Characterization of a cellobiohydrolase from the thermophilic bacterium Thermothelomyces thermophilus (*Tt*Cel7) produced in recombinant *Escherichia coli*

Chamaipon Beagbandee, Peeranat Jatooratthawichot, and James R. Ketudat Cairns*

Center for Biomolecular School of Chemistry, Institute of Science and Center for Biomolecular Structure, Function and Application, Suranaree University of Technology, Nakhon Ratchasima, 30000, Thailand.

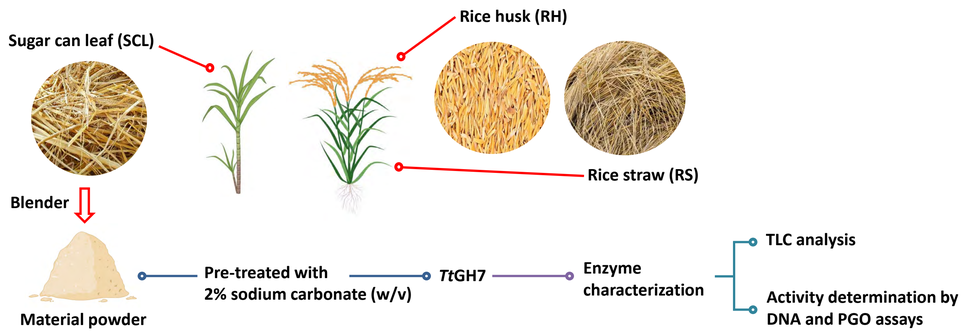
**Email: cairns@sut.ac.th*

Abstract: Cellulose is a major source of biomass monosaccharide, which may be applied as a feedstock for industrial biorefinery. Cellulose is a linear polymer with glucose monomers linked exclusively by β-1,4 glycosidic bonds, tethered to other molecules by a hydrogen bonding network that joins individual cellulose polymers to form crystalline cellulose. Cellobiohydrolases play an important role in hydrolysis of cellulose to smaller molecules, along with endoglucanases and β-glucosidases. In this study, we produced cellobiohydrolase from thermophilic *Thermothelomyces thermophilus* and its recombinant protein *Tt*Cel7A, which is a member of glycoside hydrolase family 7. *Tt*Cel7A was simply expressed in *E. coli* system (Origami(DE3)), purified and biochemically characterized. *Tt*Cel7 exhibited cellobiohydrolase activity against cellooligosaccharides (C3 to C6), microcrystalline cellulose from commercial and natural sources, respectively, under optimal conditions of 40-50 °C and pH 5.5. It retained over 80% residual activity after incubation at 60°C for 24 hours. *Tt*Cel7A has ability to work in high methanol and ethanol concentrations, and displayed over 80% residual activity after incubated at 20% ethanol or methanol for 30 min. *Tt*Cel7A is also active to release glucose and cellooligosaccharides in the presence of various metal ions. *Tt*Cel7A hydrolysis of cellotetraose releases glucose, cellobiose, and cellotriose, suggesting that cellotetraose can bind in multiple positions to produce different products. However, the breaking down of cellooligosaccharide and pre-treated agricultural biomass (rice straw, rice husk, and sugarcane leaf) confirmed that cellobiose is the main product of *Tt*Cel7 hydrolysis. The properties of *Tt*Cel7 make it a potential biocatalyst for the conversion of biomass in contaminated conditions for practical industrial applications and simultaneous saccharification and fermentation conditions to convert agricultural wastes to valuable compounds.

INTRODUCTION

Cellulose, the main component of plant cell walls, is the most abundant and renewable natural biopolymer, which is a linear polymer of D-glucose linked by beta-1,4-glycosidic linkages. In nature, cellulose can exist in ordered, crystalline, and less-ordered, amorphous forms. (Bhat and Bhat 1997) The majority of fungal genomes encode multiple forms of two types of endoglucanases, the GH7 reducing-end and GH6 nonreducing-end cleaving enzymes (Payne, C.M et al., 2015). *Tt*Cel7A containing catalytic and CBM1 domains was produced in an *E. coli* expression system. The enzyme was characterized for hydrolysis of cellooligosaccharide (C2-C6) and polysaccharides substrates, including phosphoric acid swollen cellulose (PASC), Avicel, and β-cellulose. Moreover, *Tt*Cel7 activity was observed in different concentrations of ethanol and methanol. Furthermore, the hydrolysis products from rice straw and sugarcane leaf were characterized.

METHODOLOGY



RESULTS

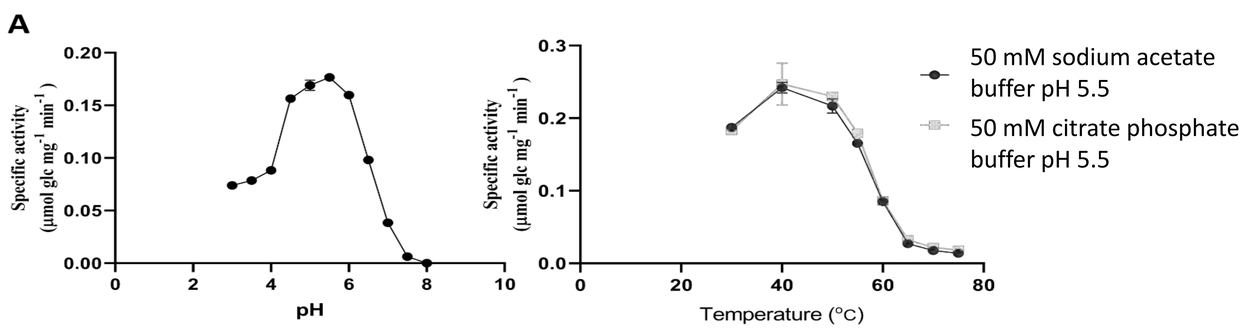


Fig. 1 Enzymatic profiles of the purified recombinant *Tt*Cel7 enzyme. The optimal pH **(A)** and temperature **(B)** of TtCel7 were assessed with cellotetraose as the substrate. The value of specific activity at each condition represents the means ± standard deviation from three replicates.

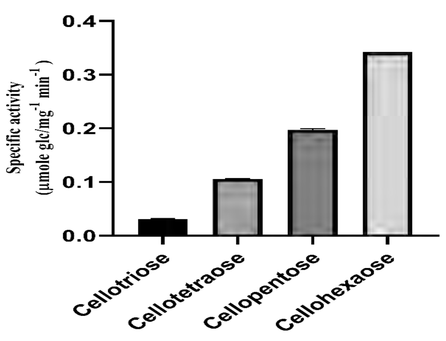


Fig. 2 Enzyme substrate specificity of TtCel7.

The specific activity on various substrate was assessed using cellooligosaccharides C2-C6 as the substrate

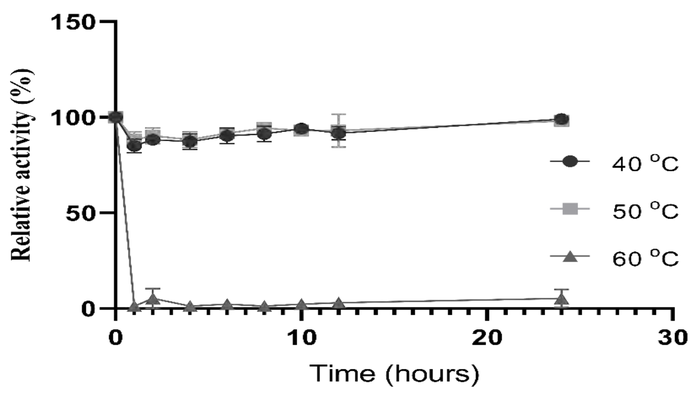


Fig. 3 Thermal stability of TtCel7.

The thermostability of *Tt*Cel7 was assessed with cellotetraose as the substrate

CONCLUSION

1. The GH7 family glycoside hydrolase (*Tt*Cel7) from thermophilic *T. thermophilus* was expressed in *E. coli*.
2. Hydrolysis of commercial and natural polysaccharides gave cellobiose as the main product.
3. *Tt*Cel7 enzyme can catalyze hydrolysis in the presence of metal ions and up to 20% ethanol and methanol up to 20% with more than 80% activity.
4. The properties of *Tt*Cel7 make it a potential biocatalyst for the conversion of biomass in contaminated conditions for practical industrial applications.

ACKNOWLEDGEMENTS

This work is supported by the Thailand Research Fund, the Synchrotron Light Research Institute and Suranaree University of Technology (grant RSA628073).

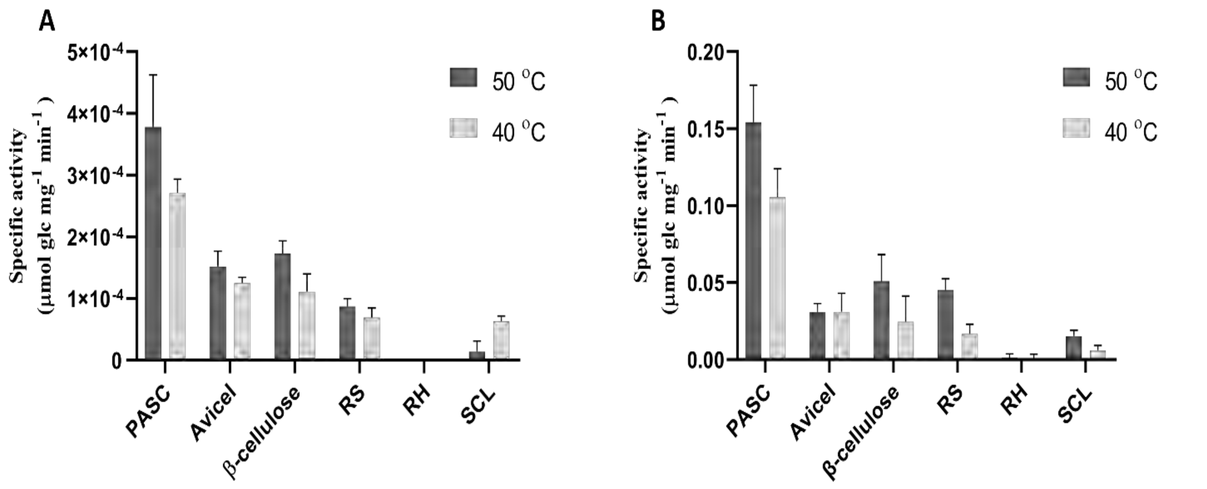


Fig. 4 The hydrolysis of polysaccharides and agricultural biomass. Each reaction initially contained 1 mg of biomass and 10 μg of *Tt*Cel7. The reaction products were determined by glucose oxidase assay **(A)** and dinitrosalicylic acid (DNS) reducing sugar assay **(B)**. RS, rice straw; RH, rice husk; SCL, sugar cane leaf.

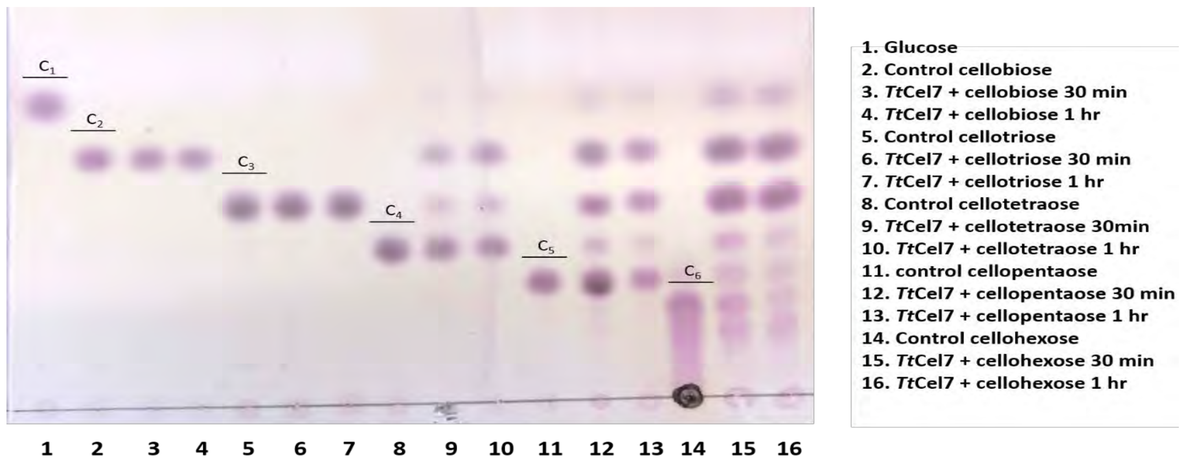


Fig. 5 Thin-layer chromatography of the hydrolysis product of *Tt*Cel7 against cellooligosaccharides. The cellooligosaccharide and C1-C6 represent the degree of polymerization of cellooligosaccharides.

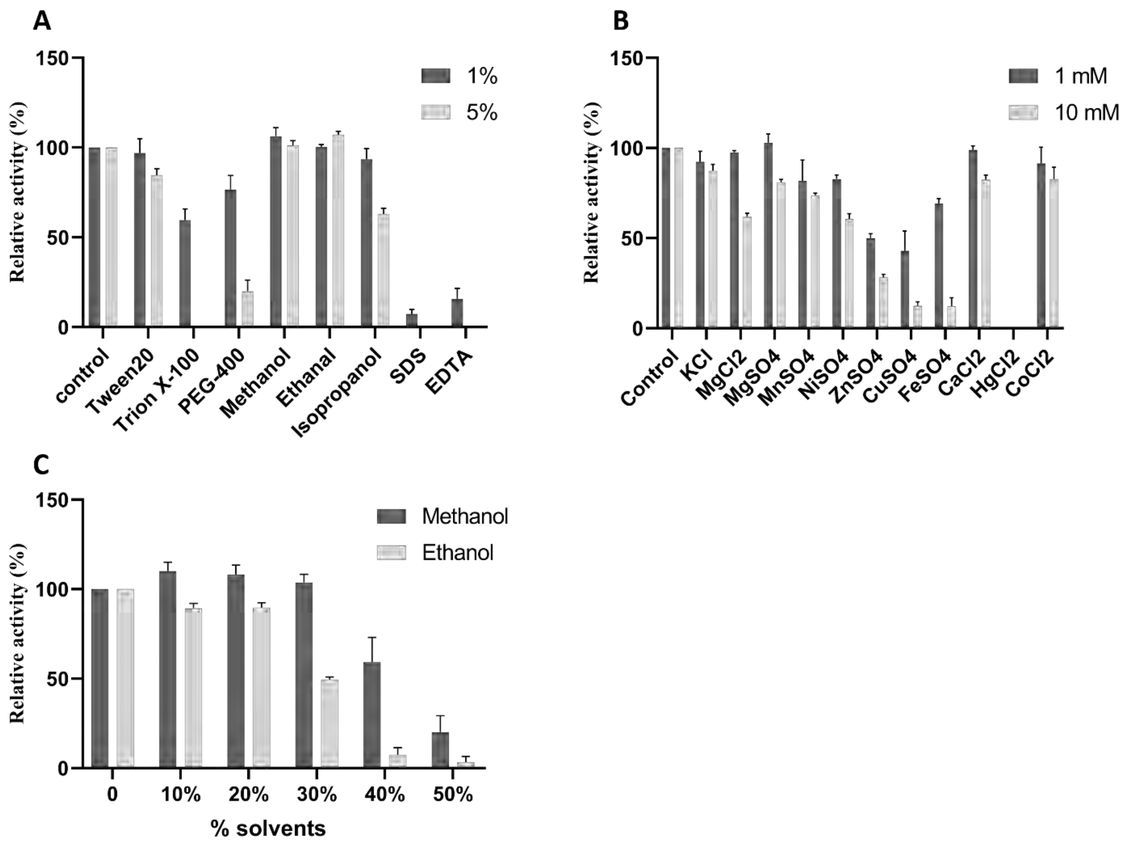


Figure 6 Enzymatic tolerant to metal ion and chemical reagent profile (**A**). The effect of organic solvents and surfactants 1% and 5% (**B**). *Tt*Cel7 activity was assessed using cellotetraose as the substrate. TtCel7 activity on various ranging from 10% to 50% of methanol and ethanol (**C**). The activities without supplementation of metal ions, organic solvents, and surfactants (control) were defined as 100%. Values are the means +/- SD of three replicated.

REFERENCES

1. Bhat, M.K. and Bhat, S. 1997.
2. Han C, Yang R, Sun Y, Liu M, Zhou L and Li D (2020)