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Overexpression and characterization of a glucose-tolerant β-glucosidase from *Thermotoga thermarum* DSM 5069T with high catalytic efficiency of ginsenoside Rb1 to Rd

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ABSTRACT

The β-glucosidase gene *Tt-bgl* from *Thermotoga thermarum* DSM 5069T was cloned and overexpressed in *Escherichia coli*. A simple strategy, induction at 37 °C with no IPTG, was explored to reduce the inclusion bodies, by which the activity of Tt-BGL was 13 U/mL in LB medium. Recombinant Tt-BGL was purified by heat treatment followed by Ni–NTA affinity. The optimal activity was at pH 4.8 and 90 °C. The activity of Tt-BGL was significantly enhanced by methanol and Al^{3+}. The enzyme was stable over pH range of 4.4–8.0, and had a 2-h half life at 90 °C. The V_{max} for *p*-nitrophenyl-β-D-glucopyranoside and ginsenoside Rb1 was 142 U/mg and 107 U/mg, while the K_m was 0.59 mM and 0.15 mM, respectively. The activity of the enzyme was not inhibited by ginsenoside Rb1 (36 g/L). It was activated by glucose at concentrations lower that 400 mM. With glucose further increasing, the activity of Tt-BGL was gradually inhibited, but remained 50% of the original value in even as high as 1500 mM glucose. Under the optimal conditions, Tt-BGL transformed ginsenoside Rb1 (36 g/L) to Rd by 95% in 1 h.

1. Introduction

Ginseng is generally known for its medical properties. Recently, pharmaceutical activities of ginseng roots have been proven by many researches, and ginseng has become a world-famous medicinal plant [1]. Ginseng roots contain various pharmaceutical components ginsenosides, polyacetylenes, polyphenolic compounds and acidic polysaccharides, and among the components, ginsenosides are the most pharmaceutically active. Until now, more than 180 ginsenosides have been isolated from ginseng roots, with five major ginsenosides (ginsenosides Rb1, Rb2, Rc, Re and Rg1) constituting more than 80% of the total ginsenosides [1,2]. The various ginsenosides have the different pharmaceutical activities because of the different skeletons and sugar moieties.

In the recent decades, many studies have focused on the pharmaceutical activities of the ginsenoside Rd, because it activity is found to be superior to those of the major ginsenosides, such as to enhance the differentiation of neural stem cells, protect neural systems against neurotoxicity by attenuating NO overproduction, decrease levels of urea nitrogen and creatinine in the kidney, and protect the kidney from apoptosis and DNA fragmentation caused by chemical drugs and cancer drugs [3–7]. Therefore, producing Rd with high-activity and low-cost has been a hot topic in the ginsenoside research. But Rd content of ginseng roots is less than those of major ginsenosides. Moreover, it is not feasible to prepare Rd by chemical synthesis because of its complex structure [8,9]. Through analyzing the structures, Rb1 has structural similarities to Rd, and Rb1 has only one more sugar residue at the C-20 position than Rd (Fig. 1). Thus, Rd can be obtained by the chemical transformation or biotransformation. In comparison with the chemical transformation, the biotransformation has more potential for conversion because of its high specificity and environmental compatibility [3,10].

β-Glucosidases (EC 3.2.1.21) does not only participate in cellulose degradation, it also play an important role in bioactive substrates liberation from these precursors, because β-glucosides constitute the majority of the known glycoconjugated the compounds [11–14]. Some studies have looked for suitable microbes or enzymes that can transform Rb1 into Rd [15–17]. However, most of β-glucosidases have been lack of high catalytic efficiency, thermostability, high glucose tolerance, high substrate tolerance, or high specificity. Thus, producing a β-glucosidase with the advantages discussed above has become important.

Abbreviations: IPTG, isopropyl-β-D-thiogalactopyranoside; GH, glycoside hydrolase; *p*NPGlu, *p*-nitrophenyl-β-D-glucopyranoside; *p*NPGal, *p*-nitrophenyl-β-D-galactopyranoside; *p*NPXyl, *p*-nitrophenyl-β-D-xylopyranoside; *p*NPAra, *p*-nitrophenyl-α-L-arabinofuranoside.

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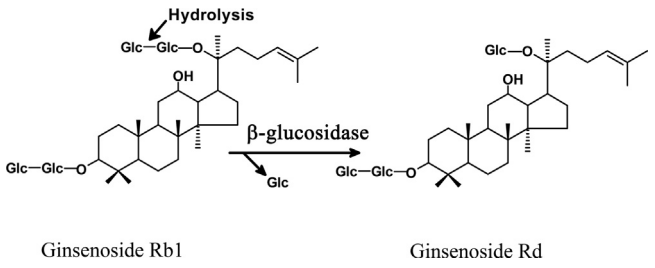

Fig. 1. Enzymatic transformation of ginsenside Rb1 to Rd.

Bacterium *Thermotoga thermarum* DSM 5069T is a strict anaerobe that grows on a variety of carbohydrates including fructose raffinose, salicin and cellulose at 80 °C, which have attracted considerable interest with regards hydrogen production and thermostable enzyme production [18]. Owing to the inherent difficulty of cultivation of *T. thermarum* DSM 5069T, it is difficult to obtain a sufficient amount of cells for large-scale enzyme production. For the production of the recombinant protein, genetic engineering is the first choice because it is easy, fast, and cheap.

In this paper, we report the sequence analysis, cloning, overexpression, and detailed biochemical characterization of the β-glucosidase from *T. thermarum* DSM 5069T. The favorable properties make the β-glucosidase a good candidate for utilization in biotechnological applications.

2. Materials and methods

2.1. Bacterial strains, plasmids, growth media

T. thermarum DSM 5069T was purchased from DSMZ (www.dsmz.de). It was grown anaerobically at 80 °C as described previously [18]. *Escherichia coli* JM109 and JM109(DE3) was grown at 37 °C in Luria-Bertani medium (LB) and supplemented with kanamycin when required. The expression vector pET-28a (Novagen) was employed as cloning vector and expression vector.

2.2. DNA manipulation

DNA was manipulated by standard procedures [19]. QIAGEN Plasmid Kit and QIAGEN MinElute Gel Extraction Kit (Qiagen, USA) were employed for the purification of plasmids and PCR products. DNA restriction and modification enzymes were purchased form TaKaRa (Dalian, China). DNA transformation was performed by electroporation using GenePulser (Bio-Rad, USA).

2.3. Plasmid constructions

The β-glucosidase gene *Tt-bgl* was amplified from *T. thermarum* DSM 5069T genomic DNA by PCR using primers bgl-1 (CCCCCATGGGTTTTCCAAAGGATTTTCT***G***TTCGG***C***GCG***AGC***ATGGC***C***-GG***C***TT***C***CAAGTTGAAATGGGATATG) and bgl-2 (CCCCTCGAGCAT-***G***CGCCA***G***AT***T***TCGTA***T***GG***GCT***TTT***C***AGGTAGTTGTGAAA***GCG***TGCCG-TTGTCCCTTCTTTG). The boldface italic nucleotides represented mutations for optimizing codons, the underlined sequences for the restriction enzyme sites. The PCR products were digested with *Nco* I and *Xho* I and inserted into pET-28a at *Nco* I and *Xho* I sites, yielding the plamid pET-28-BGL.

2.4. Expression and purification of Tt-BGL

Plasmid pET-28-BGL was transformed into *E. coli* JM109(DE3), and induced to expression recombinant Tt-BGL by adding isopropyl-β-D-thiogalactopyranoside (IPTG) to final concentration from 0 to 0.5 mM at OD_{600} about 0.7, and incubated further at 25 °C or 30 °C or 37 °C for about 6 h.

One liters of the recombinant cells carrying pET-28-BGL were harvested by centrifugation at 5000 × *g* for 10 min at 4 °C, and washed twice with distilled water, resuspended in 50 mL of 5 mM imidazole, 0.5 mM NaCl, and 20 mM Tris–HCl buffer (pH 7.9), and French-pressured for three times. The cell extracts were heat treated (75 °C, 30 min), and then cooled in an ice bath, and centrifuged (20,000 × *g*, 4 °C, 30 min). The resulting supernatants were loaded on to an immobilized metal affinity column (Novagen, USA), and eluded with 1 M imidazole, 0.5 M NaCl, and 20 mM Tris–HCl buffer (pH 7.9). Protein was examined by SDS-PAGE [20], and the protein bands were analyzed by density scanning with an image analysis system (Bio-Rad, USA). Protein concentration was determined by the Bradford method using BSA as a standard.

2.5. Determination of enzyme activities and properties

The reaction mixture, containing 50 mM imidole-potassium buffer (pH 4.8), 1 mM *p*-nitrophenyl-β-D-glucopyranoside (*p*NPGlu) or 2 mM ginsenosides Rb1 as substrates, and certain amount of β-glucosidase in 0.2 mL, was incubated for 5 min at 90 °C. The reaction was stopped by the addition 1 mL of 1 M Na_2CO_3. For *p*NPGlu, the absorbance of the mixture was measured at 405 nm. For ginsenosides Rb1, the residue was assayed by HPLC. The glucose was determined by glucose assay kit (Dingguo, China). One unit of enzyme activity was defined as the amount of enzyme necessary to liberate 1 μmol of *p*NP (or ginsenoside Rd) per min under the assay conditions.

The optimum pH for activity β-glucosidase was determined by incubation at 90 °C for 5 min in the 50 mM imidole-potassium buffer from pH 4.0 to 8.0. The optimum temperature for the enzyme activity was determined by standard assay ranging from 50 to 100 °C in the 50 mM imidole-potassium buffer, pH 4.8. The results were expressed as percentages of the activity obtained at either the optimum pH or the optimum temperature.

The pH stability of the enzyme was determined by measuring the remaining activity after incubating the enzyme (0.05 μg) at 70 °C for 1 h in the 50 mM imidole-potassium buffer from pH 4.0 to 8.0. To determine the effect of temperature on the stability of Tt-BGL, the enzyme (0.05 μg) in the 50 mM imidole-potassium buffer (pH 6.0) was pre-incubated for various times at 85 °C, 90 °C and 95 °C in the absence of the substrate. The activity of the enzyme without pre-incubation was defined as 100%.

The effects of metals and chemical agents on β-glucosidase activity of purified enzyme (0.05 μg) were determined. Fe^{2+}, Mg^{2+}, Zn^{2+}, Mn^{2+}, Ca^{2+}, K^{+}, Al^{3+}, Li^{2+}, Cu^{2+}, Co^{2+}, and Hg^{2+} were assayed at concentrations of 1 mM in the reaction mixture. The chemical agents EDTA (10 mM) were assayed. The effects of organic solvents on the enzyme were determined by adding 5%, 10%, 20%, and 30% organic solvents (ethanol or methanol) in the reaction mixture. The enzyme was incubated with each reagent for 10 min at 90 °C before addition of *p*NPGlu to initiate the enzyme reaction. Activity was determined as described above and was expressed as a percentage of the activity obtained in the absence of the chemical agents and metal cations.

The substrate specificity of the enzyme (0.05 μg) was tested by using following *p*NPGlu, ginsenoside Rb1, CMC, *p*-nitrophenyl-β-D-galactopyranoside (*p*NPGal), *p*-nitrophenyl-β-D-xylopyranoside (*p*NPXyl), *p*-nitrophenyl-α-L-arabinofuranoside (*p*NPAra), sucrose, gentiobiose, and cellobiose. Kinetic constant of Tt-BGL was determined by measuring the initial rates at various *p*NPGlu concentrations (0.2, 0.4, 0.6, 0.8, 1, 1.5, and 2 mM) or various *p*NPGal concentration (1, 1.4, 1.8, 2, 2.5. 3, 4, and 5 mM) or various cellobiose concentration (10, 20, 40, 60, 80, 120, and 160 mM) or various ginsenoside Rb1 concentration (0.072, 0.144, 0.288, 0.36, 0.72, 1.44,

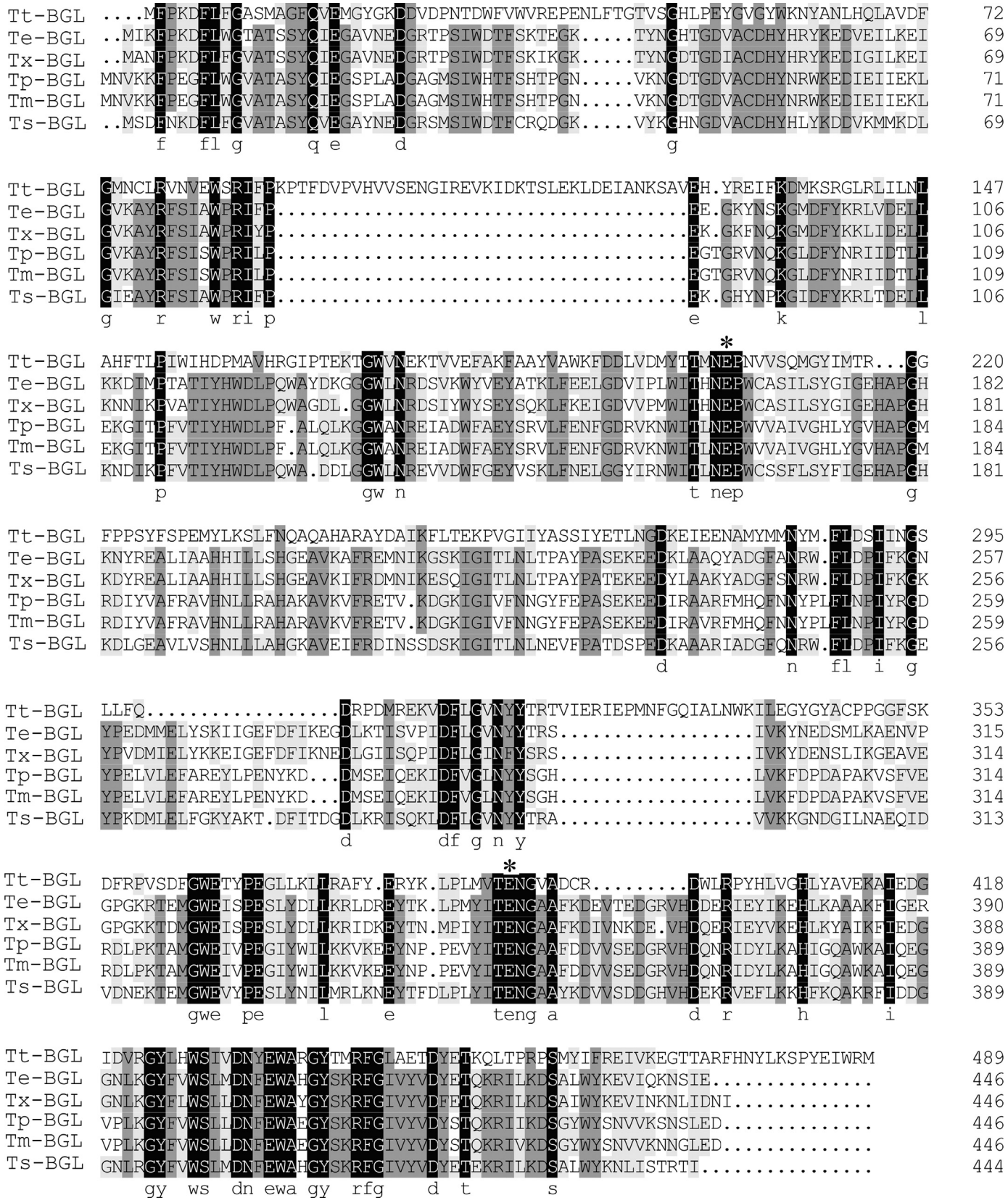

Fig. 2. Multiallignment of Tt-BGL with some GH1 family members. Sequence alignment was performed by using Clustal X2.0. The active sites are indicated as * on the top of the alignment. Tt-BGL: *T. thermarum* DSM 5069T (YP_004660190.1), Te-BGL: *Thermoanaerobacter ethanolicus* JW200 (ZP_08211795.1), Tx-BGL: *Thermoanaerobacterium xylanolyticum* LX-11 (YP_004471891.1), Tp-BGL: *T. petrophila* RKU-1 (YP_001244546.1), Tm-BGL: *T. maritima* MSB8 (AEP25088.1), Ts-BGL: *Thermoanaerobacterium thermosaccharolyticum* DSM 571 (YP_003852393.1).

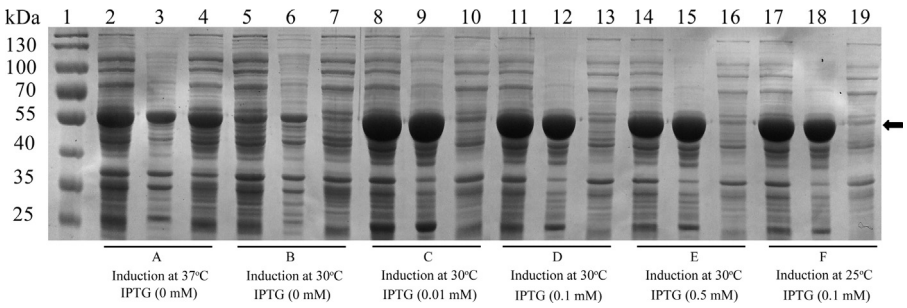

Fig. 3. SDS-PAGE analysis of the soluble Tt-BGL. Lane 1: protein marker, lane 2, 5, 8, 11, 14, 17: the total protein of JM109(DE3) harboring pET-28-BGL; lane 3, 6, 9, 12, 15, 18: the insoluble protein of JM109(DE3) harboring pET-28-BGL; lane 4, 7, 10, 13, 16, 19: the soluble protein of JM109(DE3) harboring pET-28-BGL. Closed arrow indicates recombinant Tt-BGL.

and 2.88 mM) under standard reaction conditions. The K_i value of glucose was defined as amount of glucose required for inhibiting 50% of the β-glucosidase activity and was given as the averages of three separate experiments performed in duplicate.

2.6. Analysis of ginsenoside Rb1 degradation

The ginsenoside Rb1 was treated with purified Tt-BGL, and the degradation was subjected to analysis of HPLC. The reaction mixture (100 μL) contained 50 mM imidole-potassium buffer (pH 4.8), 36 g/L ginsenoside Rb1, and 1.2 U/mL Tt-BGL. The reaction performed for various times at 90 °C, and stopped by the ice bath. The reaction was determined by HPLC at various times.

2.7. HPLC analysis

HPLC analysis of ginsenosides was performed using an HPLC 1200 system (Agilent, USA) and a C18 (150 mm × 4.6 mm; i.d., 5 μm) column with acetonitrile (*A*) and distilled water (*B*) at *A*/*B* ratios of 32:68, and 50:50 and run times of 0–13, and 13–14 min, respectively. The flow rate was 1 mL/min, and detection was performed by monitoring absorbance at 203 nm.

2.8. Sequence analysis of Tt-BGL

The condon usage preference of *E. coli* in translation initiation region of pET-20-BGL was analyzed by using codon usage tool (http://gcua.schoedl.de/). The potential ORF of *Tt-bgl* was searched using the ORF search tool provided by the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov). Database searching was performed with Blast at NCBI and against CAZy (www.cazy.org). The multiple sequence alignment tool Clustal X2.0 was used for multiple protein sequence alignment [21].

3. Results and discussion

3.1. Cloning and sequence analysis of Tt-bgl

By analysis of the genome sequence of the *T. thermarum* DSM 5069T, a protein (Theth_1013), defined as β-glucosidase in GenBank, consists of 1.470-bp fragment encoding 490 amino acids, which belonged to family 1 of the glycoside hydrolases. It shares the highest sequence similarity with that of the β-glucosidase from *T. lettingae* TMOT (72% identity, GenBank no. ABV33892.1), and the β-glucosidase of *Fervidobacterium pennivorans* DSM 9078T (56% identity, GenBank no. AFG35894), which were revealed by whole-genome sequencing, but has not been biochemically characterized. Alignment of the Tt-BGL cluster with several thermophilic bacteria members of GH1 indicated that they share some conserved peptide motifs Asn-Glu-Pro (residues 204–206) and Thr-Glu-Asn-Gly (residues 388–391), in which the Glu (E205, E389) residues are typical catalytic residues of the GH1 enzyme (Fig. 2). These results show that Tt-BGL is a bacteria member of the GH1 family, but homology analysis between the Tt-BGL and β-glucosidases from other thermophiles showed that Tt-BGL was distant with others (Fig. 2).

In order to increase the expression level of Tt-BGL in *E. coli*, we designed and performed to optimize codons of Tt-BGL for *E. coli* expression system. The DNA fragment of *Tt-bgl* gene was amplified from genomic DNA of *T. thermarum* DSM 5069T with site-directed primers (bgl-1 and bgl-2) in which rare condons for the N, C-terminal amino acid residues were replaced by optimal codons in *E. coli* without any change of amino acid sequence. Then the *Tt-bgl* gene was inserted into pET-28a at *Nco* I and *Xho* I to generate plasmid pET-28-BGL.

3.2. Overexpression of Tt-BGL

The recombinant Tt-BGL was overexpressed by adding 0.5 mM IPTG at 30 °C for about 6 h. But the overexpression of the Tt-BGL resulted in the production of large amount of inclusion bodies with a weak β-glucosidase activity of about 0.02 U/mL (Fig. 3, lanes 14–16). It is a common problem for recombinant protein aggregates to form inclusion bodies when some genes from thermophilic bacterial and fungal were over-expression in *E. coli* [22]. This complex phenomenon has many contributing factors: insolubility of the product at the concentrations being produced, inability to fold correctly in the bacterial environment, or lack of appropriate bacterial chaperone proteins [23]. To reduce inclusion body formation of Tt-BGL, we expressed the gene using different strategies. These included inducing at 25 °C and adding different concentrations of IPTG. Only small proportion of Tt-BGL was soluble in the cell-free extracts and most of the enzyme was in inclusion bodies under the induction at 25 °C with IPTG to final concentrations from 0.01 mM to 0.5 mM (Fig. 3). However, expression at 37 °C with no IPTG decreased the inclusion body formation. About 50% of the Tt-BGL (13 U/mL) was found in soluble fraction by leaky expression. Interestingly, only 0.5 U/mL β-glucosidase activity was detected by inducing at 30 °C with no IPTG (Fig. 3). These results provided a potential method for producing low-cost thermostable β-glucosidase because the enzyme was expressed without adding the expensive IPTG.

3.3. Purification and characterization of recombinant Tt-BGL

The protein in the cell-free extract was purified to gel electro homogeneity after a heat treatment and a Ni–NTA affinity. The final preparation gave a single band on SDS-PAGE gel and the molecular mass of the enzyme was estimated to be 55 kDa (Fig. 4, lane 4).

The biochemical properties of Tt-BGL were investigated by using the purified recombinant Tt-BGL. The optimal pH of the Tt-BGL was

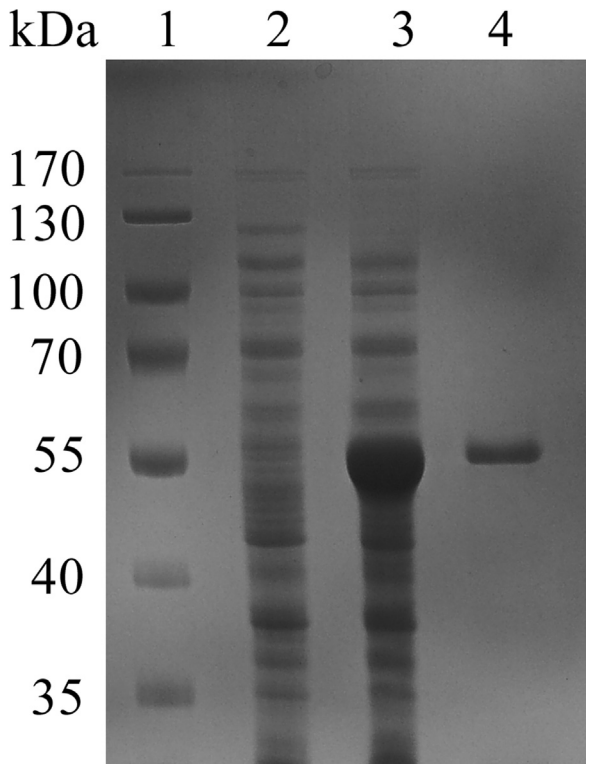

Fig. 4. SDS-PAGE analysis of recombinant BGL in *E. coli* JM109(DE3). Lane 1: protein marker, lane 2: the total protein of JM109(DE3) harboring pET-28a, lane 3: the total protein of JM109(DE3) harboring pET-28-BGL, lane 4: purified Tt-BGL (2 μg).

Table 1
Effects of cations and reagents on purified Tt-BGL activity.

Cation of reagent[a]	Residual activity (%)
Control	100
Fe^{2+}	77
Fe^{3+}	102
Mg^{2+}	102
Zn^{2+}	82
Mn^{2+}	106
Ca^{2+}	104
K^{+}	101
Al^{3+}	126
Li^{+}	103
Cu^{2+}	21
Hg^{2+}	0
Co^{2+}	97
Ag^{2+}	19
EDTA (10 mM)	105

[a] Final concentration, 1 mM or as indicated. Values shown are the mean of duplicate experiments, and the variation about the mean was below 5%.

determined to be 4.8 (Fig. 5a), while the β-glucosidase activity was higher than 50% of the maximum activity at the pH range from 4.0 to 8.0. The enzyme was stable for about 1 h at pH 4.4–8.0 at 80 °C in the absence of the substrate (Fig. 5c). The optimal temperature of the enzyme was 90 °C, which the β-glucosidase activity was higher than 60% of the maximum activity at the temperature range from 75 to 100 °C (Fig. 5b). Thermostability assays indicated that its residual activity was more than 50% after being incubated at 90 °C for 2 h (Fig. 5d). In practical applications, the high thermostability of the enzyme is desired because the longer active life means the less consumption of the enzyme.

The effects of metal ions and some chemicals on the enzyme activity were shown in (Table 1). In various assays, the Tt-BGL activity was significantly enhanced by Al^{3+}, and completely inactivated

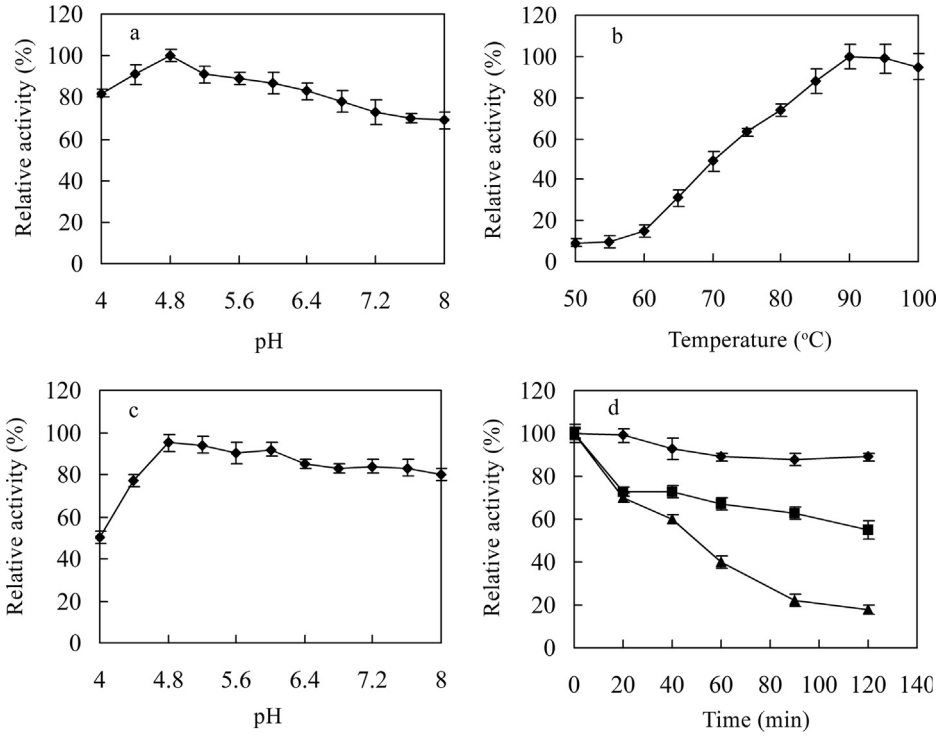

Fig. 5. The effects of pH and temperature on the activity and stability of the recombinant Tt-BGL. (a) Effect of pH on Tt-BGL activity; (b) effect of temperature on Tt-BGL activity; (c) the pH stability of the enzyme; (d) the thermostability of the enzyme. The residual activity was monitored, while the enzyme was incubated at 85 °C (filled diamonds), 90 °C (filled squares), and 95 °C (filled triangles). The initial activity was defined as 100%.

Table 2
Effects of ethanol and methanol on purified Tt-BGL activity.[a]

Final concentration of organic solvent (%)	Residual activity	
	Ethanol	Methanol
0	100	100
5	99	111
10	102	123
20	90	130
30	30	128

[a] Values shown are the mean of duplicate experiments, and the variation about the mean was below 5%.

Table 3
Substrate specificity of Tt-BGL.

Substrate	Enzyme activity (%)
p-Nitrophenyl-β-D-glucopyranoside (1 mM)	100
p-Nitrophenyl-β-D-galactopyranoside (1 mM)	70
p-Nitrophenyl-α-L-arabinofuranoside (1 mM)	0
p-Nitrophenyl-β-D-xylopyranoside (1 mM)	0
Ginsenoside Rb1 (1 mM)	92
Ginsenoside Rd (1 mM)	0
CMC (0.5%)	0
Gentiobiose (1 mM)	28
Cellobiose (1 mM)	<1
Sucrose (1 mM)	0

by Cu^{2+}, Ag^{2+}, or Hg^{2+}. The effects of Mg^{2+}, Ca^{2+}, K^{+}, Li^{+}, Co^{2+}, or EDTA (10 mM) on the enzyme activity were not so significant. The chelating agent EDTA displayed no influence on the β-glucosidase activity, indicating that the enzyme is not a metalloprotein. However, the β-glucosidase activity was greatly stimulated by Al^{3+}, which implied that Al^{3+} is required for the maximal activity of Tt-BGL. These results distinguished the enzyme from the other bacteria β-glucosidase [24].

The enzyme activity was unaffected by the concentration of alcohol below 20% and was significantly enhanced by the concentration of methanol not exceeding 30% (Table 2). The results showed the enzyme can be effectively applied in the organic biotransformation, because some ginsensides only dissolved in organic solvent.

3.4. Effect of glucose on Tt-BGL activity and substrate specificity

β-Glucosidases may be divided into three groups on the basis of their substrate specificity. The first group is known as aryl-β-glucosidases owing to strong affinity to aryl-β-glucose. The second group consists of cellobiases that hydrolyze oligosaccharides only. The third group is broad specific β-glucosidases that exhibit activity on a wide range of substrates, and are the most commonly observed form of β-glucosidases [25]. The enzyme was able to hydrolyze *p*NPGlu, cellobiose, gentiobiose, ginsenoside Rb1, and *p*NPGal, while no activity was detected upon *p*NPAra, *p*NPXyl, CMC, ginsenoside Rd, and sucrose (Table 3). These results indicated that Tt-BGL belonged to the third group.

The dependence of the rate of the enzymatic reaction on the substrates concentration followed Michaelis–Menten kinetics, with K_m and V_{max} values of 0.59 mM and 142 U/mg for *p*NPGlu, for *p*NPGal 3.54 mM and 250 U/mg, for cellobiose 35.5 mM and 19 U/mg, for ginsenoside Rb1 0.15 mM and 107 U/mg under optimal conditions. The V_{max}/K_m value of 713 U/mg mM for ginsenoside Rb1 was more than those for other substrates. The data indicates that Tt-BGL is not only specific for β-glucoside, but also especially fond of some aglycones. Moreover, β-glucosidases are frequently very sensitive to glucose inhibition and substrate inhibition in the enzymatic hydrolysis [24,26]. The enzyme activity was enhanced by the concentrations of glucose below 400 mM, and the enzyme activity was increased 136% when adding 200 mM glucose into reaction mixtures (Fig. 6a). When glucose was increased, the enzyme activity of Tt-BGL was gradually inhibited, with a K_i of 1500 mM glucose (Fig. 6a).

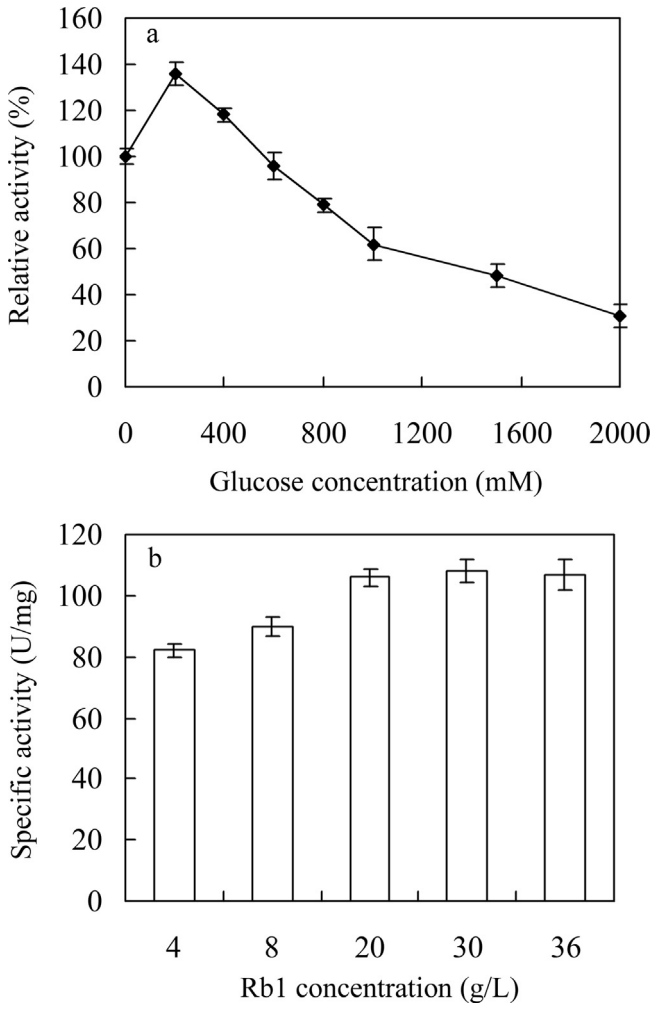

Fig. 6. The effects of glucose or ginsenoside Rb1 on Tt-BGL activity. (a) Influence of glucose on enzyme activity with *p*NPGlu as the substrate and (b) influence of ginsenoside Rb1 on enzyme activity.

3.5. Analysis of ginsenoside Rb1 degradation

To verify ginsenoside Rb1 biotransformation by Tt-BGL, ginsenoside Rb1 was incubated with Tt-BGL. As shown in Fig. 7, the reaction mixture (100 μL), containing 50 mM imidole-potassium buffer (pH 4.8), 36 g/L ginsenoside Rb1, and 1.2 U/mL Tt-BGL in 0.1 mL, was incubated for 60 min at 90 °C. Only one product was detected and identified as ginsenoside Rd by a yield of 95% (Fig. 7), while no relevant product was detected in negative control (data not shown). We also used ginsenoside Rd as a substrate to verify the specificity of Tt-BGL. As expected, the enzyme did not hydrolyze Rd after incubation at 90 °C for 1 h (Table 3), just like the phytopathogenic fungus *Cladosporium fulvum* [27]. But the catalytic efficiency of *C. fulvum* was much lower than that of Tt-BGL.

To verify the effect of ginsenoside Rb1 on Tt-BGL, different concentrations of ginsenoside Rb1 (from 4 g/L to 36 g/L) was incubated with Tt-BGL. The activity of Tt-BGL was not inhibited by ginsenoside Rb1 (36 g/L) (Fig. 6b). The K_{cat} was 101 s^{-1} at the concentration of ginsenoside Rb1 36 g/L, which was identical to the theoretical value (102 s^{-1}).

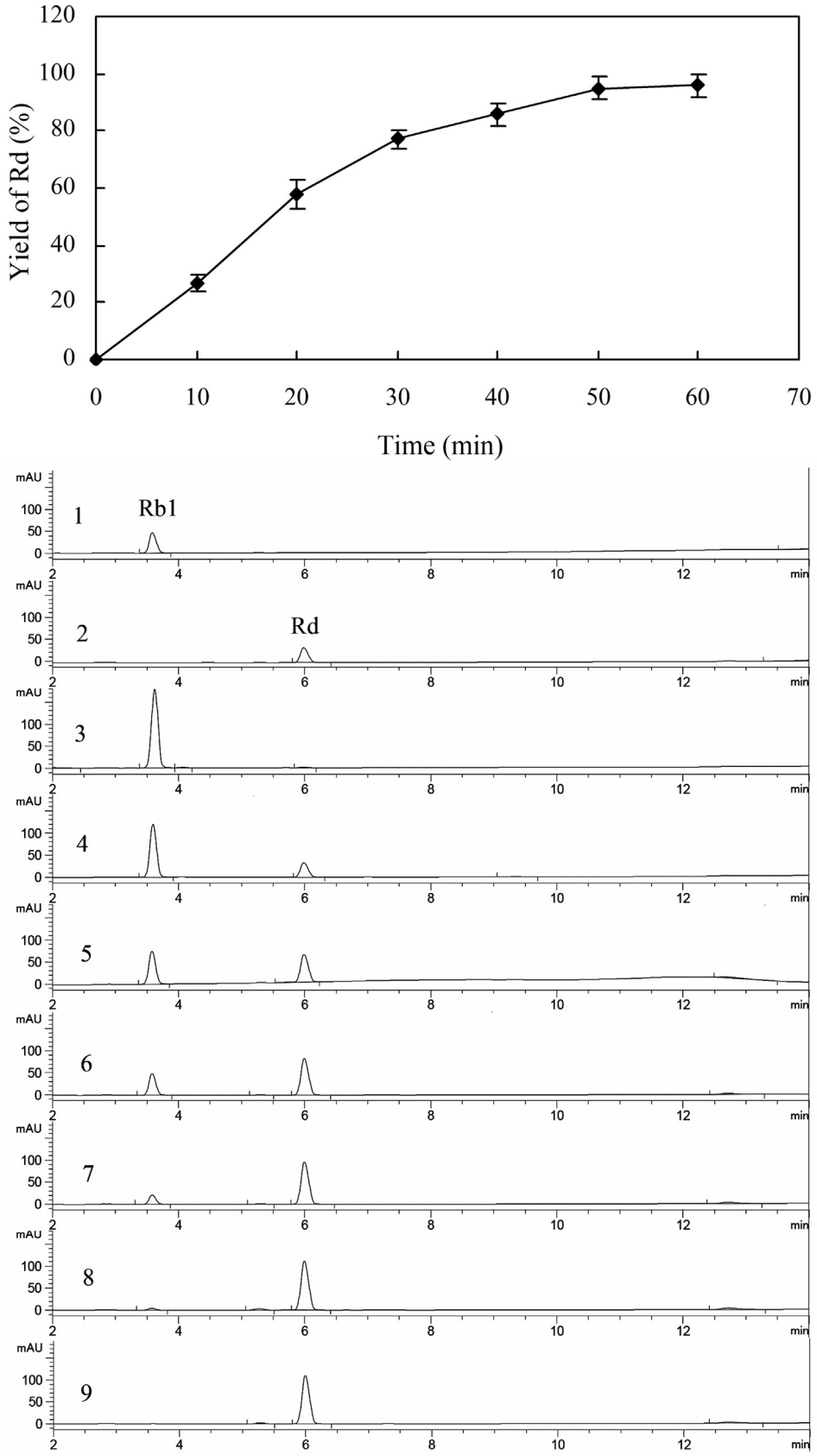

Fig. 7. HPLC analysis of ginsenoside Rb1 hydrolysis by Tt-BGL. 1: ginsenoside Rb1, 2: ginsenoside Rd, lane 3, 4, 5, 6, 7, 8, 9: ginsenoside Rb1 (36 g/L) incubated with Tt-BGL (1.2 U/mL) for 0, 10, 20, 30, 40, 50, and 60 min, respectively.

4. Conclusions

With this study, we successfully overexpressed and characterized the β-glucosidase gene *Tt-bgl* from *T. thermarum* DSM 5069T in *E. coli*. A simple procedure was explored to overcome inclusion-body formation observed. As compared on the enzyme properties, Tt-BGL was higher tolerant to glucose and ginsenoside Rb1, more efficient and selective in biotransformation ginsenoside Rb1 to Rd, and more thermal stability than β-glucosidases from other microorganisms. Thus, this study provides a useful β-glucosidase, which may be used to improve the enzymatic conversion of ginsenoside Rb1 to Rd.

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