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Original article

Heterologous expression and characterization of glycoside hydrolase with its potential applications in hyperthermic environment

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ABSTRACT

With the progressive focus on renewable energy via biofuels production from lignocellulosic biomass, cellulases are the key enzymes that play a fundamental role in this regard. This study aims to unravel the characteristics of *Thermotoga maritima* MSB8 (*Tma*) (a hyperthermophile from hot springs) thermostable glycoside hydrolase enzyme. Here, a glycoside hydrolase gene of *Thermotoga maritima* (*Tma*) was heterologously expressed and characterized. The gene was placed in the pQE-30 expression vector under the T5 promotor, and the construct pQE-30-*Gh* was then successfully integrated into *Escherichia coli* BL21 (DH5α) genome by transformation. Sequence of the glycoside hydrolase contained an open reading frame of 2.124 kbp, encoded a polypeptide of 721 amino acid residues. The molecular weight of the recombinant protein estimated was 79 kDa. The glycoside hydrolase was purified by Ni^{+2}-NTA affinity chromatography and its enzymatic activity was investigated. The recombinant enzyme is highly stable within an extreme pH range (2.0–7.0) and highly thermostable at 80 °C for 72 h indicating its viability in hyperthermic environment and acidic nature. Moreover, the Ca^{2+} and Mn^{2+} introduction stimulated the residual activity of recombinant enzyme. Conclusively, the thermostable glycoside hydrolase possesses potential to be exploited for industrial applications at hyperthermic environment.

1. Introduction

In the last couple of decades, due to global warming along with the rapid depletion of fossil fuel resources, the energy paradigm is shifting towards renewable form of energy (Souza, et al. 2015). A major concern in this regard is the production and utilization of environment friendly second-generation biofuel from agricultural waste (Bernardi et al., 2018). Lignocellulose biomass transformation into small fragments for biofuel production got special attention in this regard (Vianna Bernardi 2019). Cellulose, β-1,4-linked polymer of glucose subunits is the primary constituent of lignocellulose biomass and most plentiful polysaccharide on earth as part of the plant cell wall (Kuhad 2016; Ansari et al. 2012; Ansari et al. 2013). Its microbial degradation revealed of enormous economic potential especially for the biofuel production such as ethanol along with other high-value by-products (Su 2016).

Cellulases are a group of enzymes that induces the depolymerization of cellulose in a synergistic mode of action. This mode of action employs exoglucanase [EC 3.2.1.91], β-glucosidase [EC3.2.1.21] and glycoside hydrolase [EC 3.2.1.4] (Aich 2017; de Gouvea 2018; Rigoldi 2018). Glycoside hydrolase belongs to the

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glycoside hydrolase (GH) family of cellulases and initiate the cleavage of internal glycosidic bonds of cellulose randomly creating free ends. Exoglucanase and β-glucosidase hydrolysis leads to the production of cellobiose and glucose respectively (Berto et al., 2019; Seneesrisakul 2017). Lignocellulosic biomass conversion into reducing sugars normally entails the pretreatment method that improves the enzymatic activity on the available substrate (Christakopoulos 2013; Olga V. Moroz 2015).

In industrial applications of thermozymes, reactions at high temperature demands for thermostable enzymes potent at elevated temperature and resistant to chemical denaturation (Akram 2018; Dhawan 2016; Li 2016). Moreover, with broad range of pH stability, solvent concentration and resistant to product inhibition (Berto et al., 2019; Liu 2019; Liu 2015b; Seneesrisakul 2017; Vanessa O. A. Pellegrini 2015) and resistant to unwanted microbial contamination (Ebaid 2019). Furthermore, thermozymes exhibit low fluid viscidity with high grade of saccharification and production yield with reduced energy consumption for cooling process thereby enhancing the cost-effectiveness of bioprocess conversion (Arora 2015; Blumer-Schuette 2014).

Thermostable cellulases are secreted by several bacterial and fungal genera. A few fungal genera *Trichoderma, Rhizopus, Sclerotium, Aspergillus,* and *Sporotrichum thermophile* have been reported for thermophilic and mesophilic cellulases production (Barnard et al., 2010). Additionally, different bacterial genera including *Bacillus, Geobacillus, Caldocellum, Caldibacillus, Clostridium* and *Thermotoga* are also known to exhibits thermophilic cellulases (Bischoff 2007; Rastogi 2010; Shi 2013). In particular, *Thermotoga maritima* (*Tma*), has gained tremendous popularity as its members are able to produce industrially important thermozymes that can thrive at high temperature of ∼80 °C (Bhalla et al., 2013; Myung 2010). Some of their previously isolated and characterized enzymes are D-glyceraldehyde −3- phosphate dehydrogenase, 4-α-glucotransferase, lactase dehydrogenase, glucose isomerase and glycoside hydrolase (YI-MING TAO 2010).

Considering the diverse applications of cellulases in different industries, at the heterologous expression level, thermotolerant cellulases enzymatic activities need to be explored. Therefore, further investigation may unravel hidden potentials of thermostable enzyme for a number of industrial processes. This study was designed for the heterologous expression and characterization of glycoside hydrolase obtained from thermophilic *Thermotoga maritima* (*Tma*) MSB8 and attained in *Escherichia coli* using the pQE-30 vector. Further, the recombinant protein was purified followed by its characterization and evaluation at different temperature and pH conditions.

2. Materials and methods

2.1. Bacterial strains and reagents

The Isopropyl thiogalactoside (IPTG), 5-Bromo-4-chloro-indolyl-β-D galactopyranoside (X-Gal), Avicel, carboxy methyl cellulose (CMC) and antibiotic (ampicillin) were purchased from Sigma Aldrich (St. Louis, USA). The restriction and DNA modifying enzymes purchased from Fermentas (Lithuania USA). All other chemicals of analytical grade were being used in this study. The *Thermotoga maritima* (*Tma*) MSB8 strain was purchased from the American Type Culture Collection (ATCC, Parkville – USA), and was cultured in Thermotoga basal medium (TMB) with 0.5% glucose added (Jiang 2006). The host *E. coli* DH5α competent cells were obtained from Promega Corporation (Madison, WI, United States), whereas the pQE-30 vector was bought from QIAGEN products by Edificio Alba 28,290 Las Matas (Spain).

2.2. Cloning and recombinants identification

The gene TM0305 (accession no. MZ229329 as Submitted to NCBI) was amplified by using the *Thermotoga maritima* MSB8 genomic DNA as a template with Econo Taq PCR Master Mix using TM*Gh* forward primer F 5′ TGGAGCAACT**GCATGC**-ATGTGGAAATCGGTGG-3′ and the reverse primer R 5′-ACGGCGTT**AAGCTT**CAATCATTCCTCCTTC-3′ with *Sph*1 and *Hind*III restriction sites in Thermal cycler (BIO-RAD, T100), using the following program: an initial denaturation for 2 min at 94 °C followed by 35 cycles of incubation for 1 min at 94 °C, annealing at 58 °C for 1 min, and extension for 3 min at 72 °C repetition for 35 cycles, proceeds to final 10 min extension at 72 °C. The PCR product was then digested with *Sph*1and *Hind*III and purified by gel electrophoresis using Axygen AxyPrep DNA.

Gel Recovery Kit (Corning, NY, United States) according to the manufacturer's instructions, and then ligated with pQE-30 (3461 bp) expression vector to produce the recombinant plasmid using Takara Bio DNA Ligation Kit (Kyoto, Japan). Subsequently, the resultant pQE-30-*Gh* construct was transformed into *E. coli* (DH5α) by electroporation and spread on Luria–Bertani (LB) agar plates for selection, supplemented with 100 μg/mL ampicillin followed by incubation at 30 °C for overnight. The positive clones were screened and confirmed by colony PCR and plasmid DNA (Miniprep kit, Invitrogen) digestion with *Sph*1and *Hind*III restriction enzymes.

2.3. Expression and protein purification

The transformed cells were cultured in LB media (200 ml) supplemented with ampicillin of 100 mg/mL and placed at 30 °C in a shaker incubator at 200 rpm until reach an OD_{600nm} of 0.8. The enzyme expression was being induced by 1 mM IPTG (Isopropyl β-D-Thiogalactoside) at optical density (OD_{600nm}) of 0.8 as calculated and continued incubation for 8 h. The culture was harvested by centrifugation at 11,000*g* for 10 min at 4 °C and the enzyme supernatant was concentrated to 10-fold by ultra-filtration (10 kDa MW membrane cut-off Amicon, Beverly, MA, USA). The cell pellet was resuspended in the binding buffer (5 mM imidazole, 0.5 M NaCl and 20 mM Tris) at pH 8.0.

The cells were disrupted by sonication for 30 min (8 s on and 6 s off). The cytoplas mic extract was proceeded for heat treatment for 20 min at 70 °C. The crude enzyme was retrieved from pellet by centrifugation at 10,000*g* for 30 min at 4 °C in order to remove the cell debris and denatured proteins. The crude enzyme was retrieved from pellet by the removal of cell debris via centrifugation. The supernatant was loaded onto the immobilized Ni^{+2}-NTA column (Invitrogen Corp., Carlsbad, USA) equilibrated with the binding buffer, followed by elution suing elution buffer (500 mM imidazole, 0.5 M NaCl, and 20 mM Tris–HCl) at pH 8.0. The purity of recombinant glycoside hydrolase was evaluated by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) (12%) and dialyzed against phthalateimidazole (PI) buffer at pH 6.8 for salt removal and subsequent analysis. The theoretical molecular weight of glycoside hydrolase was calculated using the online tool available at website (http://pir.georgetown.edu/cgi-bin/comp_mw.pl). The purified enzyme was preserved in the stock solution (PI buffer of 25 mM at pH 6.8, 20% glycerol, and 0.02% sodium azide). The purified enzyme protein concentration was determined by spectrophotometer at the absorbance at 280 nm.

2.4. Enzyme activity and substrate specificity

A 2 μL of inoculum specked on LB-ampicillin agar plate with 1% (w/v) carboxymethyl cellulose (CMC) and 1 mM IPTG incubated at

37 °C for 24 h. Congo red reagent 1% (w/v) staining for 10 min followed by de-staining with 1 M sodium chloride solution. The calculated clear zone diameter divided by the diameter of colony attributes the hydrolytic capacity value of the recombinant enzyme.

The glycoside hydrolase activity was determined by DNS (3, 5-Dinitrosalicylic acid) method for reducing sugars measurement on substrate. Glycoside hydrolase activity was evaluated by mixing 1 ml enzyme with 1 ml of substrate solubilised in 50 mM sodium phosphate buffer (pH 7.0) incubated at temperatures ranging from 50 °C for 1 h. The reaction was subsequently stopped by the addition of an equal volume of DNS reagent followed by heating at 95 °C for 5 min and let it cooled on ice to room temperature. Hydrolysis of the soluble substrate into reducing sugars was determined by measuring the absorbance at 540 nm. Enzyme activity referred to as one unit (U) as the amount of enzyme required to produce 1 μ mol of reducing sugar per minute (Ghose, 1987). All the assays were performed in triplicate, and data were shown as the mean ± standard deviation.

Enzymatic activities of the recombinant TM*Gh* against different substrates was analyzed by incubating enzyme for 30 min at 70 °C with CMC-Na, avicel, Barely β-Glucan, xylan, lichenan, chitin and filter paper in sodium phosphate buffer (pH 6.0). The reaction contains 500 μL of enzyme with equal volume of phosphate buffer containing respective substrate. The quantity of reducing sugars released was determined using the dinitrosalicylic acid (DNS) reagent.

2.5. Biochemical characterization of recombinant TMGh

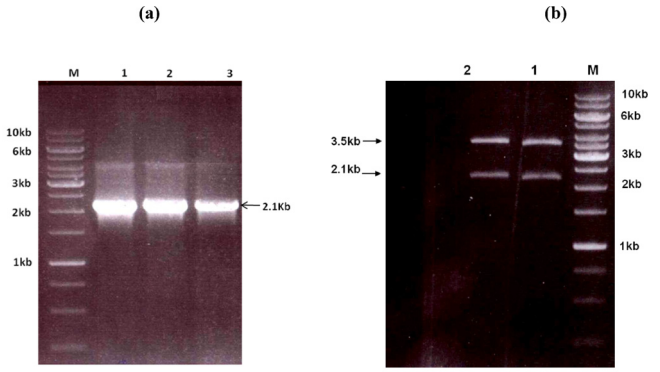
The enzymatic characteristics of the enzyme TM*Gh* were evaluated. The optimum temperature was determined at different temperatures from 40 to 90 °C by following the assay condition as mentioned earlier in 50 mM sodium phosphate buffer pH 7.0. Thermostability of the enzyme was analyzed by incubating enzyme at temperature of 60, 70, 80 and 90 °C in the absence of substrate in sodium phosphate buffer (pH 7.0) for up to 4 h and the reducing activity was calculated by following the assay as described prior. The optimum pH for the purified enzyme activity was observed over the pH range of 2 to 8 using citrate–phosphate buffer at the optimal temperature. The enzyme pH stability was evaluated by its residual enzymatic activity determined at optimum conditions by the enzyme incubation in citrate–phosphate buffer for pH 3–6, whereas pH of 7–8 was obtained by using Tris–HCl buffer at the optimal temperature for 1 h in the absence of substrate.

Additionally, the influence of different metal ions i.e. Mg^{2+}, Ca^{2+}, Fe^{3+}, Cu^{2+}, Al^{3+}, Mn^{2+} and DTT on the recombinant TM*Gh* activity was examined by adding 1 mM of concentration of each ion in the reaction mixture. The enzymatic activity was evaluated at the optimal temperature and pH conditions and noted as the percent activity of enzyme observed with the respective no metal ion added reaction mixture. The metal ion with inducing effect on activity and its required optimal concentration in order to increase the enzymatic activity was determined. The enzyme activity was obtained at the metal ion increasing concentration level (0.0–2.5 mM) under optimal conditions.

3. Results

3.1. Cloning of TMGh gene

The polymerase chain reaction amplification of glycoside hydrolase gene from *Thermotoga maritima* (*Tma*) MSB8, American Type Culture Collection (ATCC) genomic DNA resulted in a 2.124-kbp single fragment (Fig. 1a). The PCR product was ligated with the pQE-30 (Qaigen, 3.4kbp) expression vector and the construct pQE-30-*Gh* was transformed into *E. coli* BL21 (DH5α). The expression was induced with IPTG supplementation. After incubation on LB-ampicillin agar media at 37 °C for one day the positive transformants confirmed by colony PCR and double digestion with *Sph*1 and *Hind*III released the gene fragment of 2.1kbp (Fig. 1b).



of pQE-30-TM*Gh* construct with *Sph*1 and *Hind*III, *Lane M* DNA Ladder, *Lane 1, 2* restriction products.

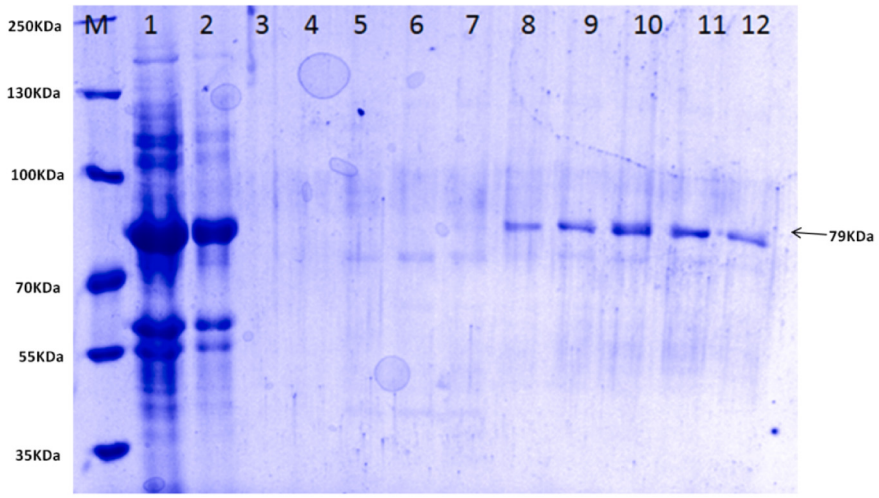
Fig. 1. Agarose gel electrophoresis map **(a)** *Lane M* DNA ladder, *Lane 1, 2,3* PCR products. **(b)** Double digestion of pQE-30-TM*Gh* construct with *Sph*1 and *Hind*III, *Lane M* DNA Ladder, *Lane 1, 2* restriction products.

3.2. Expression and purification of TMGh

The recombinant pQE-30-*Gh* was expressed in *E. coli* in LB-ampicillin with IPTG induction for expression. The soluble fraction of cells debris after sonication was proceeded for purification via heat treatment and affinity chromatography. The enzyme was purified to near homogeneity as shown in SDS-PAGE analysis. The supernatant and pellet samples were detected on 12% SDS-PAGE gel stained with Coomassie Brilliant Blue R-250 (Bio-Rad). Further purification with Ni^{+2}-NTA column affinity chromatography the corresponding recombinant enzyme molecular mass was observed at 79 kDa in (Lane 8–12) as same to the predicted (ExPasy online portal) molecular mass of 79 kDa. (Fig. 2). In the control (*E. coli* carrying empty vector) no significantly visible band was observed (Lane 3 and 4). The recombinant protein elution was maximum with 80 mM imidazole (Lane 8–12). The pellet and supernatant protein were observed in Lane 1 and 2. Furthermore, the extracellular secretion of the glycoside hydrolase was affirmed by Congo red assay. The recombinant *E. coli* harboring glycoside hydrolase plasmid observed a clear inhibition zone around on the added CMC under aerobic conditions, whereas no visible zone of inhibition was found in the control (Fig. 3).

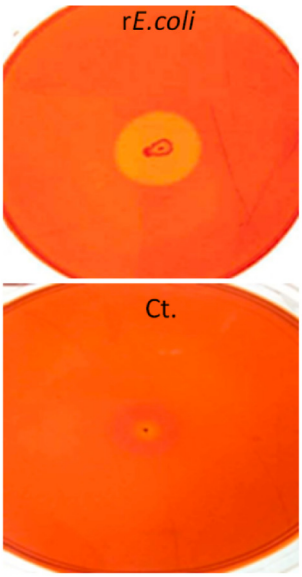
3.3. Enzymatic activity of recombinant TMGh at optimal temperature and pH

The recombinant TM*Gh* enzyme was analyzed at temperature ranging between 40 and 100 °C for the optimal temperature optimization (Fig. 4A). The result reflects that enzyme exhibits activity at a range of temperatures with maximum activity at 70 °C and partially reamained stable upto 80 °C and then drastically declines at 90 °C. The thermostability experiments revealed that recombinant TM*Gh* retained >50% of its activity after pre-incubation at 80 °C for 5 h without substrate and >45% of activity after 4 h pre-incubation at 90 °C (pH 6.8) (Fig. 4C). The optimal pH of purified TM*Gh* was determined by measuring the activity at a pH range of 2–8. The enzyme exhibited around 70% of its activity at a range of pH 5.0 to pH 7.0 and gained an optimum pH of 7.0. The maximum activity was detected at pH 7.0. (Fig. 4B).



5-9 fraction after 0, 5, 10, 20, 40 mM imidazole. *Lane 10-12* 80mM imidazole purification with Ni^{+2}-NTA column.

Fig. 2. SDS-PAGE gel electrophoresis, *Lane 1* pellete protein, *Lane 2* Supernatent protein, Lane 3, 4 control, *Lane 5–9* fraction after 0, 5, 10, 20, 40 mM imidazole. *Lane 10–12* 80 mM imidazole purification with Ni^{+2}-NTA column.



1mM of IPTG concentration. *E. coli* with empty vector was used as control (Ct) produced no clear zone. A significant yellow halo around functional recombinants was observed indicating the glycoside hydrolase activity.

Fig. 3. Recombinants of *TMGh* screening after 24 h incubation on 1 % (w/v) of CM-Cellulose plate induced with 1 mM of IPTG concentration. *E. coli* with empty vector was used as control (Ct) produced no clear zone. A significant yellow halo around functional recombinants was observed indicating the glycoside hydrolase activity.

3.4. Biochemical properties of glycoside hydrolase

The relative activity of the purified recombinant TM*Gh* were determined by using different substrates (Table 1). The glycoside hydrolase showed highest activity in producing reducing sugars with barely β-glucan and CMC, whereas, Chitin, Lichenan and Xylan appeared to be not suitable substrates for glycoside hydrolase. These finding reflects the glycoside hydrolase ability to hydro-

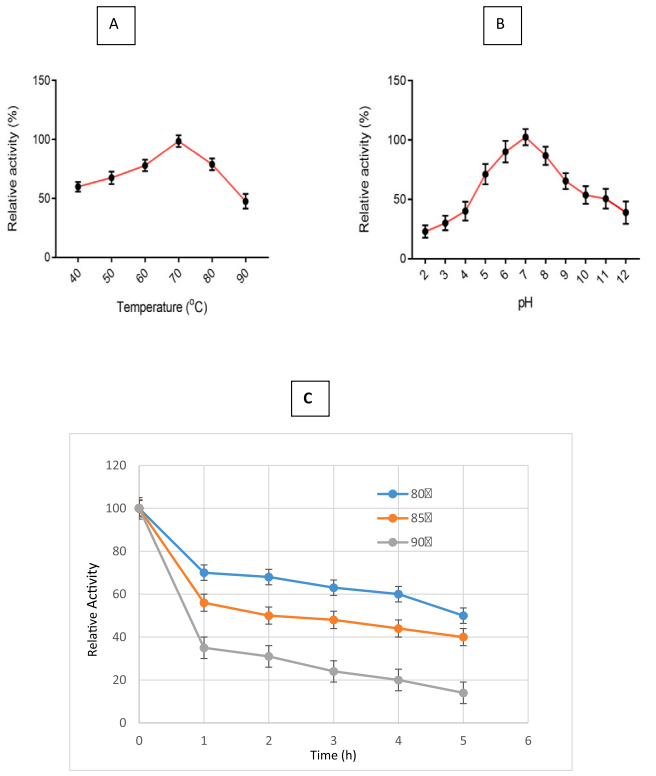


Fig. 4. Evaluation of the optimal temperature (A) and pH (B) for recombinant glycoside hydrolase (TM*Gh*). (C) Thermostability of TM*Gh*. The enzyme at concentration of (0.286 mg/mL) was incubated at 80, 85, and 90 °C for 5 h without substrate, and optimal conditions were used for the final residual activity.

Table 1
Relative activity of recombinant TM*Gh* on different substrates.

Substrate	Relative activity (U/mg)
CMC	261
Avicel	12.6
Barely β-Glucan	58
filter paper	52
xylan	ND
lichenan	ND
Chitin	ND

Reactions were conducted at pH 6.0 and 70 °C, ND = not detected

lyze the amorphous cellulose substrate into reducing sugars while inactive against the crystalline form of cellulosic substrate.

The introduction of metal ions at concentration of 5 mM reveal differently each as shown in (Table 2). An inhibitory effect was employed by metallic ions (Fe^{3+}, Cu^{2+} and Al^{3+}) on recombinant TM*Gh* as reported for glycoside hydrolases in previous studies (Eckert et al., 2002). On the other hand, when Mn^{2+} and Ca^{2+} were employed, an increase of 147% and 132% in the enzymatic activity was observed respectively. These results proposed a strong stimulatory effect of Ca^{2+} and Mn^{2+}on the recombinant TM*Gh* activity. This finding can be deduced as the Mn^{2+} and Ca^{2+}-binding resulting in the enzymatic stability of glycoside hydrolase (Karnchanatat, et al. 2008). The divalent cations Mn^{2+} and Ca^{2+} found having stimulatory effect on various glycosyl hydrolases (Béra-Maillet et al., 2000). On the other hand, the trivalent cations Al^{3+} and Fe^{3+} found to have an inhibitory effect on TM*Gh* activity.

The K*m* and V*max* values for the recombinant enzyme were calculated at the optimum temperature and pH conditions with 1 mM of Mn^{2+} with the increasing concentration of the substrate, CMC. The K*m* and V*max* values were determined to be 5.1 mg/mL and 682 U/mg, respectively.

Table 2
Effects of metal ions (5 mM) on TM*Gh* activity.

Metal ions (5 mM)	Relative activity (%)
Mg^{2+}	103.8 ± 2.5
Ca^{2+}	132.3 ± 0.4
Fe^{3+}	61.4 ± 0.9
Cu^{2+}	47.3 ± 1.7
Control	100 ± 1
Al^{3+}	52.6 ± 8.1
Mn^{2+}	147.2 ± 4.0
DTT	111.2 ± 1.3

Results here as means ± SD (n = 3). Control was taken as 100 %.(without ions), ND = Not detected.

4. Discussions

With the extensive industrial applications in various fields, cellulases found a predominant place in textiles for bio stone washing, waste paper processing and as detergents (Bhat, 2000). Over the last couple of decade characterization of cellulases for biofuel production from lignocellulosic biomass got special attention (Kumar, et al. 2008). In this regard, research focuses on the production and evaluation of stable enzymes being compatible to high temperature and low pH conditions (Jørgensen 2003).

Molecular approaches for the stable and robust enzymes production in heterologous hosts has been developed (Percival Zhang 2006). The genome of *Tma* exhibits the maximum (7%) number of glycosyl hydrolases among all the thermophilic sequences reported till now. However, a number of these putative Lignocellulosic biomass degrading enzymes have not been further characterized potentially. In this study, we report that TM0305 gene from *Tma* encodes for a glycosyl hydrolase TM*Gh*, a cellulose degrading enzyme. In this work our results revealed that the recombinant enzyme from gene TM0305 has been found to exhibit its cellulase activity on various substrates including the Carboxymethyl cellulose, Avicel and other polysaccharides of β-1, 4-glycosidic bond linkages at high temperature (Table 3). These findings reflect a hyperthermophilic enzyme potentially applicable for the lignocellulosic biomass degradation (Table 1).

Thermophilic microorganisms exhibits thermostable enzymes at extreme temperature and pH conditions (Vieille 2001). Recombinant DNA technology as a tool has conveniently increased the heterologous expression of enzymes into easily cultivable and industrially suitable microorganisms (Formantici 2003). The heterologous expression of glycoside hydrolase from *Thermotoga maritima* (*Tma*) MSB8 (hyperthermophile) into *E. coli* was made possible through recombinant DNA technology.

The enzymatic activity of recombinant TM*Gh* at optimal temperature and pH reflects that enzyme is active at various temperatures with maximum activity at 70 °C closely similar to as reported previously from thermophilic *Geobacillus sp.* 70PC53 and thermoacidophile *Alicyclobacillus acidocaldarius* ATCC27009 (Eckert 2002; Ng 2009). The optimum temperature reported here is relatively lower than related glycosyl hydrolases as reported previously (Chhabra 2002; Sadaqat 2021). The enzyme partially reamained stable upto 70 °C and then activity declines as increase in temperature. In this study, recombinant thermostable enzyme optimal activity was significantly higher than glycoside hydrolase from *Bacillus subtilis* UMC7 and *Trichoderma virens* (Kelvin Swee ChuanWei 2015; Zeng 2016).

Table 3
A comparison of biochemical properties of TM*Gh* with some previously reported thermophilic and hyperthermophilic Glycosyl hydrolases.

Specimen/Gene	Optimum temperature (°C)	Optimum pH	Temperature and pH stability	Km value mg/ml	Specific activity (U/mg)	Molecular mass (kDa)	References
Thermotoga maritima MSB8	70	7	5 h 80 °C, 5–8	5.1	261	79	This study
Thermotoga maritima TM1752	85	5.5	5 h 85 °C, 5–8	4.5	416	38	(Sadaqat 2021)
Thermotoga neapolitana TN5068	91	7.1	NG	NG	1.23	55	(McCutchen 1996)
Thermotoga maritima TM1227	90	7	NG	NG	72	76.9	(Chhabra 2002)
Dictyoglomus turgiduma	70	5.4	2 h 70 °C, 5–9	4.70	216.96	40	(Fusco 2018)
Bacillus sp. N16-5	70	9.5	2 h 60 °C, 8.5–10	NG	NG	50	(Zheng 2016)
Phialophora sp. *P13*a	60	1.6	2 h 55 °C, 1.5–7	2.5	851	44.2	(Zhao 2010)
Bacillus subtilis YH12	55	6.5	NG	30	7302.4	40	(Liu 2015a)
Bacillus circulans CGMCC 1416a	58	7.6	1 h 50 °C, 7–9	NG	NG	31	(Li 2008)

The optimal pH of purified TM*Gh* was determined by measuring the activity at a pH range of 2.0–8.0 (Fig. 4). The highest activity was observed at pH 7.0. The optimum pH results find similarity to findings reported previously (Chhabra 2002; Eckert 2002; Ng 2009). The enzyme also exhibits activity at pH of 4.0 and 5.0 reflecting its marginally acidophilic nature (Fig. 4b). The introduction of metal ions at concentration of 5 mM reveals differently each as shown in (Table 2). An inhibitory effect was employed by metallic ions (Fe^{3+}, Cu^{2+} and Al^{3+}) on recombinant TM*Gh* as reported for glycoside hydrolases in previous studies (Eckert 2002). The glycoside hydrolase showed highest activity in producing reducing sugars with barely β-glucan and CMC, whereas, Chitin, Lichenan and Xylan appeared to be not suitable substrates for glycoside hydrolase. These finding reflects the glycoside hydrolase ability to hydrolyze the amorphous cellulose substrate into reducing sugars while inactive against the crystalline form of cellulosic substrate.

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In the current situation, a substantial search for the industrial processes at elevated temperature demands for potent hyperthermostable enzymes. A thorough investigation into the activity of enzymes at different elevated temperature and pH conditions revealed that recombinant TM*Gh* activity at optimum 70 °C and is being potentially active at 80 °C. TM*Gh* can probably find industrial application in the area where thermostable enzyme for longer duration is needed. It can play a key role in achieving the goal of biofuel by degrading cellulosic materials. However, the enzyme itself appeared to be a bit sensitive to acidic pH (below 4), losing most of its activity. This work can lead to further evaluation and enzymatic stability for industrial applications in future.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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