

Applied Microbiology and Biotechnology

Hydrolysis of ionic liquid treated substrate with an *Iocasia fonsfrigidiae* strain SP3-1 endoglucanase

Sobroney Heng^{1,7}, Sawannee Sutheeworapong², Chinnapong Wangnai³, Verawat Champreda⁴, Akihiko Kosugi⁵,
Khanok Ratanakhanokchai^{1,6}, Chakrit Tachaapaikoon^{1,6*}, and Ruben Michael Ceballos^{7,8,9*}

¹School of Bioresources and Technology, King Mongkut's University of Technology Thonburi, Bangkok 10150, Thailand

²Systems Biology and Bioinformatics Laboratory, Pilot Plant Development and Training Institute, King Mongkut's University of Technology Thonburi, Bangkok 10150, Thailand

³Pilot Plant Development and Training Institute, King Mongkut's University of Technology Thonburi, Bangkok, Thailand

⁴National Center for Genetic Engineering and Biotechnology, 113 Thailand Science Park, Paholyothin Road Klong Luang, Pathumthani 12120, Thailand

⁵Biological Resources and Post-harvest Division, Japan International Research Center for Agricultural Sciences, Ibaraki, Japan

⁶Excellent Center of Enzyme Technology and Microbial Utilization, Pilot Plant Development and Training Institute, King Mongkut's University of Technology Thonburi, Bangkok 10150, Thailand

⁷Department of Biological Sciences, University of Arkansas, Fayetteville, AR, USA 72701

⁸Arkansas Center for Space & Planetary Sciences, University of Arkansas, Fayetteville, AR, USA 72701

⁹Molecular and Cell Biology Department, University of California – Merced, Merced, CA, USA 95343

* Correspondence:

Ruben Michael Ceballos

rceballos@ucmerced.edu

1	<u>M</u> <u>I</u> <u>M</u> <u>L</u> <u>N</u> <u>D</u> <u>D</u> <u>Y</u> <u>F</u> <u>L</u> <u>M</u> <u>N</u> <u>N</u> <u>V</u> <u>W</u> <u>N</u> <u>K</u> <u>E</u>
121	TGGGGA <u>ATG</u> ATCATGCTGAATGACGATTACTTTTTTAATGAATAACGTCTGGAATAAAGAG
19	A <u>A</u> <u>S</u> <u>C</u> <u>V</u> <u>Y</u> <u>Q</u> <u>Q</u> <u>R</u> <u>I</u> <u>F</u> <u>Q</u> <u>E</u> <u>N</u> <u>I</u> <u>N</u> <u>G</u> <u>K</u> <u>P</u> <u>A</u>
181	GCTGCCTCCTGCGTTTACCAGCAAAGAATCTTTCAAGAGAACATCAATGGCAAACCAGCA
39	I <u>G</u> <u>W</u> <u>Q</u> <u>W</u> <u>Q</u> <u>W</u> <u>P</u> <u>Y</u> <u>S</u> <u>V</u> <u>N</u> <u>V</u> <u>V</u> <u>A</u> <u>Y</u> <u>P</u> <u>E</u> <u>V</u> <u>I</u>
241	ATTGGCTGGCAATGGCAATGGCCGTATAGTGTTAATGTAGTTGCTTATCCAGAGGTTATT
59	Y <u>G</u> <u>D</u> <u>K</u> <u>P</u> <u>W</u> <u>D</u> <u>K</u> <u>S</u> <u>L</u> <u>G</u> <u>L</u> <u>V</u> <u>A</u> <u>D</u> <u>F</u> <u>P</u> <u>F</u> <u>K</u> <u>A</u>
301	TATGGCGATAAGCCTTGGGACAAGTCACTGGGACTGGTGGCAGACTTTCCATTTAAGCC
79	G <u>S</u> <u>K</u> <u>Q</u> <u>V</u> <u>T</u> <u>A</u> <u>D</u> <u>F</u> <u>D</u> <u>I</u> <u>K</u> <u>I</u> <u>Q</u> <u>A</u> <u>T</u> <u>G</u> <u>T</u> <u>Y</u> <u>N</u>
361	GGTTCTAAGCAAGTGACTGCTGATTTTGATATTAAGATAACAAGCTACCGGTACTTATAAT
99	M <u>A</u> <u>F</u> <u>S</u> <u>L</u> <u>W</u> <u>A</u> <u>I</u> <u>T</u> <u>D</u> <u>P</u> <u>A</u> <u>N</u> <u>P</u> <u>K</u> <u>K</u> <u>T</u> <u>I</u> <u>S</u> <u>H</u>
421	ATGGCTTTTTTCACTATGGGCAATCACCGATCCTGCTAATCCGAAAAAACCATCAGCCAT
119	E <u>I</u> <u>M</u> <u>I</u> <u>W</u> <u>N</u> <u>V</u> <u>N</u> <u>H</u> <u>N</u> <u>M</u> <u>T</u> <u>P</u> <u>A</u> <u>G</u> <u>Q</u> <u>R</u> <u>K</u> <u>E</u> <u>T</u>
481	GAAATCATGATCTGGAATGTCAATCATAATATGACACCTGCGGGCCAAAGGAAAGAGACT
139	I <u>T</u> <u>V</u> <u>S</u> <u>G</u> <u>H</u> <u>V</u> <u>F</u> <u>D</u> <u>V</u> <u>Y</u> <u>V</u> <u>K</u> <u>N</u> <u>S</u> <u>H</u> <u>G</u> <u>D</u> <u>D</u> <u>S</u>
541	ATTACGGTTAGTGGGCACGTTTTTCGATGTTTATGTAAAAAACAGTCATGGTGACGATTCTG
159	G <u>A</u> <u>N</u> <u>A</u> <u>N</u> <u>I</u> <u>W</u> <u>T</u> <u>Y</u> <u>I</u> <u>A</u> <u>F</u> <u>S</u> <u>P</u> <u>R</u> <u>K</u> <u>S</u> <u>I</u> <u>F</u> <u>K</u>
601	GGAGCAAACGCCAATATTTGGACTTATATTGCGTTTTCCCTCGAAAAATCAATCTTCAAA
179	G <u>P</u> <u>L</u> <u>D</u> <u>I</u> <u>S</u> <u>A</u> <u>F</u> <u>I</u> <u>D</u> <u>Y</u> <u>L</u> <u>I</u> <u>D</u> <u>Q</u> <u>E</u> <u>I</u> <u>L</u> <u>T</u> <u>S</u>
661	GGGCCACTGGATATCAGCGCTTTCATTGATTATCTCATCGATCAAGAAATATTAACAAGC
199	A <u>N</u> <u>Y</u> <u>I</u> <u>T</u> <u>S</u> <u>I</u> <u>E</u> <u>L</u> <u>G</u> <u>N</u> <u>E</u> <u>I</u> <u>V</u> <u>T</u> <u>G</u> <u>K</u> <u>G</u> <u>I</u> <u>T</u>
721	GCAAATTATATCACTAGTATCGAACTTGGAATGAAATTGTCACCGGAAAGGGAATCACA
219	E <u>I</u> <u>S</u> <u>N</u> <u>Y</u> <u>A</u> <u>I</u> <u>V</u> <u>I</u> <u>K</u> <u>N</u> <u>K</u> <u>Q</u> <u>*</u>
781	GAAATCAGTAATTATGCGATTGTTATTAAAAATAAACAATAA

Note: predicted molecular weight of IfCelS12A is ~30.1 kDa (calculated above sequences using a Peptide and Protein Molecular Weight Calculator (<https://www.aatbio.com/tools/calculate-peptide-and-protein-molecular-weight-mw> on 07/18/2023)).

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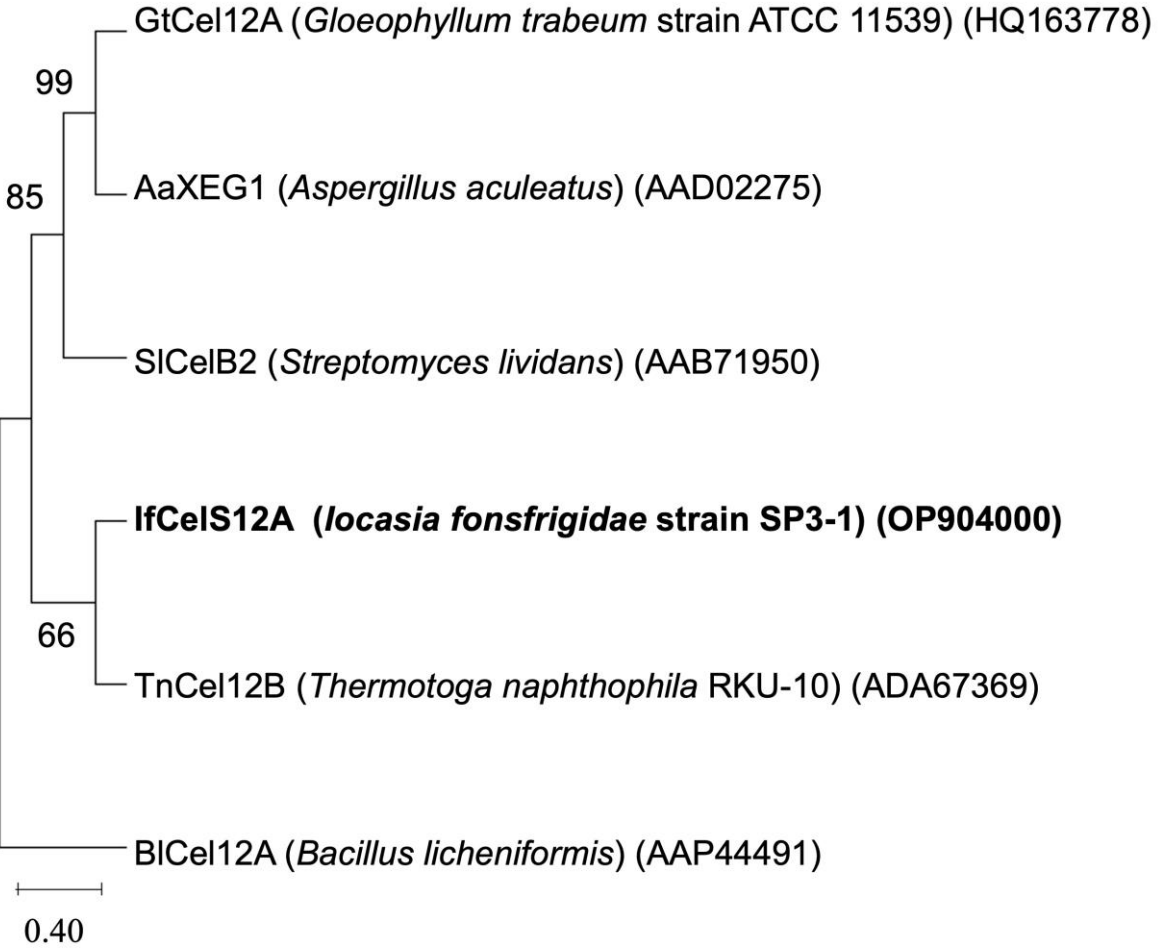
57 **Supplementary Fig. S1** Nucleotide and amino acid sequences of *IfCelS12A*. The signal peptide is underlined. The letter in
58 the box of ATG represents the start codon and the translational stop codon (TAA) is exhibited by an asterisk.

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Supplementary Fig. S2 Modular structure of the *IfCelS12A* gene. The modular structure of *IfCelS12A* shows a signal peptide sequence upstream from the primary sequence of the glycoside hydrolase, which is characteristic of the GH12 family of enzymes. Positions 1-23 represent the commonly found GH12 enzyme signal peptide sequence. Positions 23-50 and 260-273 are non-specific hits. Positions 50-260 are the core sequence for glycoside hydrolase of the GH12 family of enzymes.

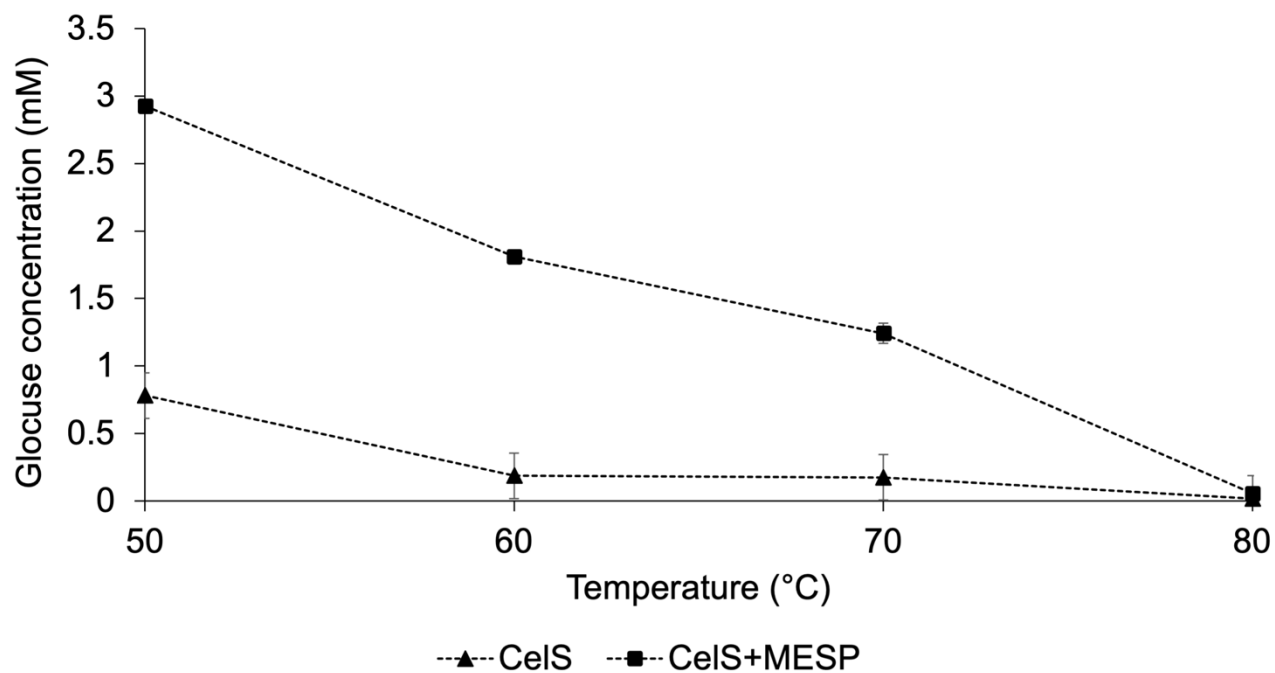
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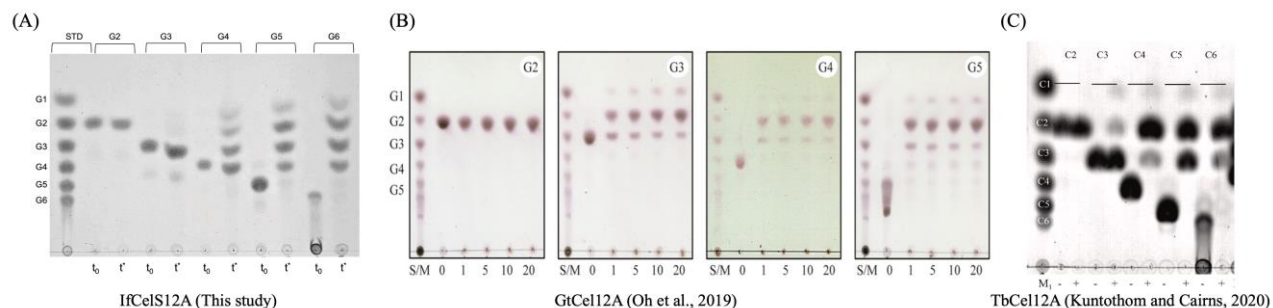
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132 **Supplementary Fig. S3** Phylogenetic analysis of the catalytic domain of IfCelS12A. A phylogenetic analysis of IfCelS12A
133 with other GH12 family members: *G. trabeum* (GtCel12A) strain ATCC 11539 (HQ163778), *T. naphthophila* (TnCel12B2)
134 RKU-10 (ADA67369), *A. aculeatus* (AaXEG) (AAD02275), *S. lividans* (SIcelB2) (AAB71950), *B. licheniformis*
135 (BIcel12A) (AAP44491) – reveals that the catalytic domain of IfCelS12A is nested within a cluster of previously describe
136 GH12 hydrolases. (The phylogenetic was constructed using the neighbor-joining method and a bootstrap analysis of 1000
137 replicates in the MEGA 11 Program).

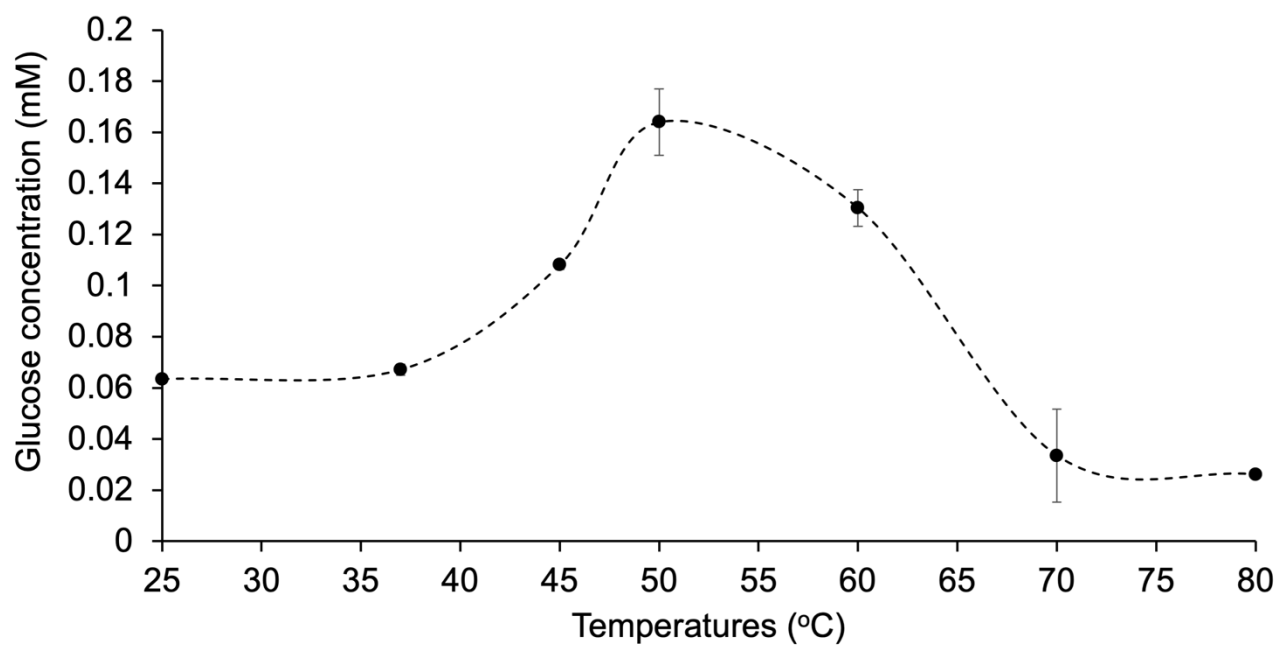
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Supplementary Fig. S4 IfCelS12A hydrolytic activity in the absence or presence of MESP at elevated temperatures. Optimal enzymatic activity is observed at 50°C for both IfCelS12A alone and IfCelS12A with MESP. However, a noted increase in hydrolytic activity on the substrate (i.e., CMC) is noted when MESP is employed. Furthermore, IfCelS12A in the presence of MESP promotes increase glucose release at higher reaction temperatures. Incubation time for each temperature trial was 60 min on 1.0% (w/v) CMC.



Supplementary Fig. S5 Comparison of TLC product release data between IfCelS12A data and previously reported GH12 hydrolase activity. **A** IfCelS12A hydrolysis of cellooligosaccharide (present study). **B** TLC yields from GtCel12A-mediated hydrolysis of cellooligosaccharides (Oh et al. 2019). **C** TLC profile from TbCel12A-mediated hydrolysis of cellooligosaccharide (Kuntothom and Cairns 2020).



Supplementary Fig. S6 Temperature profile for IfCelS12A-mediated hydrolysis of substrate. IfCelS12A-mediated hydrolysis of CMC was examined at different temperatures. Optimal temperature for hydrolysis is 50°C. Incubation time for each temperature trial was 30 min on 0.5% (w/v) CMC.

Table S1 Comparison of hydrolysis ability on cellooligosaccharide of IfCelS12A with other GH12 family

Substrate	Hydrolysis ability (binding)		
	IfCelS12A (present study)	GtCelS12A (Oh et al. 2019)	TbCel12A (Kuntothom and Cairns 2020)
Cellobiose (G ₂)	–	–	–
Cellotriose (G ₃)	–	+	+
Cellotetraose (G ₄)	+	+	+
Cellopentoase (G ₅)	+	+	+
Cellohexaose (G ₆)	+	+	+

(–) : no hydrolytic activity; (+): hydrolysis detected

Table S2 Comparison of the product release from celloooligosaccharide of IfCelS12A with other GH12 family

Substrate	Product release		
	IfCelS12A (present study)	GtCelS12A (Oh et a. 2019)	TbCel12A (Kuntothom and Cairns 2020)
Cellotriose (G ₃)	ND	$G_1 \ll G_2 = G_3$	$G_1 \ll G_2 = G_3$
Cellotetraose (G ₄)	$G_1 \ll G_2 < G_3 = G_4$	$G_1 \ll G_2 \gg G_3 = G_4$	$G_1 \ll G_2 \gg G_3 = G_4$
Cellopentoase (G ₅)	$G_1 \ll G_2 \leq G_3 > G_4 = G_5$	$G_1 \ll G_2 \gg G_3 \gg G_4 = G_5$	$G_1 \ll G_2 \geq G_3 = G_5$
Cellohexaose (G ₆)	$G_1 \ll G_2 \leq G_3 > G_4 = G_6$	NR	$G_1 \ll G_2 \gg G_3 = G_6$

ND: not determined ; NR: not reported; $\ll$: less than; $\leq$: less than or equal; $\gg$: greater than; $\geq$: greater than or equal