## **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 ( $\circ$ ). 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 ( $\Delta$ ). Residues T1302–A1399 indicated CBM20 domain ( $\blacktriangle$ ). 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 ( $\bigstar$ ). The four conserved regions of the  $\alpha$ -amylase family, designated as I, II, III, and IV are shown in rectangular.

																					GTG	GGG	AGA	AGA	TGG
																					V	G	R	R	W
	AGA	AAA	GCAI	TTC	GCT	TAT	TTC	TCA	ATC	TTT	TTG	CTT	TTG	GCG	CAA	ATTG	TTT	TCA'	TTT	AGT	GCA	ATC	GTT	CGC	GCT
	R	Κ	Н	F	А	Y	F	S	Ι	F	L	L	L	А	Q	L	F	S	F	S	А	Ι	V	R	А
1	AAT	GA/	AAAC	GTG	CAA	AGC	CCI	GTC	GTA	AAC	CGGA	AAT	GAA	GTG	ACG	STTC	CGT	TAT	GTC	GGC	ACA	GGG	GAG	GAG	CAG
1	Ν	Е	Ν	V	Q	S	Ρ	V	V	Ν	G	Ν	Е	V	Т	F	R	Y	V	G	Т	G	Е	Е	Q
76	TCI	GTO	CTTA	TTA	.GCC	GGA	TCG	TTT	AAC	GGC	TGG	CAA	ACA	AGT	GGA	AGAC	AAA	AAG	ATT	'GAA	TTG	ACA	AAA	GAA	AGC
26	S	V	L	L	А	G	S	F	Ν	G	W	Q	т	S	G	D	K	K	Ι	Е	L	Т	K	Е	S
151	GAC	CAI	TAT	TGG	TCA	GTC	ACA	AAA	ACG	CTI	CCA	GAC	GGA	AAG	CAI	ATG	TAC	AAG'	TTT	GTT	GTG	GAC	GGA	AAT	TGG
51	D	Н	I	W	S	V	Т	K	Т	L	Ρ	D	G	K	Н	М	Y	K	F	V	V	D	G	Ν	W
226	AAG	GCCA	AGAT	'CCA	CTA	AAT	AAC	AAT	CAA	GTA	GAT	GAT	'GGA	TAT	GGI	GGG	AAA	AAT	AGC	GTC	GTT	GTI	GTC	GGT	CAA
76	ĸ	P	D	P	T.	N	N	N	0	v	D	D	G	Y	G	G	к	N	S	V	V	v	V	G	0
301	CCT	- GTT		CAA	CAA	CGC	ATC	GTC	- ACG	СТС	- GTT	'GGG	AAC	- 	CAA	GAT	GAG	 TTA	GGA	CAT	ACA	GGC	GAG	тGG	GAT
101	P	v	0	0	0	R	т	v	т Т	т.	v	G	N	т.	0	П	E.	т.	G	н	т Т	G	E	W	D
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1276	AAT	GCI	rgaa	TTG	TTC	CAG	CTA	ACT	GTA	TAC	GAT	CCA	AAC	TAT	AAA	ACG	CCA	GAT'	TGG	ATG	AAG	CAT	GCG	GTT	GTT
426	Ν	A	Ε	L	F	Q	L	Т	V	Y	D	Ρ	Ν	Y	Κ	Т	Ρ	D	W	М	Κ	Η	А	V	V
1351	TAT	'CAA	ATT	TTC	CCT	GAT	CGI	TTC	TTT	'AAC	CGGC	CAAT	CCA	GAC	AAT	'GAC	GAT	GCT	AAA	TCG	AAT	GCA	CGA	GGA	AAT
451	Y	Q	I	F	Ρ	D	R	F	F	Ν	G	N	Ρ	D	Ν	D	D	A	K	S	N	A	R	G	N

1426	GAACCAATTGAACATCGCGAATGGTCGCAGCTTCCAGACAATCCACGCATGAAAGGGACAGCTGGATATGAT	GGC
476	E P I E H R E W S Q L P D N P R M K G T A G Y D	G
1501	GATGGCATTTGGTCGAACGACTTTTTCCGGTGGAGATATTGCAGGAATTGAACAAAAGCTAGATTATTTACAA	TCT
501	D G I W S N D F F G G D I A G I E Q K L D Y L Q	S
1576	CTTGGGGTTAATACGATATATTTAAATCCGATTGCTAAAGCACCATCTAACCATAAATACGATGCGAATGAC	TAC
52.6	Τ, G V N T T Y T, N P T A K A P S N H K Y D A N D	Y
1651		ΔTG
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1700		M
1/26	CACITAATTITTAGACGJIGITATTTAATCACGITATCAGACGATTCCATTTATTTTGACCGITATGGAAAATAT	CCA
5/6	H L I L D G V F N H V S D D S I Y F D R Y G R Y	Р
	Region I	
1801	ACCGTCGGAGCATACGAATATTGGTCGGCTGTGTATGATTGAT	GCT
601	T V G A Y E Y W S A V Y D L M N G K G L S E E Q	A
1876	CGTGCTCAAGTTGAACAAAAGTTTAAAGCTGAGGGACAACAGTTCAGTCCGTACGGATTCCATTTATGGTTT	AAT
626	R A Q V E Q K F K A E G Q Q F S P Y G F H L W F	Ν
1951	ATCGAGAATGAAAAAGTAAACGGTGTGTACAAATATCAATCGTGGTGGGGGATTTGATAGTTTACCAGAATTT	AAG
651	I E N E K V N G V Y K Y Q S W W G F D S L P E F	K
2026	TCTGTCACTGGAACGAAAGTAAAGTATGCGAGCGAGTTAAATAACGAACAGCTTGCAAATTACATTTTTAT	GAA
676	S V T G T K V K Y A S E T, N N E O T, A N Y T F Y	E
2101	GACGATTCTGTCGCCAAAAGCTGGATTACGCGCGGAGCTTCAGGTTGGCGCTTAGACGTTGCGAATGAAGTC	GAT
701		D
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21/6	ACACAGTTTTTGGCGCGCAATTTTCGTAAAGAGTTGTTGCAAGGTGACTACGACCGTGGCCCCAACGTTGAAAAAAC	GGG
726	T Q F W R E F R K E L L Q G D Y D R G P T L K N	G
2251	GAGCAACCGTTAATTCTCGGTGAAATTTGGGATGATGCGTCAAAATACTTCCTAGGCGATCAATACGATTCA	GTG
751	E Q P L I L G <mark>E I W D</mark> D A S K Y F L G D Q Y D S	V
	Region III	
2326	ATGAACTATCGTTTCCGCGGTGCTGTGTTAGACTTTTTACGAAATGGGAAAGCGGAAGATATTGATGCCCGA	CTA
776	M N Y R F R G A V L D F L R N G K A E D I D A R	L
2401	ACAGCGATTCGTGAAGATTATCCGGAAGAAGCTTTTTTATGCGCTAATGAACTTAATTGGTTCACACGATACG	GCA
801	TAIREDYPEEAFYALMN <mark>LIGSHD</mark> T	A
	Region IV	
2476	CGCGCTGTTTTCTTACTTGGAAACGGAACGGAACGGATTCATACGAACGTGCTGAACTTGATCCGAATTATAATGAA	GAA
826	RAVFT. T. G. N. G. T. D. S. Y. F. R. A. F. T. D. P. N. Y. N. F.	E
2551		CCT
2JJ1 851		C
001		G
2020	GATGAAGCAGGAGTGACAGGTTCGAAGGATCCGGGGACGATCGTCGTCGTCGTCGTCGTGGGGAAGTGAGGGACAAG	CAA
8/6	DEAGVTGSKDPDDRRTYPWGSEDR	Q
2701	CTCATTGCACATTATCAAAAAGTAGGTGCGATTCGCACGAAGCATCAAGATGTATTAGCGAAAGGAACAATT	GAG
901	LIAHYQKVGAIRTKHQDVLAKGTI	E
2776	ACAGTATATGCGAAAGGTGATGTATACGTCTTTGCCCGTCAGTACGGAAAAGATGTTGCGCTTATTGCGGTA	AAC
926	T V Y A K G D V Y V F A R Q Y G K D V A L I A V	Ν
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2851	CGCGGCAATAGTGAACAAACGGTACAGTTGGATGTCGCTTCACTTATTCCAAATGGCATCAAATTAGTAGAT	GAA
951	R G N S E Q T V Q L D V A S L I P N G I K L V D	Е
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1026	T L T W E G D A P T Y R I Y Q S T L K G A G Y E	L
3151	GTGGAAGAAACGAGTGAAAAGACAGCTGTGATAGACAATTTGAAAAACGGTACCGCATATTACTTTGCGATT	ACA
1051	V E E T S E K T A V I D N L K N G T A Y Y F A I	Т
3226	GCTGTTGATACAAACGGAAATGAGTCGGCTAAAGTAGAAACAAGCCGCCTTGTCCCACATTATCCATTAGAA	GAA
1076	AVDTNGNESAKVETSRI, VPHYPI, E	E
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3316	GTGUAAATUGATAATGTAAUGAAACATGGGTTAGCTGACGGTTTACAAGCGGTGTTCCAAGTGAAAAAACCG	AAT
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3451	AGCGACAAATGGGAAGACATACAAGCAACGTACGACCGAC
1151	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
3526	ACTCCGCTACAAGCAGGCACGTACACGTATCGTTATGGCTTTACGACAAATCTCGGTGACAGTTGGGTGCATACA
1176	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
3601	GAAGAAAAAACGTTTACACTCACTGCAAATGAGGCAGATCAACAAGCACCAGCAAAGGGCATTCAACTTGTTCAA
1201	E E K T F T L T A N E A D Q Q A P A K G I Q L V Q
3676	${\tt CCAGACGTTGAATCCGGTCAAGTGAATTTAACGTGGTCGTTCGT$
1226	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
3751	${\tt ATCATTGAGCGTGATGGTCAAATCGTCCATACAACAACGAATATCGGTACATCATTTACTGATTATGATGTTGAA}$
1251	I I E R D G Q I V H T T T N I G T S F T D Y D V E
3826	AACGGAAAAACATATACGTATGTTGTGAAGTTGTATGACCGTGCTGGGAACGTTGTCGCATCAAATGACGTGCAA
1276	NGKTYTYVVKLYDRAGNVVASNDVQ
3901	${\tt ATTACGCCAGACATCGTGATGGTTCAAGTGACGTTTAAAGTGAAAGCACCAAGCTATACACCGTTAGATACGCGC$
1301	I T P D I V M V Q V T F K V K A P S Y T P L D T R
3976	${\tt ATTACGATTCCAAATAGTATTAACGGTTGGAATACAGGTGCATGGGAAATGACGCGTGGTGGAGCGGTCACACCT}$
1326	I T I P N S I N G W N T G A W E M T R G G A V T P
4051	GATTGGGAATTTACAACGGAGCTTCAAGAAGGCGAAACAATTACTTATAAGTATGTAAAGGGTAGCTCATGGGAT
1351	D W E F T T E L Q E G E T I T Y K Y V K G S S W D
4126	CAAGAAGGGCTAGCTGATCATACACGTGATGATCAAACAGATGATGATGTTAGCTATTATGGCTATGGTGCGATC
1376	Q E G L A D H T R D D Q T D D D V S Y Y G Y G A I
4201	GGAACGGATTTAAAAGTTACGGTACAAAATCAAGGCAATAACAAAATGATCATTCAAGATTACATTTTACGTTGG
1401	G T D L K V T V Q N Q G N N K M I I Q D Y I L R W
4276	ATCGATATGCCTGTCGTTGTTGAAGAAGAGTGAAAAAAGATGGCGATCGCGTCACGATAAAAGGAAACGCGATTAAA
1426	I D M P V V V E E V K K D G D R V T I K G N A I K
4351	GATGGCGTGTTAACGATCAATAAAGAACGTGTCACGATTCAAGACGACATGTCATTTACGTACACGTTCACACCA
1451	D G V L T I N K E R V T I Q D D M S F T Y T F T P
4426	GCAGCCAATCAAAAAGAAGTGGCCATCCACATCGAACCGTCTGAACGAAGTAAGT
14/6	
4501	GCCGGTGCCATTGCGAAAAAATACGAAAAAACTACGTACTAAATATCGAAACAAATAGCGCGGAAGGTGTGTGT
1501	
4370	
1520	
4651	AGCGGCACAACACCAGGTAGCGGCACAATGCCGGGCAGCGGCACAACACCGGGCAGTGGCACAACACCGGGCAGT
1551	S G T T P G S G T M P G S G T T P G S G T T P G S
4726	GGCACAACACCGGGCAGCGGCACAACACCAGGAAGTGGCACAACACCAGGAAGTGGCACAACACCAGTGAAGGGT
1576	<u>GTTPGSGTTPGSGTTPGSGTT</u> PVKG
4801	GAAAATGGTACGGTTGTTTTACAGCCGAAAGTAGAGACGAAAGAAA
1601	E N G T V V L Q P K V E T K E K D G K V V E K V A
4876	${\tt ACTATTTCAACAAATGAAGTTGAAGCGATTGTCAAGGAGCTGTCGAATGAAAATAAACAAGTCGTCGTCTCCCTC}$
1626	TISTNEVEAIVKELSNENKQVVVSL
4951	${\tt GGCTCGCTTCCAAAAGGTGTAGCCACAAAAGTAGATGTGCCAGCTACATTATTTACAAAAGCGGCAAATAAGCAA}$
1651	G S L P K G V A T K V D V P A T L F T K A A N K Q
5026	GGAGAAGCACGGGGGGGGGG
1676	G E A T I V S A T E Q A T Y K L P A K E V Q A S L
5101	${\tt GCAGCGATTGCAAAAACACTCGGTGCAACAGTCGAACAAGTAAACATCTCGATCGA$
1701	A A I A K T L G A T V E Q V N I S I E M K V K D A
5176	${\tt CCACAACTACGTGCGAAAGCATTATCTGATGCAGTAGAGTTTCATGTTGTGGCGAAGGCAAACGGAAAAGAACAA}$
1726	PQLRAKALSDAVEFHVVAKANGKEQ
5251	GTGATTGATCGTTTTACTCAATATGTTGAACGTGAAATCGTATTGAAGCAGGCGGTGAATGCAAGTCGTTCTATT
1751	VIDRFTQYVEREIVLKQAVNASRSI
5326	GTGGTGCGCGTAAACGATGACGGTTCCATTACACCTGTACCGACCACATTTGTTGGCAACAAAGCGGTCATCAAG
1776	V V R V N D D G S I T P V P T T F V G N K A V I K
5401	TCATTGACGAACTCGACATACGTCGTCGTTGAAGGAACACATACAT
1801	S L T N S T Y V V E G T H T F G D I E Q H W A K
E 4 9 4	
54/6	GGATATATUGAAACATTGGCAGCTAAAACAGCTTGTGAAAGGTATGACAGAAACAGCATACUGACCAAATGAGCAG
⊥α∠β	ст в т в а к у в v к с м т в т а у к Р N Е Q <b>↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ </b>

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5551
   ATGACACGTGCCCAGTTTGCTGTGTTGCTTGTACGCGCATTAGCATTGCCACATGAAACATATGACGGTCGATTT
1851
    M T R A Q F A V L L V R A L A L P H E T Y D G
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5626
   GCTGATGTAAAAAGGCACAGAATGGTTTAACAAAAACGGTGAATTAGCGGCTGCAGTCAAACTAGGTATCATTCAA
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1876
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5776
     AAGCTTTCGTTTGTTGGCTATAATGAAGCAACAAACGACAAAACGAAAAAGGTAACAGATTTCCGCGATGCA
     K L S F V G Y N E A T N D K T K K V T D F R D A
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5851 AAACAATTGCCAACATGGGCAAAACAGGCGATTGAAGCAGTATACCAAGGAGGAGTCATGCAAGGACGAGATAAT
1951
    K Q L P T W A K Q A I E A V Y Q A G V M Q G R D N
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5926
   GGAAGCTTTGATCCTACGAGCCATATGACGCGTGCCGAAATGGCGAAAGTATTGGTGGAGTTTTTAGGAAAAGTA
1976
      S F D P T S H M T R A E M A K V L
                                   VEFLGK
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   AAATTGATGTAA
2001
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**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.



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