Molecular cloning, heterologous expression, and functional characterization of a cellulolytic enzyme (Cel PRII) from buffalo rumen metagenome

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Abstract A cellulase encoding gene, *Cel PRII*, was identified from Mehsani buffalo rumen metagenome, and cloned and expressed in *Escherichia coli* BL21(DE3)-pLysS. The 1170 bp full length gene encodes a 389 residue polypeptide (*Cel PRII*) containing a catalytic domain belonging to glycosyl hydrolase (GH) 5 family. The fusion protein consisting of the Cel PRII, thioredoxin tag and 6x Histidine tag with predicted molecular weight of 63 kDa when recovered from inclusion bodies under denaturing conditions, exhibited cellulolytic activity against carboxymethyl cellulose (CMC). Recombinant Cel PRII was stable in the pH range 4.0–10.0 with pH optima 6.0. The optimal reaction temperature of Cel PRII was 30 °C with more than 50% of its activity retained at the temperatures ranging from 0 to 50 °C. Cel PRII exhibited enhanced enzymatic activity in the presence of Mn²⁺ ions and was inhibited in the presence of chelating agent EDTA. The K_m and V_{max} values for CMC were found to be 166 mg/mL and 1292 IU/mg, respectively. Cel PRII identified in the present study may act as an excellent

candidate for industrial applications, and may aid in lignocellulosic biomass conversion because of its potential cellulolytic activity, thermostability, and excellent pH stability.

Keywords Buffalo rumen · Glycoside hydrolase 5 · Metagenome mining · Protein purification · Lignocellulosic biomass degradation

Abbreviations

GH	Glycosyl hydrolase
CMC	Carboxymethyl cellulose
ORF	Open reading frame
DNS	3,5-Dinitrosalicylic acid
SDS-PAGE	Sodium dodecyl sulfate–polyacrylamide gel electrophoresis
EDTA	Ethylene diamine tetraacetic acid

Introduction

Lignocellulosic biomass, the most abundant bio-renewable polymer on earth (Jørgensen et al. 2007), consists of a mixture of various complex carbohydrates, viz., cellulose and hemicelluloses, and aromatic polymer lignin (Tomme et al. 1995). Lignocellulosic biomass is regarded as the pioneer alternative to meet the fuel and energy (Isikgor and Becer 2015; Pirzadah et al. 2014) requirement considering the fact that the world is going to encounter an acute scarcity of food, fuel, and energy in near future, compelling the mankind to search for newer alternatives. As cellulose has major share in lignocellulosic biomass composition (Anwar et al. 2014), demand for potential cellulases from

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the unexplored niches like rumen has increased tremendously. Cellulose is a homopolysaccharide composed entirely of D-glucose linked together by β -1,4-glycosidic bonds (Lynd et al. 2002). Cellulase is the class of enzymes which systemically degrades cellulose polymers. Cellulases are classified into 14 glycoside hydrolase (GH) families (GH 5, 6, 7, 8, 9, 10, 12, 26, 44, 45, 48, 51, 61, and 74), and enzymes from rumen microorganism predominantly fall within three families (GH 5, 9, and 51) (Gullert et al. 2016; Krause et al. 2003).

Cellulases have potential applications in wide spectrum of industries including traditional uses in food and brewery production, animal feed processing, detergent production and laundry, textile processing, and paper pulp manufacture (Godfrey and West 1996; Juturu and Wu 2014; Kuhad et al. 2011). Saccharification of cellulose to glucose by cellulases is a key process in the production of bioethanol from cellulosic biomass. Although a varied range of cellulases has been identified from bacteria and fungi (Bhat and Bhat 1997; Juturu and Wu 2014), there is still a need to identify new enzymes as they are unable to meet the industrial requirements due to certain limitations including stability, activity, and sensitivity to certain byproducts (Bao et al. 2011). Hence, researchers are still making efforts to mine various environmental niches for novel biocatalysts, which are multifunctional, thermostable, halostable, possess high activity, and are less sensitive to the byproducts formed during the process.

Rumen, one of the most diverse ecosystems in nature, contains microbial consortia of anaerobic prokaryotes, protozoa, fungi, and bacteriophages, which act synergistically to convert the lignocellulosic feeds into volatile fatty acids serving as an excellent energy source for host ruminants (Mackie 1997). Most of the digestion of the plant materials, the natural diet of grazing ruminants, takes place under the anaerobic conditions of rumen. Rumen microbiome is considered to be the most efficient microbial system for the degradation of lignocellulosic biomass (Flint et al. 2008), a fact that has attracted great interest in mining enzymes from this environment. Several hydrolase genes have been identified and isolated from the cultivable microbial community harbored within the rumen (Krause et al. 2003). However, it is generally accepted that the large proportion of the microorganisms remains uncultivated (Rondon et al. 1999). Even for the intensively studied microbial community, such as rumen, it is accepted that more than 85% of the microbes is still uncultivable (Krause et al. 2003). This microbial diversity represents an untapped potential source of novel and unique biocatalysts important for biomass conversion and industrial applications.

With the advent of metagenomics, i.e., direct analysis of DNA fragments from given environmental sample, many

hydrolase genes have been identified from uncultivated microorganisms (Brulc et al. 2009; Cheema et al. 2012; Ferrer et al. 2005; Gong et al. 2013; Hess et al. 2011; Rashamuse et al. 2013; Wong et al. 2010; Zhao et al. 2010). The present study aims to exploit the power of metagenomic analysis to identify and express the novel hydrolase genes from the buffalo rumen microbiome. In the present study, we report a GH5 cellulase, Cel PRII, identified using the bioinformatics approach from the Mehsani buffalo rumen microbiome having potential hydrolytic activity on carboxymethyl cellulose.

Materials and methods

Strains and samples

The experiment was performed using the metagenomic DNA isolated from the rumen liquor collected from Mehsani buffalo reared at Livestock Research Station, Sardar Krushinagar Dantiwada Agricultural University, Gujarat, India. Permission was obtained from the Committee for Purpose of Control and Supervision of Experiments on Animals (CPCSEA) before initiating the study. DNA isolation was performed by QIAamp DNA Stool Mini Kit (Qiagen GmbH, Hilden, Germany) as per the manufacturer's instruction. *Escherichia coli* DH5 α strain was used for the propagation of plasmid while *E. coli* BL21(DE3)-pLysS strain was used for the expression of the recombinant enzyme cloned in expression vector pET32a(+) (Novagen, New York, USA). The substrates and chemicals used in this study were acquired from Sigma Chemicals (St. Louis, MO, USA) and Fluka Analyticals (Switzerland). Restriction enzymes were obtained from New England Biolabs (Ipswich, MA, USA).

Amplification of GH5 encoding gene, cloning, and sequencing

Shotgun sequencing data of Mehsani buffalo rumen metagenome were analyzed by CAZyme (<http://mothra.ornl.gov/cgi-bin/cat/cat.cgi?tab=ORTHOLOGS>) database for finding the putative carbohydrate active enzymes. Gene-specific primers (EU37 primers, Table 1) were designed to amplify the desired full length gene from metagenome using the reference sequence of the organism showing the closest similarity. The amplification reaction was performed with total volume of 25 μ L containing 15 ng of template DNA (10 ng/ μ L), 12.5 μ L of 2x EmeraldAmp GT PCR master mix (Takara, Japan), 0.5 μ L of each primer (10 pmol/ μ L), and 10 μ L of nuclease-free water. The PCR amplicon was purified using QIAquick Gel Extraction Kit (Qiagen GmbH, Hilden, Germany), and

Table 1 Primer sequences used in this study

Primer name	Primer sequence (5' to 3')
EU37 F	CGCGGATCCATGA
EU37 R	GAAAAACATTTTAATGCTGGCCG
	GCGCTCGAGTTCTTCATTCCTCTCCAG
M13_Foward	GTAAACGACGGCCAGT
M13_Reverse	CAGGAAACAGCTATGAC
pET32_Foward	AACGCCAGCACATGGACAG
pET32_Reverse	CAGCTTCCTTTCGGGCTTTG

digested with *Bam*HI and *Xho*I enzymes for subsequent cloning into pET32a(+) vector and transformed into *E. coli* DH5 α cells. The recombinant clones were selected on ampicillin (50 μ g/mL) plates, and confirmed through colony PCR and Sanger sequencing using an ABI 3500 genetic analyzerTM (Applied Biosystems, USA) by vector specific pet32a_F and pet32a_R primers (Table 1). Raw sequence reads were curated and assembled in BioEdit sequence alignment editor to generate the full length sequence.

Sequence analysis

The nucleotide and protein sequence of putative glycosyl hydrolase *Cel* PR^{II}-encoding gene was analyzed for its similarity with the known sequences in the NCBI non-redundant database by BLASTn and BLASTp, respectively (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). Functional annotation of *Cel* PR^{II} protein was performed by InterPro (<http://www.ebi.ac.uk/interpro>).

Sub-family determination for GH5 was done using the method described for GH5 subfamily classification (Aspeborg et al. 2012). Briefly, GH5 catalytic module sequences obtained from CAZy database were clustered using UCLUST at 75% identity to reduce the number of similar sequences. Clustered GH5 sequences were aligned using MUSCLE (Edgar 2004) and phylogenetic analysis was conducted using MEGA7 (Kumar et al. 2016).

Over-expression and purification of enzyme

Recombinant plasmids were transformed into *E. coli* BL21(DE3)pLysS cells and cultured in 2xYT media at 37 °C until an OD₆₀₀ nm 0.6 was obtained followed by induction with 0.5 mM IPTG for 3.5 h. Protein purification was carried out under denaturing conditions followed by refolding of protein for attaining the active configuration. Briefly the pellet of induced cells was resuspended in lysis buffer (16 mM Na₂HPO₄, 572 mM NaCl, 2.8 mM KH₂PO₄ and 1% SDS), and cellular extract was freeze thawed

3–4 times for efficient lysis and protein release followed by incubation on ice for 30 min, and centrifugation at 13,000 $\times$ g for 20 min to eradicate the contamination of SDS. The supernatant containing protein was eluted in 500 mM imidazole using Clontech His 60 Ni gravity (Takara, Japan) flow column by following the manufacturer's protocol. Ten fractions each of 1 mL were collected, and checked for quantity and quality using ND1000 spectrophotometer and SDS-PAGE, respectively. The fraction containing the purified protein was desalted using PD 10 desalting column (GE Healthcare, UK) by the gravity protocol as per the manufacturer's instructions. Confirmation of full length protein expression from non-induced, induced, and purified fraction was carried out by western blotting using mouse anti-6x His tag primary antibody (HIS.H8) (Pierce, USA) and HRP-conjugated goat anti-mouse secondary antibody (Pierce, USA) followed by development with DAB-substrate kit (Pierce, USA).

Characterization of *Cel* PR^{II} enzyme

Cellulase activity of *Cel* PR^{II} was calculated by quantifying the reducing sugars released by DNS method (Miller 1959). The enzyme assay mixture consisted of 2.5% (w/v) carboxymethyl cellulose (CMC; low viscosity Sigma-Aldrich Co., St. Louis, MO, USA) dissolved in 50 mM sodium citrate buffer (pH 5.5) and 100 μ g of purified enzyme. After the incubation for 1 h, reaction was stopped by the addition of DNS reagent, boiled for 10 min in boiling water bath, and absorbance was measured at 540 nm. Glucose standards of appropriate concentrations (50–800 μ g) were used to determine the concentration of reducing sugar. One international unit (IU) of enzyme activity was defined as the amount of enzyme releasing 1 μ mol of reducing sugar in 1 min under the standard assay conditions.

Hydrolytic activity of enzyme towards Xylan, Locust Bean Gum (mannan) and Avicel (Crystalline cellulose) was determined by replacing CMC in above assay mixture with 1% of the respective substrate in the same buffer. Hydrolytic activity was also checked on the plates supplemented with 1% of respective substrate.

To determine the optimum temperature for enzyme activity, the assay mixture described above was incubated at a temperature range 0–80 °C for 1 h, and the activity was quantified by DNS method. To determine optimal pH of *Cel* PR^{II}, 2.5% (w/v) CMC substrate was dissolved in 50 mM sodium citrate buffer of pH range 2.0–10.0 and was incubated with purified enzyme for 1 h at the determined optimum temperature, and further quantified for the release of reducing sugars by DNS method as described above. Appropriate blank was kept for each reaction.

For determining pH stability of enzyme, purified enzyme was incubated in 50 mM sodium citrate buffer of pH range 2.0–10.0 for 48 h at 4 °C, and the residual activity was determined by DNS method. Enzyme preincubated in the buffers of different pH was used in the assay mixture described above. Thermostability of purified enzyme was determined by pre-incubating the purified enzyme at various temperatures, viz., 4, 30, 40, 50, 55, 60, 70, and 80 °C for 1 h followed by quantifying its residual activity using DNS method. Enzyme preincubated at various temperatures was used in the assay mixture.

Effect of metal ions, viz., Ca^{2+} , Mn^{2+} , Mg^{2+} , Na^+ , and K^+ , and chelating agent EDTA on enzyme activity was determined by adding them in the reaction mixture at 5, 10, and 15 mM concentration and quantification of cellulolytic activity using DNS method.

The kinetic parameters K_m and V_{max} for Cel PRII were determined by Michaelis–Menten equation with the help of graph pad Prism 6.0 software. The enzyme was incubated at 40 °C with the CMC substrate having concentration ranging from 5 to 50 mg/mL. The reducing sugars were determined by DNS assay.

Structure prediction for Cel PRII enzyme

The secondary protein structure prediction, binding site analysis, and structure alignment were performed using RaptorX (<http://raptorx.uchicago.edu/>) (Källberg et al. 2012).

Nucleotide sequence accession number

The nucleotide sequence for *Cel PRII* gene was deposited in GenBank under the accession number KU374969.

Results

Cloning and sequencing

Shotgun sequencing of Mehsani buffalo rumen metagenome by Ion Torrent PGM (Life Technologies) resulted in a total of 22,022,873 reads accounting for about 3.5 giga base of sequence data. Assembly of these reads using Newbler (GS De Novo Assembler v. 2.6) generated 137,210 contigs. CAZyme analysis of assembled contigs revealed a total of 2597 putative CAZymes comprising 1929 glycosyl hydrolases, 373 glycosyl transferases, 259 carbohydrate esterases, 17 pectin lyases, and 19 carbohydrate binding modules (Patel et al. 2014). GH5 cellulase *Cel PRII* amplicon of 1192 bps (Supplementary Fig. 1) was amplified using gene specific primers. The BLASTn analysis of the *Cel PRII* coding sequence retrieved after Sanger sequencing revealed 97% identity with region encoding glycosyl hydrolase family 5 of

Prevotella ruminicola (GenBank accession no. CP002006) with query coverage of 100%. The BLASTp analysis of the sequence revealed 93% identity with hypothetical cellulase-encoding protein from *Prevotella* sp. MA2016 (GenBank accession no. WP_028913243). Functional annotation of 389 amino acid long protein by InterPro Scan assigned it to IPR017853 glycoside hydrolase superfamily which has TIM barrel structure (SSF51445 13–381 amino acids) having transglycosidase activity and catalytic domain (G3DSA:3.20.20.8043–381 amino acids) having glycosidase activity. GH5 subfamily classification was carried as described above to identify the subfamily within the GH5 family that Cel PRII belongs, and analysis showed that Cel PRII belongs to subfamily 38 within the GH5 family (data are not shown).

Protein expression and purification

The extract of the induced cells was distinct from that of the non-induced cells with a specific band of about 63 kDa on 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) (Fig. 1). Western blot analysis for checking full length protein expression revealed a distinct band in case of the crude extract and purified protein in contrast to the non-induced samples (Fig. 2).

Characterization of Cel PRII enzyme

The activity of enzyme was negligible towards the substrates, such as Xylan, Avicel, and Locust Bean Gum, as tested using both plate-based and solution-based assays. Further characterization of the enzyme was carried out using CMC as substrate. The optimal temperature for Cel PRII was recorded as 40 °C (Fig. 3). In case of pH optimization, enzyme exhibited good activity in pH range 5.0–7.0, with highest activity at pH 6.0 (Fig. 4), and was stable in the pH range 4.0–10.0 (Fig. 5). Thermostability for the recombinant enzyme was recorded till 60 °C (Fig. 6). While checking the pH stability and thermostability of Cel PRII, readings obtained for pH 2 and 3, and 70 and 80 °C were below the lowest point on the linear standard curve, and hence were not considered significant for further analysis. The enzymatic activity was not significantly influenced by the presence of metal ions except for Mn^{2+} , which increased the activity of enzyme by twofold when used at concentration of 15 mM. Although at low concentration of EDTA, slight activity was observed and the increase in concentration diminished the enzyme activity completely (Fig. 7), highlighting the concentration dependent effect of EDTA on enzyme activity. Among kinetic parameters observed for Cel PRII enzyme, K_m was 166 mg/mL and V_{max} was 1292 IU/mg (Fig. 8).

Fig. 1 SDS-PAGE analysis of Cel PRII protein. *Lane 1* pageRuler un-stained protein ladder. *Lane 2* non-induced Cel PRII. *Lane 3* induced Cel PRII crude lysate showing 60 kDa band. *Lane 4* purified Cel PRII protein showing 60 kDa band

Fig. 2 Western blot analysis of Cel PRII protein. *Lane 1* EZ run pre-stained protein ladder. *Lane 2* non-induced Cel PRII. *Lane 3* induced Cel PRII crude lysate showing 60 kDa band. *Lane 4* purified Cel PRII protein showing 60 kDa band

Structure analysis for Cel PRII protein

Cel PRII protein (GenBank accession no. KU374969) from the present study and its closest homolog glycosyl hydrolase family 5 (*Prevotella ruminicola* 23) (GenBank

Fig. 3 Temperature optimization for Cel PRII

Fig. 4 pH optimization for Cel PRII

Fig. 5 pH stability for Cel PRII

accession no. ADE83057.1) were subjected to structure alignment and determination of binding site residues using RaptorX. The structure alignment revealed a length alignment score of 345 residues and TM score of 0.860.

Fig. 6 Temperature stability for Cel PRII

Fig. 7 Effect of metal ions and chelating agent on Cel PRII activity

Fig. 8 Determination of Michaelis–Menten constant (K_m) and V_{max} of Cel PRII enzyme

Binding site analysis for Cel PRII revealed 37% helices, 10% β -sheets, and 52% loops in the structure (Fig. 9a) with two binding pockets predicted. The pocket multiplicity

value was 140. The ligands envisaged were TRS (2-amino-2-hydroxymethyl-propane-1,3-diol) and BGC (beta-D-glucose). Residues for TRS ligand were Gln53, His124, Asn179, Glu180, Trp216, Tyr241, His248, Trp253, Glu321, Trp354, and Phe360 while, BGC ligand comprised Ser48, His49, Gln53, Arg61, His124, Trp253, Trp354, Asp359, and Phe360 residues.

Binding site analysis for homolog ADE83057.1 depicted 37% helices, 11% β -sheets, and 51% loops (Fig. 9b) with one binding pocket in its structure. The pocket multiplicity value was 135 with the presence of BGC(beta-D-glucose)-binding ligands consisting of His50, Gln54, His125, Asn180, Glu acid181, Trp217, Tyr242, His249, Trp254, Glu322, Trp355, and Phe361 positioned residues.

Multiple sequence alignment of *Cel PRII* (GenBank accession no. KU374969) with AEV59731.1 (cellulase from uncultured bacterium), WP_022411268.1 (endoglucanase from *Ruminococcus* sp), WP_013065043.1 (family 5, glycosyl hydrolase, *Prevotella ruminicola*), and ADX05725.1 (putative carbohydrate-active enzyme from uncultured bacterium) (Fig. 10) illustrated that the amino acid residues His(H), Ser(S), Gln(Q), Glu(E), Trp(W), and Tyr(Y) at positions 50, 53, 54, 61, 125, 322, 355, and 357 present in the ligand binding sites of these proteins are conserved.

Discussion

Metagenomics, a tool developed and widely used for identification of microbial diversity of uncultivable organisms, has now been employed to mine genes for novel and industrially important biocatalysts from different environments (Daniel 2005; Lorenz and Eck 2005). Mining of metagenomes for potentially important enzymes has been successful in many studies, thus demonstrating the importance and efficacy of the technique for identification of new enzymes (Bashir et al. 2014; Ferrer et al. 2016). The present study unites modern metagenomics with conventional molecular cloning for identification and characterization of potent fibrolytic enzymes from dynamic microbiome of buffalo rumen.

In the present study, Mehsani rumen metagenome dataset was analyzed for the presence of carbohydrate-active enzymes. GH5 family cellulase showing the closest similarity to *P. ruminicola* 23 D5EU37 was amplified using designed gene-specific primers. GH5 family cellulases have been identified as the most dominant cellulases both in the cultured strains, such as *Fibrobacter succinogens*, where they consist of 50% of total cellulases (Krause et al. 2003), and in uncultured microbes (Ferrer et al. 2005; Palackal et al. 2007). Many of the metagenomic cellulases cloned and characterized from other environments belong

Fig. 9 **a** Structure of Cel PRII protein (GenBank accession no. KU374969). **b** Structure of Cel PRII closest homolog glycosyl hydrolase, family 5 (*Prevotella ruminicola* 23) (GenBank accession no. ADE83057.1) as predicted by RaptorX

to GH5 family (Feng et al. 2007; Healy et al. 1995; Voget et al. 2006). Expression of recombinant GH5 cellulase Cel PRII from buffalo rumen metagenome in BL21(DE3)-pLysS expression strain yielded a purified protein of 63 kDa with an activity of 190 IU/mg. Endoglucanase isolated from Korean native goat rumen had specific activity of 56.7 U/mg of protein (Kim et al. 2016). Specific activity of Umcel5N (Liu et al. 2009) isolated from uncultured bacterium from buffalo rumen was 0.67 IU/mg against CMC. Higher specific activity of Cel PRII compared to other reported endoglucanases from the rumen fluid suggests its candidacy for industrial applications. The activity of enzyme decreased gradually as the acidity of the reaction increased with highest activity recorded at pH 6.0 and a twofold decrease in activity under alkaline conditions. The results of our experiments correspond with earlier findings, which indicate that the pH optima of cellulases obtained from buffalo rumen range from pH 4 to 7 (Duan et al. 2009; Pol et al. 2012). This is also in correlation with the physiological conditions of rumen, where the pH generally fluctuates in the range 5.0–7.0. During production of fermented acid after feeding, the growth of cellulolytic bacteria is repressed and the fiber degradation is decreased. At these times, acidic cellulases are needed to ensure the digestion of fiber at proper rate under the given conditions. The enzyme exhibits pH stability in a broad range of pH 4.0–10.0. Approximately, 90% of the enzymatic activity is retained till pH 10.0 indicating the stability of the enzymes at higher pH, and thus its possible applications in industry where higher pH is required.

In terms of temperature profile, optimum temperature was observed to be 40 °C, and at higher temperatures 50% of the activity was retained. The enzyme exhibited good thermostability till 60 °C, but activity was decreased by twofolds at increased temperatures and gets completely inactivated at 80 °C. The results obtained correlate with the

previous studies, where the enzyme activity is said to diminish at higher temperatures (Andreaus et al. 1999; Yang and Dang 2011). Umcel5N (Liu et al. 2009) and Umcel5G (Feng et al. 2007) showed thermostability at 50 °C, but were inactivated quickly at temperatures greater than 60 °C and exhibited less than 20% of initial activity after incubation for 1 h.

The enzyme activity of Cel PRII was enhanced in the presence of Mn^{2+} . Most endoglucanases expressed in previous studies also showed enhanced activity in the presence of Mn^{2+} ions (Feng et al. 2007; Franco-Cirigliano et al. 2013; Voget et al. 2006); however, endoglucanases isolated from *Bacillus licheniformis* JK7, *Bacillus amyloliquefaciens* DL3, and *Bacillus flexus* showed strong inhibitory effect in the presence of Mn^{2+} ions. It is assumed that Mn^{2+} may act as a bridge between the substrate and active site of enzyme linking them together, resulting in increased enzyme activity. It might also be due to the response of Mn^{2+} to certain amino acid residues in the active site of protein resulting in favorable conformation change in protein, and thus an increase in activity. Other divalent cations, such as Ca^{2+} , Mg^{2+} , Na^{+} , and K^{+} ions, did not influence the enzymatic activity significantly. Chelating agent EDTA has an inhibitory effect on enzymatic activity as it might trap the ions and co-factors required by the enzyme. The V_{max} value of Cel PRII was 1292 IU/mg as determined from the Lineweaver–Burk plot. Other cloned endoglucanases from buffalo rumen metagenome, such as C67-1, Umcel5K, and Umcel5N (Duan et al. 2009; Liu et al. 2009), were having significantly lower V_{max} values of 367.7, 152.4, and 286.5 IU/mg, respectively.

The structure alignment of Cel PRII and its closest homolog ADE83057.1 illustrated a TM score of 0.860. TM score or template modeling score is a measure of similarity between two structures, and in present study TM score of

ecologically rich niche rumen in the present study can act as potential candidate for commercialization to meet the increasing industrial demands and for lignocellulosic biomass conversion due to its good thermostability and excellent pH stability.

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Compliance with ethical standards

Conflict of interest The authors declare no conflicts of interest.

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