## Supplementary Information

Figure S1. Nucleotide and deduced amino acid sequences of ApuASK. Numbers on left side indicate the positions of nucleotide and amino acid, respectively. Residues from -29 to 0 are signal peptide (underlined). Residues A336-T414 constituted CD and pullulan-degrading enzymes N -terminus domain (○). Residues Q452-R912 indicated alpha-amylase catalytic domain (grey highlight). Residues D917-L1001 represented amyC domain ( $\bullet$ ). Residues T1006-L1092; Q1214-T1302 constituted two fibronectin type III (FnIII) domains ( $\triangle$ ). Residues T1302-A1399 indicated CBM20 domain ( $\mathbf{\Delta}$ ). Residues P1531-T1596 represented nine-repeated sequence of PGSGTT with a PGSGTA and PGSGTM (double underlined). Residues E1811-R1874; F1875-A1936; V1944-M2003 indicated three consecutive repeats of S-layer homology (SLH) domain ( $\star$ ). The four conserved regions of the $\alpha$-amylase family, designated as I, II, III, and IV are shown in rectangular.


2476 CGCGCTGTTTTCTTACTTGGAAACGGAACGGATTCATACGAACGTGCTGAACTTGATCCGAATTATAATGAAGAA

2551 CTCGGTAAAAAGCGATTAAAACTCGCTGCTATTTTACAAATGGGTTATCCAGGTGCGCCGACAATCTACTATGGT

2626 GATGAAGCAGGAGTGACAGGTTCGAAGGATCCGGACGATCGTCGTACGTATCCGTGGGGAAGTGAGGACAAGCAA

2701 CTCATTGCACATTATCAAAAAGTAGGTGCGATTCGCACGAAGCATCAAGATGTATTAGCGAAAGGAACAATTGAG
901

ACAGTATATGCGAAAGGTGATGTATACGTCTTTGCCCGTCAGTACGGAAAAGATGTTGCGCTTATTGCGGTAAAC

2851 CGCGGCAATAGTGAACAAACGGTACAGTTGGATGTCGCTTCACTTATTCCAAATGGCATCAAATTAGTAGATGAA
R G N S E Q T V Q L D V A S L I P N G I K L V D E

AGCGACAAATGGGAAGACATACAAGCAACGTACGACCGACAAGATGGCGATGCGAATGTATTCCGTGCATCGTTO$\begin{array}{llllllllllllllllllllllllll}S & D & K & W & E & D & I & Q & A & T & Y & D & R & Q & D & G & D & A & N & V & F & R & A & S & F\end{array}$ACTCCGCTACAAGCAGGCACGTACACGTATCGTTATGGCTTTACGACAAATCTCGGTGACAGTTGGGTGCATACA$\begin{array}{lllllllllllllllllllllllll}T & P & L & Q & A & G & T & Y & T & Y & R & Y & G & F & T & T & N & L & G & D & S & W & V & H & T\end{array}$GAAGAAAAAACGTTTACACTCACTGCAAATGAGGCAGATCAACAAGCACCAGCAAAGGGCATTCAACTTGTTCAA

$\Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta$ CCAGACGTTGAATCCGGTCAAGTGAATTTAACGTGGTCGTTCGTAGATCGAGATGACAATGATGCCTATATGCTT P D V E S G Q V N L T W S F V D R D D N D A Y M L $\Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta$ ATCATTGAGCGTGATGGTCAAATCGTCCATACAACAACGAATATCGGTACATCATTTACTGATTATGATGTTGAA
 $\Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta$ AACGGAAAAACATATACGTATGTTGTGAAGTTGTATGACCGTGCTGGGAACGTTGTCGCATCAAATGACGTGCAA
 $\Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta \Delta$ ATTACGCCAGACATCGTGATGGTTCAAGTGACGTTTAAAGTGAAAGCACCAAGCTATACACCGTTAGATACGCGC
 ATTACGATTCCAAATAGTATTAACGGTTGGAATACAGGTGCATGGGAAATGACGCGTGGTGGAGCGGTCACACCT
 GATTGGGAATTTACAACGGAGCTTCAAGAAGGCGAAACAATTACTTATAAGTATGTAAAGGGTAGCTCATGGGAT
 CAAGAAGGGCTAGCTGATCATACACGTGATGATCAAACAGATGATGATGTTAGCTATTATGGCTATGGTGCGATC
 GGAACGGATTTAAAAGTTACGGTACAAAATCAAGGCAATAACAAAATGATCATTCAAGATTACATTTTACGTTGG
 ATCGATATGCCTGTCGTTGTTGAAGAAGTGAAAAAAGATGGCGATCGCGTCACGATAAAAGGAAACGCGATTAAA
 GATGGCGTGTTAACGATCAATAAAGAACGTGTCACGATTCAAGACGACATGTCATTTACGTACACGTTCACACCA
 GCAGCCAATCAAAAAGAAGTGGCCATCCACATCGAACCGTCTGAACGAAGTAAGTCAGACATCTTTAAAAATGAT
 GGCGGTGCCATTGCGAAAAATACGAAAAACTACGTACTAAATATCGAAACAAAACAATTGCGCGAAGGTGTGTTG
 CAAGAGGATACAACACCGGGCAGTGGCACAACACCAGGCAGCGGTACAACACCAGGCAGTGGCACAGCACCAGGT


AGCGGCACAACACCAGGTAGCGGCACAATGCCGGGCAGCGGCACAACACCGGGCAGTGGCACAACACCGGGCAGT GGCACAACACCGGGCAGCGGCACAACACCAGGAAGTGGCACAACACCAGGAAGTGGCACAACACCAGTGAAGGGT
 GAAAATGGTACGGTTGTTTTACAGCCGAAAGTAGAGACGAAAGAAAAAGACGGCAAAGTAGTAGAAAAAGTGGCA
 ACTATTTCAACAAATGAAGTTGAAGCGATTGTCAAGGAGCTGTCGAATGAAAATAAACAAGTCGTCGTCTCCCTC
 GGCTCGCTTCCAAAAGGTGTAGCCACAAAAGTAGATGTGCCAGCTACATTATTTACAAAAGCGGCAAATAAGCAA
 GGAGAAGCAACGATTGTGAGTGCAACTGAGCAGGCGACATACAAATTGCCAGCAAAAGAAGTACAAGCGTCTCTG
 GCAGCGATTGCAAAAACACTCGGTGCAACAGTCGAACAAGTAAACATCTCGATCGAAATGAAAGTAAAAGATGCT
 CCACAACTACGTGCGAAAGCATTATCTGATGCAGTAGAGTTTCATGTTGTGGCGAAGGCAAACGGAAAAGAACAA
 GTGATTGATCGTTTTACTCAATATGTTGAACGTGAAATCGTATTGAAGCAGGCGGTGAATGCAAGTCGTTCTATT
 GTGGTGCGCGTAAACGATGACGGTTCCATTACACCTGTACCGACCACATTTGTTGGCAACAAAGCGGTCATCAAG
 TCATTGACGAACTCGACATACGTCGTCGTTGAAGGAACACATACATTTGGCGACATCGAGCAACATTGGGCGAAA


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 GGATATATCGAAACATTGGCAGCTAAACAGCTTGTGAAAGGTATGACAGAAACAGCATACCGACCAAATGAGCAG
5551 ATGACACGTGCCCAGTTTGCTGTGTTGCTTGTACGCGCATTAGCATTGCCACATGAAACATATGACGGTCGATTT

5851 AAACAATTGCCAACATGGGCAAAACAGGCGATTGAAGCAGTATACCAAGCAGGAGTCATGCAAGGACGAGATAAT

6001 AAATTGATGTAA
2001 K L M
$t \boldsymbol{t} \boldsymbol{t} \boldsymbol{t} \boldsymbol{t}$

Figure S2. The comparison of conserved regions of various $\alpha$-amylase (Amy), cyclodextrin glucanotransferase (CGTase), type I pullulanase (Pul), and amylopullulanase (Apu) using WebLogo 3.3.


Figure S3. (a) SDS-PAGE (12\%) and (b) Native-PAGE (12\%) of the purified ApuASK. Zymogram for (c) pullulytic activity and (d) amylolytic activity of the purified ApuASK. Lane 1, standard molecular weight marker; Lane 2, purified ApuASK. Arrows pointing the band correspond to the purified ApuASK.
a

b


C
d

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