, such as feed additives. Interestingly, treating bgl T2 under pH 5.0 for one hour gives it 60% more activity. It seems like that the McIlvaine buffer of pH 5.0 somehow enhance the enzyme protein. This might be because that the bgl T2 favourably crystallized under pH 5.0 by its dimer(s), being the preferred biological arrangement in the asymmetric unit [24]. It is hard to get a convincing explanation.

Metal ions and chemicals may affect the activity of an enzyme. This study tested the effect of eleven common chemicals on the bgl T2. bgl T2 showed a stable property to these chemicals, which only zinc sulphate and cobalt sulphate inhibited about 7% of the bgl T2 activity.

Conclusion

This paper successfully uncovered a novel β -1, 4-glucosidase bgl T2 and its ORF from *Aspergillus fresenii* under the help of high throughput sequencing of mRNA technique. This method is more convenient than

the traditional method to obtain a new enzyme and its genetic information as explained in the discussion section. The optimized *bgl* T2 gene was heterologously expressed by *K. phaffii* X33. The properties of *bgl* T2 were tested including optimal catalysis pH and temperature, pH tolerance, thermostability, effects of common chemicals, and kinetic properties against pNPG.

Materials And Methods

Strains, vectors, media and chemicals

The *Aspergillus fresenii* (JCM 01963) was purchased from RIKEN BioResource Center, Japan Collection of Microorganisms. *Escherichia coli* TOP 10 and *K. phaffii* X-33 (Invitrogen, USA) were used as host strains. The *bgl* T2-opt gene was synthesized according to the codon bias of *K. phaffii* and constructed into the pPICZαA vector (Invitrogen, USA) with the *Eco*RI and *Xba* I restriction enzyme sites. The *p*-nitrophenyl-β-D-glucopyranoside (pNPG) was purchased from Sigma (USA). Other chemicals that not specifically mentioned were of analytical grade and were available on commercial supplier.

Enzyme assay

The enzyme activity of β-1, 4-glucosidase was assayed according to the description of Parry *et al.* [25] with some modifications. Using pNPG as the hydrolytic reaction substrate, the release of *p*-nitrophenol per minute determined stands for the activity of β-1, 4-glucosidase. The reaction mixture contained 100 μL of pNPG (10 mM) in McIlvaine buffer, 100 μL of enzyme solution. After incubating the reaction mixture for 10 min, the reaction was stopped by adding 800 μL of 1.0 M sodium carbonate. The absorbance of the final reaction solution was read at 405 nm, and the reading was calculated to know the amount of *p*-nitrophenol generated according to the standard graph which prepared under the same conditions.

One β-1, 4-glucosidase enzyme unit (U) was defined as the amount of β-1, 4-glucosidase that released 1 μmol of *p*-nitrophenol out of pNPG per minute.

Identification of the β-1, 4-glucosidase *bgl* T2

The *A. fresenii* (JCM 01963) was cultured on the induction plate (replace the sucrose in Czapek–Dox medium by Avicel PH-101, Sigma) and control plate (replace the sucrose in Czapek–Dox medium by glucose) for 7 days. The mycelium of the *A. fresenii* on both plates were collected. Their RNA were extracted and broken down into small fragments. Their cDNA were synthesized by reverse transcriptase reactions using random hexamers and double-stranded DNA were synthesized by polymerase chain reaction (PCR). After purification, selection, and amplification of their double-stranded DNA obtained from mRNA, the library for high throughput sequencing was constructed. The library was sequenced by Illumina HiSeq 4000, PE150. Clean reads were obtained at least 6GB for each treatment and assembled by Trinity [26].

The genomic DNA of *A. freseenii* was extracted as the template to amplify the *bgl* T2 gene. The primers designed based upon the assembled mRNA result were *bgl* T2-F (5'-ATGAAGTTTGGTTGGTTCGAGGCGGCG-3') and *bgl* T2-R (5'-TTAAACCACCACGGGCAACGAGCCCTG-3'). The PCR mixture contained 5 µl of 5 × HF buffer, 4 µl of 10 mM dNTPs mix, 0.5 µg of genomic DNA of *A. freseenii*, 0.5 µL of 10 pmol/µL each primers, and 0.5 U of Phusion DNA polymerase (Thermo Scientific) in a total volume of 50 µL. The conditions and procedures of the *bgl* T2 gene amplifications were set as following: one initialization step at 98 °C for 5 min, 30 times of the amplification cycles (denaturation at 95 °C for 30 s, annealing at 52 °C for 30 s, extension at 72 °C for 2 min), and one final elongation step at 72 °C for 10 min. The DNA sequencing of amplification products were detected by chain-termination methods [27]. The results were compared within National Center for Biotechnology Information (NCBI) databases as well as the assembled mRNA result for similarity analysis and intron detection.

Once the encoding sequence of *bgl* T2 was confirmed, its amino acids sequence was also compared within NCBI protein BlastX for the similarity study [28].

Construction of the expression plasmid and strain

The *bgl* T2 coding sequence (CDS) was optimized according to the code bias of (*K.phaffii*) and synthesized with a 6×His-tag and a restriction sites of *Eco*RI at 5' end while a restriction sites of *Xba* I at 3' end. Plasmid of pPICZαA and optimized *bgl* T2 coding sequence were double digested with *Eco*RI and *Xba* I. The digested products were purified and ligated as *bgl* T2 opt-pPICZαA recombinant plasmid. The recombinant plasmid of *bgl* T2 opt -pPICZαA were transformed into TOP 10 *E. coli* competent cells by chemical methods [29]. A zeocin-resistant colony was confirmed by sequencing harboring the recombinant plasmid and used to reproduce the recombinant plasmid. 5 µg of the recombinant plasmid was linearized with *Sac* I, purified and transformed in *K.phaffii* X-33 strain by electroporation. The positive expression strains were selected by zeocin-resistance (1000 µg/ml). The recombinant transformant that presented highest β-1, 4-glucosidase activity, called *bgl* T2-7, was used for enzymatic characters study.

Expression *bgl* T2 by recombinant *K.phaffii* X-33 and purification

The recombinant *K.phaffii* X-33 of *bgl* T2-7 was inoculated into a 250 ml flask containing 25 ml of buffered glycerol-complex medium (BMGY: 1 % yeast extract, 2 % peptone, 1 % glycerol, 0.1 M potassium phosphate at pH 6, 1.34 % Yeast Nitrogen Base with ammonium sulfate without amino acids, and 4 µg biotin) and incubated at 28 °C with 250 rpm shaking. When the culture reached an OD₆₀₀ of 4, the yeast was harvested by centrifuging at 3,000 g for 5 min at room temperature. Supernatant was decanted. The cell pellet was re-suspended to an OD₆₀₀ of 1.0 using buffered methanol-complex medium (BMMY: 1 % yeast extract, 2 % peptone, 0.1 M potassium phosphate at pH 6, 1.34 % Yeast Nitrogen Base with ammonium sulfate without amino acids, 4 µg biotin, and 0.5 % methanol) and the culture was incubated in a 500 mL flask with 2 layers of sterile gauze cover at 28 °C with 250 rpm shaking. 100 % methanol was supplied every 24 hours to maintain a final concentration of 0.5 % methanol to induce expression.

bgl T2 was also expressed in a 5 L fermentation vessel to exam its productivity. After culturing 30mL *bgl* T2-7 in YPD medium (1 % yeast extract, 2 % peptone, and 2 % dextrose) to an OD₆₀₀ of 1.0, it was transferred into a 500 mL flask with 250 mL YPD medium and incubated it until its OD₆₀₀ reached to 2.0 with 250 rpm shaking as the start yeast for the fermentation. For the first 48 h biomass enrichment phase, *bgl* T2-7 was cultivated in pH 5.5 fermentation medium (5 % glucose, 0.5 % monopotassium phosphate, 0.1 % calcium sulphate, 1.8 % potassium sulphate, 0.8 % magnesium sulphate, 5 % ammonium di-hydrogen phosphate, 0.15 % potassium hydroxide, and 4.5 mL/L trace salt solution[1]) with 350 rpm. Glycerol supplementation was applied when the dissolved oxygen reached to 60 %. Before inducement phase, a hunger period of 2 hours was meant to consume all the carbon sources in the vessel. Methanol was added to keep the dissolved oxygen maintaining at 20 % to 60 % during whole 96 hours inducement phase.

The supernatant of *bgl* T2-7 fermentation was collected by centrifugation. It was purified by Ni-NTA magnetic beads. The purified *bgl* T2 enzyme activity was tested and its protein concentration was determined by the BAC protein assays kit (ThermoFisher scientific, USA).

Character study of *bgl* T2

The expression culture was centrifuged at 12,000 rpm (13,105 g, rcf). Its supernatant was diluted and used for the character study of *bgl* T2. The optimal pH of *bgl* T2 was performed every 0.5 pH in a range of pH 2.5 to pH 8.0 with Mcllvaine buffer. The optimal temperature of *bgl* T2 was performed every 5 °C from 25 °C to 80 °C. To test the resistance to different pH, ultra-filtrated *bgl* T2 was treated in Mcllvaine buffer from pH 2.5 to pH 8.0 for one hour, and then diluted 100 times by the Mcllvaine buffer at optimal pH 5.5, while the control *bgl* T2 was mixed with relative Mcllvaine buffer and diluted right before the enzymatic activity residue assay. The thermostability of *bgl* T2 was tested at five time points to calculate the half-life of *bgl* T2 under different temperature, 50 °C, 55 °C, 60 °C, and 65 °C. *bgl* T2 mixed with final concentration of 10mM of sodium sulphate, copper sulphate, calcium chloride, ammonium sulphate, potassium chloride, sodium chloride, magnesium chloride, sodium nitrate, manganese sulphate, zinc sulphate, and cobalt sulphate to understand the effects of those chemicals toward *bgl* T2 activity. Determining the activity of *bgl* T2 against 2.5 mM, 2 mM, 1.5 mM, and 1.2 mM *p*NPG under optimal reaction condition, the K_m and V_{max} were calculated according to Eadie-Hofstee plots [30].

[1] trace salt solution : 6.0 g CuSO₄·5H₂O, 0.09 g KI, 3.0 g Mn₅O₄·H₂O, 0.2 g Na₂MoO₄·2H₂O, 0.02 g boric acid, 0.5 g CoCl₂·6H₂O, 20 g ZnCl₂, 65 g FeSO₄·7H₂O, 5 mL sulphuric acid, and 0.2 % biotin.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

All authors of this paper declare that they have no competing interests.

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Authors' contributions

YY did most of the experiments, data analysis, and the manuscript writing. JW quantified the *bgl* T2 protein. HG helped the SDS-PAGE. YC supervised this study. All authors read and approved the manuscript.

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Figures

BGL_T2	MKFGWFEEAALTAASVYSA.....ODDLAFSPPPYPSFWANGOGEW	41
XP_024702113.1A.....	41
SFJJ_AK---Y---F---D---	22
F48825.1	---LS-L-----A-E---F---	40
BOXPE1.1	---R---L-V-----AN-QVFDMSHGNGE-----F---D---	50
BGL_T2	ADAYKRAVDLVSQMTLAEKVNLTGTGWSLDRKCVGQTGSVFRGLLS.LC	90
XP_024702113.1	-E-----I-----T-----Q-ER-----NIP---	90
SFJJ_A	-EV-----I-----T-----Q-ER-----NIP---	71
F48825.1	-E-Q---AI-----D-----E-M-----G---NIGG.M-	89
BOXPE1.1	--HR---EI-----EM-R-----INWG--	100
BGL_T2	LQDSPGIRPFDYNSAFPAQVNVAAATWTKTLAYLRGKMGQEFSDRGIDV	140
XP_024702113.1	-----S-----Q---E-----	140
SFJJ_A	-----S-----Q---E-----	121
F48825.1	-----DS-----N-----Q-----	139
BOXPE1.1	G-----S-L-----T-----E--N--V-I	150
BGL_T2	QLGPAAGPLGRHPDGRNWGFSPPDPLSGVLFAETIRGIQEGVIATAK	190
XP_024702113.1	-----S-K---DA-----	190
SFJJ_A	-----A-----T-----K---DA-----	171
F48825.1	-----S-----T-----K---DA--V---	189
BOXPE1.1	L-----KY---I-----T-----K---DA-----	200
BGL_T2	HFILNEQEQFRQVPEAAGFGFNISDTLSMLDDKTMHLYLWPFADAVRA	240
XP_024702113.1	--V---H-----Y-----V-----	240
SFJJ_A	-Y-M---H---Q---Y---V---S---V-----	221
F48825.1	-Y---H---A---Y---I---V---I---M-----	239
BOXPE1.1	-Y---H---G---Q-Y-Y-TE-I-V-----	250
BGL_T2	GVGAIMCSYQINNSYGCQNSSETLNKLLKALGFGQFVMSDWSAHSVG	290
XP_024702113.1	-----V---N-----	290
SFJJ_A	-----V---N-----E-----T-----	271
F48825.1	-----N-----Y-----G-----	289
BOXPE1.1	-----V---N-----Q-----	300
BGL_T2	SALAGLMSMGDIAFNGLSYWGTNLTIGVLMGTVPQWRVDMAVRIMA	340
XP_024702113.1	---M-----VS---T-F---V-----	340
SFJJ_A	A-----VT-DS-T-F-A---V---I-----	321
F48825.1	-----T-DSAT-F---A-----	339
BOXPE1.1	A-----S-D---F---VS---A-----T	350
BGL_T2	AYYKVGDRDLHTPPFSWTTDEYGFHFVAVSEGAYERVNHVVDVQRDHA	390
XP_024702113.1	-----N---R---F---N-----QL-----	390
SFJJ_A	---TKY---R---A-NH---E---V---N	371
F48825.1	-----YQ-N---R---KY-YQ-P-K---N--N-S	389
BOXPE1.1	-----HR---N---R---W-S---WTK--D--N--S-S	400
BGL_T2	EIIRRVGADSIIVLLKNGALPLTGKRVALLGADGNSFKGANGCADRG	440
XP_024702113.1	D-----I---Q-T-----SR--KL--L-----SW---D---	440
SFJJ_A	DL--I--Q-T-----SR--KL--L-----SW---D---	421
F48825.1	-V--KL--T-----NN-----SY---S---	439
BOXPE1.1	Q---ET--A-T---T-----V--GV-----M---P---	450
BGL_T2	CNNGTLNAMNGSGTTEFPYLVTEQAIQNKVLQGRGVFVSIIDNKALDOV	490
XP_024702113.1	-----A-----R--K-----V-H---K---	490
SFJJ_A	-----AN-----E-----AV--S---KI	471
F48825.1	-----A-----AE--KHK-S-YA-----S--	489
BOXPE1.1	-----AN-----RE-LSNG---AV--G--S-M	500
BGL_T2	AKLASQSSVSLVFNADAGEGFIIVDENRGRKGLTLWKNQDNLIKTAQ	540
XP_024702113.1	-----LS--G-E---N-----E-----Q	540
SFJJ_A	---AR--R---S---YLS--G-E--N---I---V---N	521
F48825.1	ET--K-A-----S---Y-S--G-E--N-----A--N	539
BOXPE1.1	-DV-----H-----S---S--G-E-----EAV-D-VVS	550
BGL_T2	NCNNTIVVHSGVPLVDEWYHFNVTGILNAGLPGQESGNSLTDVLYGL	590
XP_024702113.1	---V---I-----I-----D-----IA---R	590
SFJJ_A	---V-I-----I-----D-----IA---R	571
F48825.1	H-----I-R--DN---A-I-----V---R	609
BOXPE1.1	H-----I-R--DN---A-I-----V---R	600
BGL_T2	VNPGGKTEFTWTKRKSYSQALLTKPNNGGAPQEDFEEGVFIDYRHFDK	640
XP_024702113.1	-----A-S-----E---S--VRDA-----S--TQ-----	640
SFJJ_A	-----A-S-----EA--DY-VREL-----D--S-----G--	621
F48825.1	-----A-S-----EA--DY-VREL-----D--S-----G--	639
BOXPE1.1	---SA-----E-----E-----D--N-----	650
BGL_T2	RNDTPIYEFVGLSYTTFAYSGLIQISLN..APKYIPTSGKTKAAQTFGK	688
XP_024702113.1	-E-----G-----S---T-----F---	688
SFJJ_A	F-E-----H---EL-D-HV-P---,---SR-T---M-E--KN--E	669
F48825.1	-E-----H---EL-D-HV-P---,---SR-T---M-E--KN--E	687
BOXPE1.1	-E-----H---EL-D-HV-P---,---SR-T---M-E--KN--E	700
BGL_T2	TDEASDLVYPDELQRIHQFIYFVWLNSTDLKKSQDPHYGKDSKYPEDA	738
XP_024702113.1	-K---F---H---EY-----E-----R---	738
SFJJ_A	IGD--EY--EG-E---E---I-----A--D-SH--E---G-	719
F48825.1	VGN--Y--EG-T--SK-----A---Y--VDTAEHV--G-	737
BOXPE1.1	IGS-A-YL--EG-K--TK-----ED--D--N--E-E--G-	750
BGL_T2	TDGSPQDLLEASGGPGGNGLYEELVWVSNITNTGNIAGDEVPQLYVSL	788
XP_024702113.1	---A--P---A-----D--F---KVK--V---V---	788
SFJJ_A	---A--P---A-----D--F---KVK--V---V---	769
F48825.1	---P---G---S---R--D--I---TVK--RV--A---	787
BOXPE1.1	R---P--K-G-A---T--QD---AT---V---Y---	800
BGL_T2	GGPNEPPKVRKFDRLHLGFSQEVVWATLIRDLNSWDAQDQVITKY	838
XP_024702113.1	---R-----IR---EST--T--N-----	838
SFJJ_A	---KV---E-I--A---A--T-----A---S---TV-P-	819
F48825.1	---KV---E-I--A---A--T-----A---S---TV-P-	837
BOXPE1.1	---RV---E-I--A---A--T-----A---S---TV-P-	850
BGL_T2	PKKVFVGSRRKPLQSSLPVVV	861
XP_024702113.1	---TIY--N-----A---KAQ	861
SFJJ_A	---TIY--N-----A---KAQ	842
F48825.1	---H---Q---HAA--K-Q	860
BOXPE1.1	---H---Q---HAA--K-Q	873

Figure 1

Alignment of amino acid sequences of bgl T2, *A.steynii*, *A.oryzae*, *A.aculeatus*, and *Aspergillus fumigatus*. Underline indicated the predicted signal peptide.



Figure 2

SDS-PAGE of the recombinant bgl T2. Lane M: molecular weight markers; Lane 1: heterologous expressed bgl T2.

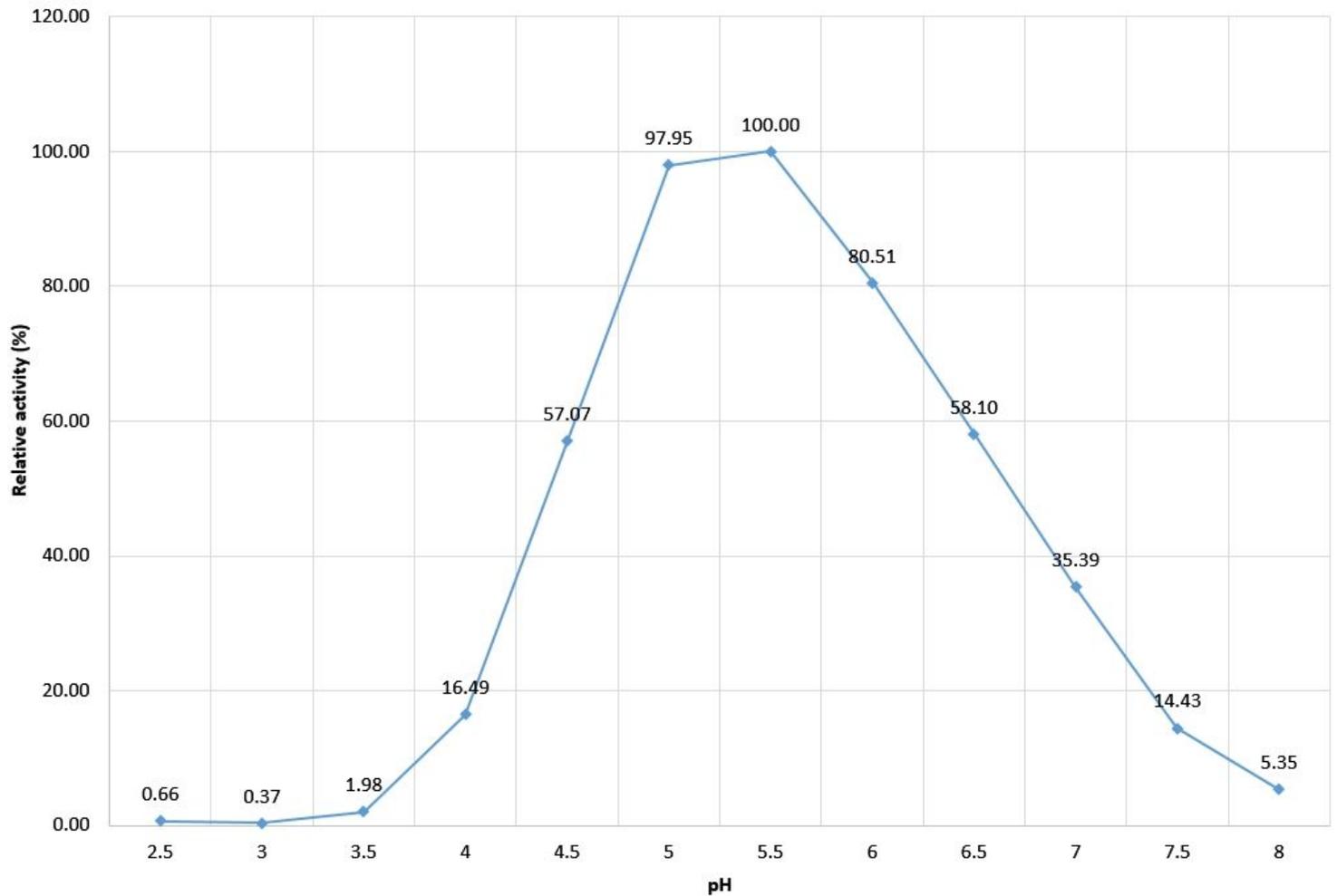


Figure 3

the dynamic graph between pH and bgl T2 activity. Results are the mean of three replicates.

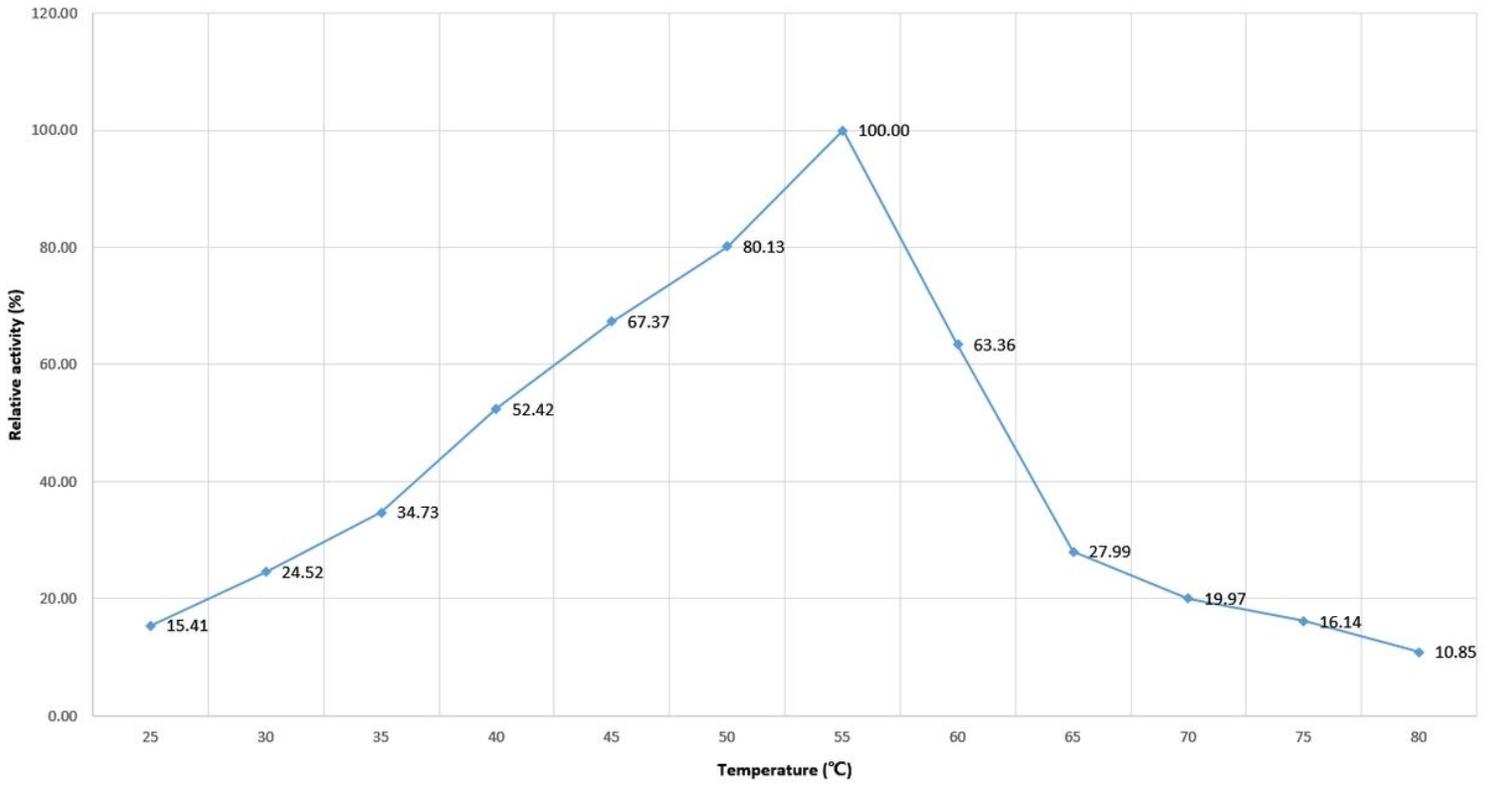


Figure 4

the dynamic graph between temperature and bgl T2 activity Results are the mean of three replicates.

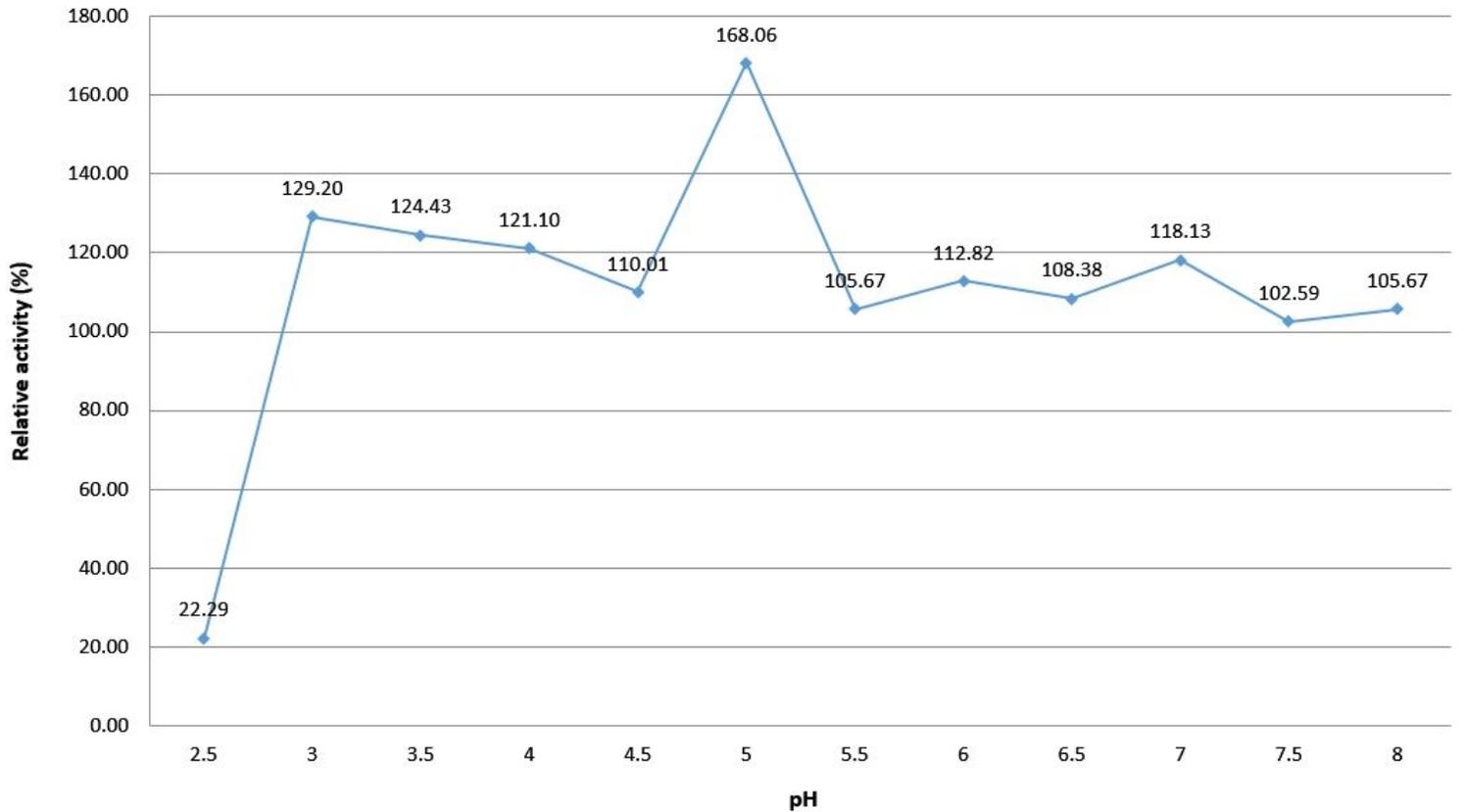


Figure 5

the tolerance of bgl T2 against pH value Results are the mean of three replicates.

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