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Full Length Article

A Novel Application Potential of GH6 Cellobiohydrolase CtCel6 from Thermophilic *Chaetomium thermophilum* for Gene Cloning, Heterologous Expression and Biological Characterization

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Abstract

Chaetomium thermophilum is a thermophilic fungus expressed a series of glycoside hydrolases. Genome sequence analysis of *C. thermophilium* revealed that *ctcel6* gene encoded a putative cellobiohydrolase which composed of 397 amino acid residues including a predicted signal peptide sequence. Ctcel6 gene was cloned, heterologously expressed in *Pichia pastoris* and purified by Ni^{2+} affinity chromatography. Sequence alignment indicated that CtCel6 enzyme belonged to glycoside hydrolase family 6 (GH6) and the molecular mass of purified recombinant enzyme CtCel6 was 42 kDa by SDS-PAGE analysis. Characterization of recombinant CtCel6 exhibited high hydrolysis activity and excellent thermostability. The optimum reaction temperature and pH was 70ºC and pH 5, respectively. The bivalent metallic cations Mg^{2+} and Ca^{2+} significantly enhanced the activity of CtCel6. The specific activity of CtCel6 enzyme was 1.27 U/mg and K_m value was 0.38 mM on β-D-glucan. The substrate specificity and hydrolysis products insisted that CtCel6 was an exo-/endo-type cellobiohydrolase. The biochemical properties of recombinant CtCel6 made it potentially effective for bioconversion of biomass and had tremendous potential in industrial applications such as enzyme preparation industry and feed processing industry. © 2017 Friends Science Publishers

Keyword: Glycoside hydrolase family 6; Cellobiohydrolase; Heterologous expression; Thermostable enzyme; Bioconversion

Introduction

Lignocellulosic biomass, the most abundantly renewable and available carbohydrate resource on the earth, has been regarded as an available feedstock for biochemical and biotechnological applications to produce biofuels and chemicals (Margeot *et al.*, 2009). Since the global energy crisis and environmental pollution are intensifying, it has attracted extensive attention to the utilization of lignocellulosic biomass. Cellulose is the major composition of lignocellulosic biomass and the bioconversion of cellulose needs a combined effect of three classes of hydrolytic enzymes, including endoglucanases (EC 3.2.1.4), cellobiohyrolases (EC 3.2.1.176; EC 3.2.1.91) and β-glucosidases (EC 3.2.1.21) (Lynd *et al.*, 2002; Sánchez, 2009). Cellobiohyrolases are a group of glycoside hydrolases, which hydrolyzed oligosaccharides of assorted lengths generated by endoglucanases to cellobiose (Ragauskas *et al.*, 2006). According to the classification of the carbohydrate-active enzymes database (CAZy), cellobiohyrolases are assigned to 5 glycoside hydrolase families: GH5-7, GH9 and GH48 (Cantarel *et al.*, 2009). In particular, the cellobiohydrolases of GH6 are widely believed to act processively from the non-reducing terminal of cellulose chains to generate cellobiose. GH6 family members are mainly produced by bacterial and fungal sources, and the hydrolysis mechanism is inverting. GH6 family includes both endoglucanases and cellobiohydrolases, many GH6 endoglucanases have been reported, such as *Thermobifida fusca* Cel6A (Ali *et al.*, 2015), *Thermobifida halotolerans* GH6 endoglucanase (Yin *et al.*, 2015), and *Cellulosimicrobium funkei* CelL (Kim *et al.*, 2016). However, the study of GH6 cellobiohydrolases has been reported rarely.

Chaetomium thermophilum is a thermophilic fungus living in the high temperature environment up to 60ºC belonged to the phylum Ascomycota. By now, many glycoside hydrolases have been isolated from *C. thermophilum*, such as a GH55 β-1,3-glucanase (Papageorgiou and Li, 2015), a β-glucosidase (Xu *et al.*, 2011) and a cellobiohydrolase II (Wang *et al.*, 2013). Generally, *C. thermophilum* glycoside hydrolases are thermostable and have a high optimal reaction temperature based on the previous researches. Thermostable enzymes have potential advantages in lignocelluloses conversation, on account of effectively improving hydrolysis efficiency and reducing the possible contamination at high temperature in industrial processes (Huy *et al.*, 2016).

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Currently, many microorganism-derived enzymes are produced by fermentation processes in vitro (Villatte *et al.*, 2001). *Pichia pastoris*, as a convenient production system of yeast that could heterologously express proteins in high amounts, has been wildly used as a heterogeneous expression system (Li *et al.*, 2014; Zhao *et al.*, 2015). To satisfy the strong demand for the thermostable hydrolase production in enzyme preparation industry, heterologous expression using *P. pastoris* has become the primary way which significantly improves the protein expression level (Formighieri and Melis, 2016). However, as a significant part of glycoside hydrolases of thermophilic *C. thermophilum*, there are few reports about the thermostable GH6 cellobiohydrolases from *C. thermophilum*.

In this study, a novel GH6 cellobiohydrolase gene *ctcel6* was first cloned from *C. thermophilum*. The gene *ctcel6* was heterologously expressed in *P. pastoris* system, and the corresponding recombinant enzyme CtCel6 was purified and characterized. The thermostable of CtCel6 was investigated. Moreover, we found it could effectively degrade crystalline cellulose and soluble cellulose at high temperature and the hydrolysis products were mainly cellobiose. The effect of cations and the substrate specific activity of recombinant CtCel6 were also determined. These biochemistry characterizations suggested that recombinant CtCel6 had a great prospect of commercial application in bioconversion of lignocellulosic biomass.

Materials and Methods

Materials

Chaetomium thermophilum HSAUP072651 was isolated from bovine feces at Tengchong (Yun'nan, China). The strain was deposited in China General Microbiological Culture Collection Center (CGMCC; Beijing, China) with accession No. 3.17990. *Escherichia coli* T1 (TransGen, China) was used for nucleotide sequenced. *Pichia pastoris* strain GS115 (Invitrogen, USA) was used as heterologous expression system. The pMD18-T vector (Takara, China) and the pPIC9K vector (Invitrogen, USA) were used for clone system and expression system, respectively. Primers were synthesized by Sangon Bitech Co. Ltd. (Shanghai, China). RNA extraction kit and reverse transcription kit were purchased from TransGen Company. Sodium carboxymethyl cellulose (CMC-Na), β-D-glucan, pectin, chitin, xylan, amylose and saccharose were purchased from Sigma-Aldrich. Phosphoric acid swollen cellulose (PASC) was prepared from Avicel according to the method of Wood (1988). Cello-oligosaccharides (Megazyme, Wicklow, Ireland) were used for the hydrolysis products analysis.

Gene Cloning and Sequence Analysis

After cultured three days with cellulose induced at 50°C, total RNA of *C. thermophilum* was isolated from the mycelia as described in the manufacturer's instructions (TransGen, China). Based on the RNA template, reverse transcription was carried out using reverse transcription kit (TransGen, China). The *ctcel6* gene was identified from the preview research of genome of *C. thermophilum* (http://ct.bork.embl.de/) (Amlacher *et al.,* 2011). The gene sequence was amplified using the primers as follows: 5'-CCTACGTAGCCCCCAACCCAACCCA-3', and 5'-GGCCTAGGTTAGTGGTG GTGGTGGTGGTGGAACGGAGGGTTGGCA-3'. The designed primers contained a C-terminal 6x His-tagged sequence for the purification of expressed product and the compatible restriction sites of *Sna*BI and *Avr*Π, respectively. PCR reaction program was as follows: 94°C, 5 min; (94°C, 30 s; 53°C, 30 s; 72°C, 60 s) □ 30 cycles; 72°C, 10 min. The amplified product was connected to pMD18-T vector, and the recombinant plasmid was transformed to *E. coli* T1. The screened positive transformants were gene sequenced to ensure that the cloned gene was correct.

The signal peptide sequence of CtCel6 protein was predicted using the online software SignalP 4.1 (http://www.cbs.dtu.dk/services/SignalP/). The characteristic analysis of CtCel6 amino acid sequence was carried out using the online ProtParam (http://web.expasy.org/protparam/). Multiple sequences alignment was based on ClustalW2 (http://www.simgene.com/ClustalW). Glycosylation sites analysis was performed using NetNGlyc 1.0 (http://www.cbs.dtu.dk/services/NetNGlyc/) for N-linked glycosylation sites and NetOGlyc 4.0 (http://www.cbs.dtu.dk/services/NetOGlyc/) for O-linked glycosylation sites, respectively.

Construction of Expression System

The cloned gene *ctcel6* was digested with *Sna*BI and *Avr*Π, as well as pPIC9K vector. Then, the digested *ctcel6* was ligated to pPIC9K vector to produce the secretion expression plasmid pPIC9K/*ctcel6*, which was confirmed by DNA sequencing and restriction analysis. The recombinant plasmid pPIC9K/*ctcel6* was preserved and prepared for the next step.

Transformation and Expression of the Recombinant Enzyme in *Pichia pastoris*

The recombinant expression plasmid pPIC9K/*ctcel6* was linearized using the restriction enzyme *Sac*I. After digestion process, pPIC9K/*ctcel6* transformed to *P. pastoris* GS115. Transformants were screened on MD and MM plates at 28°C to streak single colony. The multi-copy transformants were picked up from YPD plates with different concentrations of G418 containing 1, 2, 3 or 4 mg/mL. After incubation 12 h in YPD liquid medium, genomic DNA extraction was carried out from the selected transformants. A pair of AOX1 sequencing primers (5'-

GACTGGTTCCAATTGACAAGC-3' and 5'-GCAAATGGCATTCTGACATCC-3') was used for PCR amplification with the extracted genomic DNA. The heterogeneous expression program of recombinant CtCel6 was performed in *P. pastoris* as described by Li *et al*. (2009).

Purification and SDS-PAGE Analysis

The recombinant CtCel6 was purified from the fermentation liquor using Ni^{2+} affinity chromatography (HisTrap^{TM} FF crude, GE Healthcare). The molecular mass of purified recombinant CtCel6 was confirmed by 12% (w/v) SDS-PAGE. The spots of enzyme were stained using Coomassie brilliant blue R-250 and PierceR Glycoprotein Staining Kit (Thermo Scientific, USA), respectively.

Enzyme Assay

The CtCel6 hydrolysis activity was measured according to the amount of reducing sugar using Nelson-Somogyi method (Miller, 1959). The reaction mixture was composed of 100 μL 0.1% (w/v) β-D-glucan in 50 mM pH buffer and 100 μL diluted enzyme solution. After reaction incubated for 30 min, the hydrolysis reaction was terminated by adding 200 μL 3,5-dinitrosalicylic acid reagent at boiling water within 10 min. After cooled down to ambient temperature, the absorbance was measured at 540 nm. One unit (U) of enzyme activity was defined as the amount of enzyme that catalyzed the liberation of reducing sugar equivalent to 1 μmoL glucose per minute under assay condition.

Characterization of the Purified CtCel6

The optimal pH was determined in 50 mM different buffer solutions ranging from pH 3 to pH 9. The different pH buffer solutions were acetate buffer (pH 3–6), sodium phosphate (pH 6–8) and Tris-HCl (pH 8–9). The optimal temperature was evaluated at 40–90°C in 50 mM optimal pH buffer. Thermal stability was estimated by detecting the residual activity after the enzyme was incubated at 40, 50, 60, 70, 80 and 90°C for 1 h, respectively. The effect of different metal cations on CtCel6 activity was performed. Substrate specificity was investigated using β-D-glucan, chitin, pectin, saccharose, xylan, amylose, PASC, CMC-Na, filter paper and wheat straw. K_m was determined on β-D-glucan under the optimum condition and calculated by Michaelis-Menten equation. All assays were performed in triplicate.

The function mechanism of CtCel6 hydrolyzed PASC, CMC-Na and cellooligosaccharides was researched by analyzing the released products. The reaction system on PASC (or CMC-Na) contained of 0.5% (w/v) PASC (or CMC-Na) and 0.2 mg/mL CtCel6 in 200 μL of reaction mixture at 50°C for 3 h for complete reaction. To detect the hydrolytic products on oligosaccharides, the 200 μL reaction mixture containing 0.2 mg/mL purified CtCel6 and 0.5 mg/mL oligosaccharide was incubated at 50°C for 3 h for complete reaction. Hydrolytic products were detected using thin layer chromatography. The reaction products and the oligosaccharides mixture standard (Gentaur, Kampenhout, Belgium) solution were applied onto silica plate (Merck, Germany) and subsequently developed with the developing solvent as ethyl acetate-methanol-water-acetic acid (in the ratio of 4:2:1:0.5, v/v/v/v). After completely dried, the developed silica plate was visualized by dipping in a mixed solution containing 2% (w/v) N-phenylaniline, 2% (v/v) phenylamine and 85% (v/v) phosphoric acid in acetone, followed by heating at 85°C for 15 min.

Results

Sequence Characteristics of CtCel6

A putative cellobiohydrolase gene *ctcel6* (GenBank NO. XM_006694845.1) was isolated using RT-PCR. The gene sequence was 1194 bp and the open reading frame sequence encoded a mature polypeptide, which contained 397 amino acid residues. The calculated molecular mass of the mature polypeptide was 41.8 kDa and the pH value was 5.7 according to the ProtParam tool. Sequence similarity analysis indicated that CtCel6 belonged to the glycoside hydrolase family 6 compared with other published GH6 family enzymes (Fig. 1). There was a potential signal peptide contained 17 amino acid residues (MKLTTSTLLSLATAALA) in CtCel6 predicted by Signal P 4.1 Server, indicated that it was an exocrine enzyme. Glycosylation sites analysis showed that one N-linked glycosylation sites (N20) and eight O-linked glycosylation sites (T22, T24, T25, S33, S38, T41, T46 and S269) were found in the amino acid sequence according to NetNGlyc 1.0 Server and NetOGlyc 4.0 Server, respectively. It seemed that CtCel6 enzyme could be glycosylated.

Heterologous Expression and Purification

The expression process of *ctcel6* gene in *P. pastoris* was carried out as described in Pichia Expression Kit (Invitrogen, USA). Transformants were screened and selected the highest producer for further characterization after inducted with methanol. To obtain the purified CtCel6 protein, the screened transformant was cultured under the optimum shake-flask culture condition at 30°C for 7 days. The recombinant CtCel6 protein was produced with a C-terminal histidine tag and purified by Ni^{2+} affinity chromatography. SDS-PAGE analysis indicated that the molecular mass of CtCel6 was 42 kDa closed to the predicted value (Fig. 2). After SDS-PAGE, glycoprotein staining indicated that CtCel6 was a glycoprotein accorded exactly as the prediction (Fig. 2).

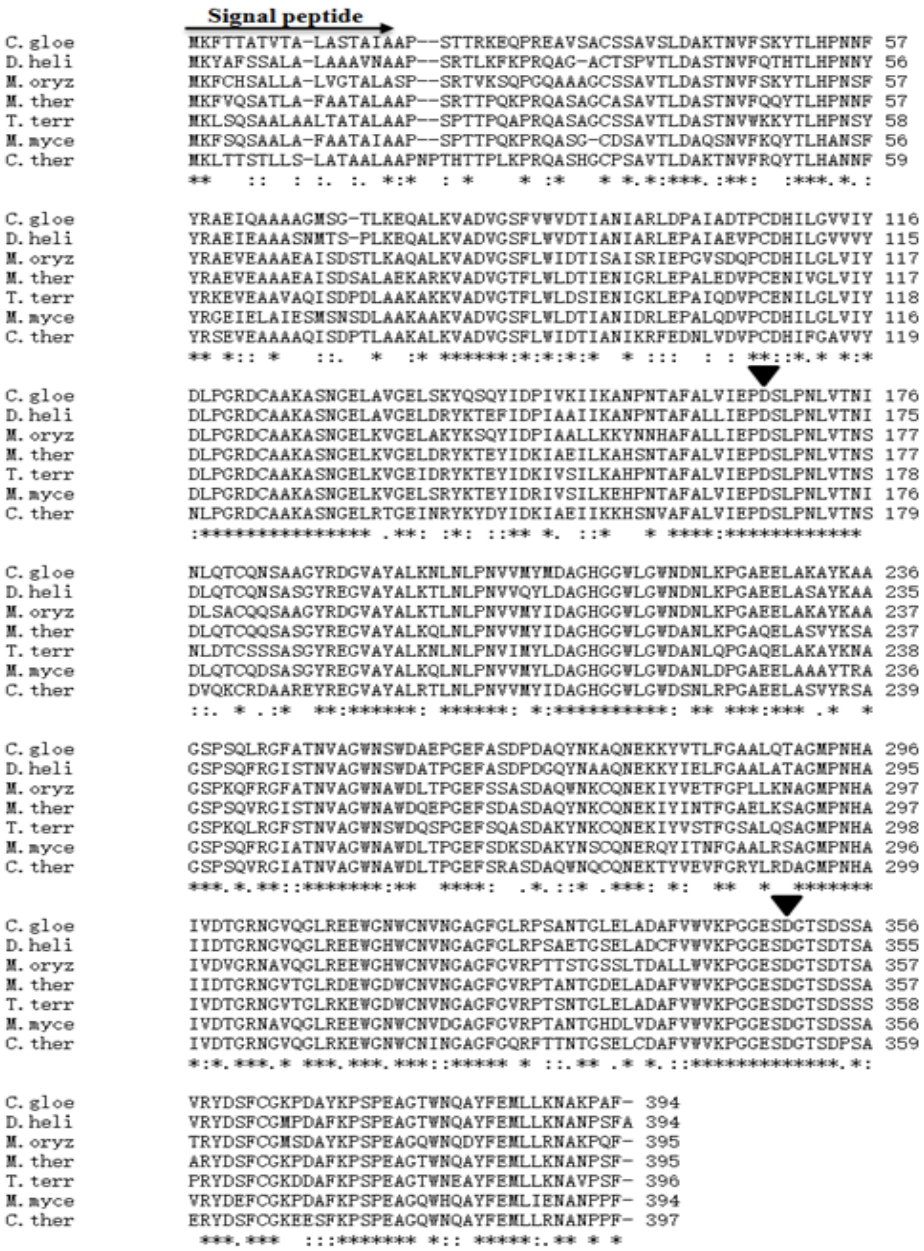

Fig. 1: The sequence alignment of *C. thermophilium* CtCel6 with other GH6 cellobiohydrolases from *Colletotrichum gloeosporioides* Cg-14 (EQB48544), *Diaporthe helianthi* (OCW35304), *Magnaporthe oryzae* 70-15 (XP_003710956), *Myceliophthora thermophile* ATCC 42464 (XP_003664525), *Thielavia terrestris* NRRL 8126 (XP_003650908) and *Madurella mycetomatis* (KXX74427) using ClustalW2. Asterisk indicates the positions which have a single, fully conserved residue. Colon indicates the strongly similar parts among homologous sequences and period means the weakly similar parts among homologous sequences. Two conserved aspartates are noted by closed inverted triangles as the catalytic residues. The potential signal peptide is signed with black arrow

Activity Assay and Characterization of CtCel6

To characterize the optimal activity condition of CtCel6 enzyme, the optimum temperature and the optimal reaction pH were measured. The optimum temperature and pH was 70°C (Fig. 3A) and pH 5 (Fig. 3B), respectively. The thermostability of CtCel6 was also investigated. After incubated 1 hour at different temperatures from 40°C to 50°C, there was little impact on the hydrolysis activity of CtCel6 enzyme. However, after the treatment of 60°C, 70°C and 80°C for 1 h, the enzyme activity retained 90.5%, 71.3% and 46.8%, respectively. When pretreated at 90°C for

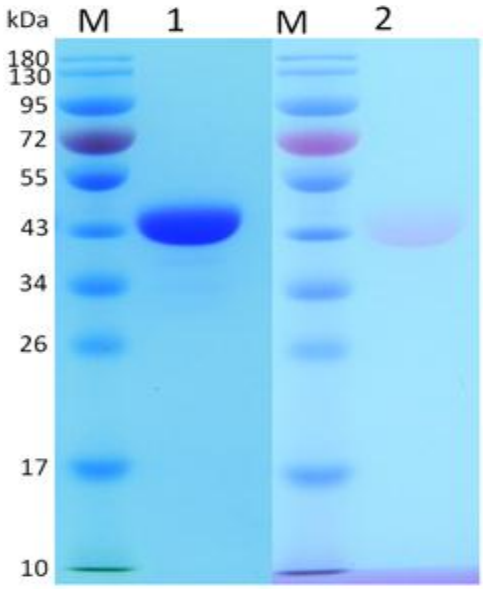

Fig. 2: SDS-PAGE analysis of purified recombinant CtCel6. Lane M, molecular mass marker; lane 1, Coomassie brilliant blue staining; lane 2, carbohydrate staining

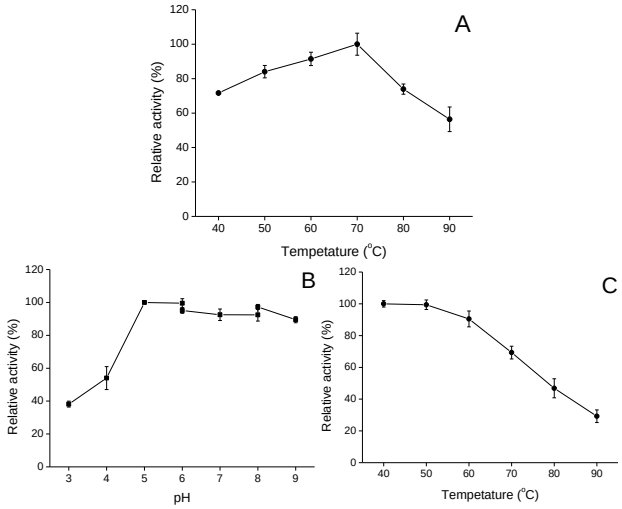

Fig. 3: The optimal temperature (A) and optimal pH (B) and thermostability (C) of purified recombinant CtCel6. The CtCel6 activity was assayed as described in Materials and Methods. The highest activity was defined as 100%. Values are means ±SD of three replications

1 h, only 29.2% residual activity was detected (Fig. 3C). The effects of various 1 mM metal ions on enzyme activity were also tested. The activity of CtCel6 was significantly enhanced with Ca^{2+} and Mg^{2+} by 151.6% and 149.9%, respectively. In contrast, Hg^{2+}, Mn^{2+}, Cu^{2+} and Fe^{3+} reduced the enzyme activity at different degrees. Monovalent cation K^+ and Na^+ were detected no obvious effect, as well as Zn^{2+}

Table 1: Effect of cations on the activity of purified CtCel6

Metal ions	Relative activity (%)
Control	100.0±2.5
Na^+	100.5±5.4
K^+	96.1±4.2
Zn^{2+}	100.6±11.2
Mg^{2+}	149.9±4.8
Hg^{2+}	31.9±3.6
Ca^{2+}	151.6±2.9
Mn^{2+}	39.9±5.6
Cu^{2+}	38.4±4.5
Fe^{3+}	65.7±6.7
Al^{3+}	103.3±3.9

Control is measured without metal ions. The final concentration of each the metal ions is 1 mM. The activity was determined as described in Materials and Methods. Values are means ±SD of three replications

Table 2: Substrate specific activity of CtCel6

Substrate	Specific activity (U/mg)
β-D-Glucan	1.27±0.10
Pectin	1.90±0.07
PASC	0.95±0.06
Filter paper	0.39±0.01
Wheat straw	0.37±0.01
CMC-Na	0.11±0.03
Chitin	ND
Saccharose	ND
Xylan	ND
Amylose	ND

ND means not detected

The concentration of β -D-glucan is 0.1% (w/v), as well as pectin. PASC, CMC-Na, saccharose, xylan and amylose are used as 0.5% (w/v). Chitin, filter paper and wheat straw are 20 mg in each reaction. PASC was prepared from Avicel as described in Materials and Methods. Wheat straw is not pretreatment. Values are means ±SD of three replications

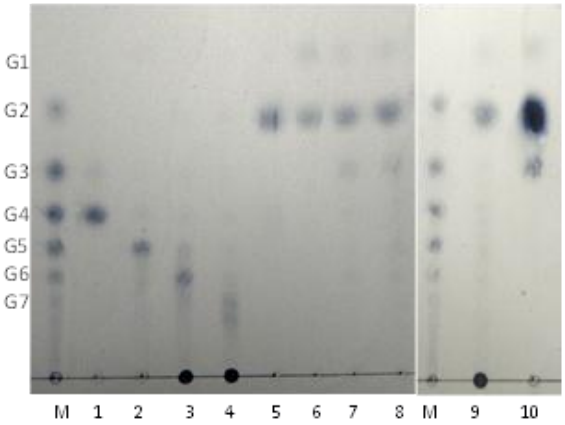

Fig. 4: Analysis of hydrolytic products by thin layer chromatography. Assay methods are described in the part of Materials and Methods. Lane M, the oligosaccharides mixture standard; lane 1, cellotetraose; lane 2, cellopentaose; lane 3, cellohexaose; lane4, celloheptaose; lane 5 to lane 8 mean the hydrolytic products on cellotetraose, cellopentaose, cellohexaose and celloheptaose, respectively. Lane 9, the hydrolytic products on CMC-Na; Lane 10, the hydrolytic products on PASC. G1to G7 means the degree of polymerization of cellulose

and Al^{3+} (Table 1). The observation of substrate specificity showed that CtCel6 displayed high activity towards β-D-glucan with 1.27 U/mg, lower activity on PASC with 0.95 U/mg and slight activity on CMC-Na. Almost no detectable activity was found on chitin, saccharose, xylan and amylose (Table 2). The corresponding kinetic parameter K_m was 0.38 mM on β-D-glucan.

The hydrolysis products were mainly cellobiose released from PASC, and lower amounts of cellotriose and glucose were liberated. The similar result was detected on CMC-Na, but the amount of reaction products relatively lower than those on PASC. After reacted at 50°C for 3 h, CtCel6 hydrolyzed cello-oligosaccharides completely and each substrate disappeared from the reaction. The sole product on cellotetraose was cellobiose. The end products on cellopentaose were mainly cellobiose and low amounts of glucose. CtCel6 converted cellohexaose and celloheptaose into cellotriose, cellobiose and glucose under react condition (Fig. 4).

Discussion

By now, many GH6 family enzymes have been isolated and experimentally characterized from multiplying fungi (Zhang *et al.*, 2000; Tao *et al.*, 2014; Kim *et al.*, 2016). However, as an important part of cellobiohydrolases, only a few reports on the GH6 cellobiohydrolases from thermophilic fungi (Thompson *et al.*, 2012). Herein, a new cellobiohydrolase gene *ctcel6* was first cloned from thermophilic *C. thermophilum* and heterologous expressed in *P. pastoris* system. Blastp analyzed the amino acid sequence similarity of CtCel6 protein, the result revealed a relatively high degree of identity with other glycoside hydrolase family 6 members. CtCel6 shared 78% identity with *Myceliophthora thermophile* GH6 protein (XP_003664525), 73% identity with *Thielavia terrestris* GH6 protein (XP_003650908) and 72% identity with *Magnaporthe oryzae* exoglucanase-6A (XP_003710956). Some aromatic amino acid residues were found in CtCel6, which were supposed to be cellulose substrate-binding sites of GHs (Takashima *et al.*, 2007). Two conserved Asp residues D170 and D352 were observed (Fig. 1), suggesting that they are likely to be involved in catalytic activity as proton donor and nucleophile. Moreover, SDS-PAGE analysis showed CtCel6 was a glycoprotein (Fig. 2). Glycosylation, as a common post-translational modification, has important function in structure and function of enzymes. Generally, sugar residues are linked to Ser, Thr, Hyl and Asp residues in the way of O-linked and N-linked. Recent evidences in the literature suggested that the enzymatic glycosylation might be concerned with stability (Chen *et al*., 2014; Tao *et al.*, 2014).

The result showed that CtCel6 has high thermostability and a high temperature activity (Fig. 3A, C), similar to other enzymes of thermophilic fungi (Li *et al*., 2011; Shi *et al*., 2014). Thermostable CtCel6 should play a major role to adapt the high temperature environment for *C. thermophilum*. These properties indicated that thermostable cellobiohydrolase CtCel6 has practical value and great commercial application potential. Generally, in most operational situations, the optimal temperature of enzymatic action is 40–60°C, it is considered that high temperature reaction activity and thermostable enzyme is favorable (Aditya *et al.*, 2014; Huy *et al.*, 2016). In addition, the purified recombinant CtCel6 exhibited excellent activity in a range of pH 5 to pH 9 with the maximum hydrolysis activity at pH 5 as showen in Fig. 3B, which was similar to cellobiohydrolases from other thermophilic fungi, such as *Fusarium chlamydosporum*, *Thermoascus aurantiacus* and *Thielavia terrestris*. (Qin *et al.*, 2010; Hong *et al.*, 2003; Woon *et al.*, 2016). The bivalent metallic cations Mg^{2+} and Ca^{2+} significantly enhanced the activity of CtCel6, while the monovalent cations K^+ and Na^+ were not detected with obvious effect (Table 1). Similar conclusion was reported on GH9 endoglucanase CenC from *Clostridium thermocellum* and β-glucosidase Bgl4 from *Penicillium funiculosum* (Haq *et al.*, 2015; Ramani *et al.*, 2015), owing to bivalent metallic cations could increase the stability and cause conformational changes of catalytic center of cellulose. The observation of substrate specificity showed that CtCel6 displayed high activity towards β-D-glucan with 1.27 U/mg, which was higher than two cellobiohydrolase (CBHI, CBHII) from *Trichoderma viride* (Song *et al.*, 2010), but lower than cellobiohydrolase PcCel6A from *Paenibacillus curdlanolyticus* (Baramee *et al.*, 2016). PASC could also be hydrolyzed by CtCel6 as showed in Table 2. According to CAZy enzyme classification, cellobiohydrolases act on the terminal of cellulose chains to remove cellobiose, while the role of endoglucanases is to produce a series of compounded cellooligosaccharides. During the hydrolysis reaction for 3 h, the major product was cellobioses on each cellooligosaccharides by thin layer chromatography analysis. This result indicated that CtCel6 was a cellobiohydrolase, instead of an endoglucanase (Fig. 4). The hydrolyzed products on PASC and CMC-Na were mainly cellobiose (Fig. 4). As previously reported, some cellobiohydrolases of GH6 were detected to hydrolyze CMC-Na, as well as PASC, which were defined as the exo-/endo-type cellobiohydrolases (Wang *et al.*, 2013; Baramee *et al.*, 2016). In exo-/endo-type cellobiohydrolases, the flexible tunnel-like active sites are the reason that causing exo-type action connected with endo-type action. Nevertheless, true exo-type cellobiohydrolases appear to hydrolyse crystalline cellulose, but CMC-Na is not their substrate. Consequently, the analysis of substrate specificity and hydrolysis products highlighted that CtCel6 was an exo-/endo-type cellobiohydrolase (Table 2; Fig. 4). The majority of crystalline cellulose degradation is performed by exo-type cellobiohydrolases, while soluble cellulose could be

hydrolyzed by endo-type cellobiohydrolase. As a result, exo-/endo-type cellobiohydrolases are necessary for lignocellulosic biomass biodegradation in industry, especially the thermostable cellobiohydrolases.

Conclusion

We cloned a new GH6 cellobiohydrolase gene *ctcel6* from thermophilic *C. thermophilum* and heterologously expressed in *P. pastoris* system. The corresponding recombinant protein CtCel6 was purified and detected its characteristics. These results demonstrated that the recombinant CtCel6 had high hydrolysis activity and excellent thermostability at elevated temperature. These properties are considered essential conditions for enzymic preparation which make CtCel6 act as an interesting potential candidate for biochemical and bioindustrial applications.

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