

SUPPLEMENTARY MATERIALS TO

**Insight into the Thermophilic Mechanism of a Glycoside Hydrolase Family 5
 β -Mannanase**

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Yao).

19 **TABLE S1.** The primers used in this study.

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Primer	Sequence ^a
<i>PMan5A_f</i>	GCGAATTCGCAAATTGGGGTCAGTGTGGTG
<i>PMan5A_r</i>	AATGCGGCCGCTCAAGCCGAGTCAATTGCACTAACATG
<i>M1_f1</i>	GCGAATTCGTTGCCAACTATGGCCAATGTGGTGGAC
<i>M1_r1</i>	GGCATTGTTCTGTGAGGAACGGGAGCCAGTA
<i>M1_f2</i>	TACTGGCTCCCGTTCCTCACGAACAATGCC
<i>M1_r2</i>	AATGCGGCCGCTCAAGCCGAGTCAATTGCACTAACATGATCC
<i>M2_f1</i>	GCGAATTCGTTGCCAACTATGGCCAATGTGGT
<i>M2_r1</i>	CATTCCAATTGTTTACAAAGTTAATGATCAGTTTG
<i>M2_f2</i>	CAAACGTATCATTAACCTTTGTGAACAATTGGAATG
<i>M2_r2</i>	AATGCGGCCGCTCAAGCCGAGTCAATTGCACTAACATG
<i>M3_f1</i>	GCGAATTCGTTGCCAACTATGGCCAATGTGGTGGAC
<i>M3_r1</i>	ATGCCCTCATCTCCGATGCAGACCATGC
<i>M3_f2</i>	GCATGGTCTGCATCGGAGATGAGGGCAT
<i>M3_r2</i>	AATGCGGCCGCTCAAGCCGAGTCAATTGCACTAACATGATCC
<i>M4_f1</i>	GCGAATTCGTTGCCAACTATGGCCAATGTGGTGGAC
<i>M4_r1</i>	CAGGAGGCAGGGTTTGCCGGCGTTCTT
<i>M4_f2</i>	AAGAACGCCGGCAAACCTGCCTCCTG
<i>M4_r2</i>	AATGCGGCCGCTCACTGAACTGGCTAATATGATTCGTCAC
<i>ΔN1_f</i>	GCGAATTCACCCAAACGACCACTCTGACGACG
<i>ΔN1_r</i>	AATGCGGCCGCTCAAGCCGAGTCAATTGCACTAACATGA
<i>ΔN1N2_f</i>	GCGAATTCCTCTATACCCAGCAAGAATGGTCTCAAG
<i>ΔN1N2_r</i>	AATGCGGCCGCTCAAGCCGAGTCAATTGCACTAACAA
<i>ΔN1N2N3_f</i>	GCGAATTCGCAGGCACCAACACCTACTGGCTT
<i>ΔN1N2N3_r</i>	AATGCGGCCGCTCAAGCCGAGTCAATTGCACTAACATGA
<i>PMan5A(ΔN3)_f1</i>	GCGAATTCGCAAATTGGGGTCAGTGTGGTGGCGAAAAC CGTCAATGGTAAACTTGAGACCATTCCTTGCTGGGTATAGAGCCGGC
<i>PMan5A(ΔN3)_r1</i>	A
<i>PMan5A(ΔN3)_f2</i>	ACGGCAAGACCGCCTACTATGCAGGCACCAACACCTACTGGCTTCC
<i>PMan5A(ΔN3)_r2</i>	AATGCGGCCGCTCAAGCCGAGTCAATTGCACTAACATGATCC
<i>M1-C1_f1</i>	GCGAATTCGTTGCCAACTATGGCCAATGTGGT
<i>M1-C1_r1</i>	GGAGCTACAGTGATTAGTTGAACCATA
<i>M1-C1_f2</i>	TATGGTTCAACTAATCACTGTAGCTCC
<i>M1-C1_r2</i>	AATGCGGCCGCTCAAGCCGAGTCAATTGCACTAACATG
<i>M1-C2_f1</i>	GCGAATTCGTTGCCAACTATGGCCAATGTGGTGGAC GAAACTATCCGCGGCGATACCGTTCGTGCTGAGAGCGGTCGACTGC
<i>M1-C2_r1</i>	C GGCAGTCGACCGCTCTCAGCACGAACGGTATCGCCGCGGATAGTTT
<i>M1-C2_f2</i>	C
<i>M1-C2_r2</i>	AATGCGGCCGCTCAAGCCGAGTCAATTGCACTAACATGATCC
<i>M1-C3_f1</i>	GCGAATTCGTTGCCAACTATGGCCAATGTGGT
<i>M1-C3_r1</i>	ACTACTACCATAGTAAATGGTATACCCGTCATTCGGAGACTGTCCA

	GT
	ACTGGACAGTCTCCGAATGACGGGTATACCATTTACTATGGTAGTA
M1-C3_f2	GT
M1-C3_r2	AAT <u>GCGGCCGCT</u> CAAGCCGAGTCAATTGCACTAACATGATCC
M1-E411G_f	CAGTCTGCACATGACGGGTACAGCATC
M1-E411G_r	CCGTCATGTGCAGACTGTCCAGTACTC
M1-S412T_f	GCACATGACGAGTACACCATCTTCTAT
M1-S412T_r	GTGTACTCGTCATGTGCAGACTGTCCA
M1-F415Y_f	GACGAGTACAGCATCTACTATGGTAGT
M1-F415Y_r	TAGATGCTGTACTCGTCATGTGCAGAC
H112Y_f	GACGGTAAAAAGGGGTACTTTGCTGG
H112Y_r	ACCCCTTTTTACCGTCAATCACGAAG
F113Y_f	GTAAAAAGGGGCACTATGCTGGTACA
F113Y_r	TAGTGCCCTTTTTACCGTCAATCACG
H112Y/F113Y_f	GACGGTAAAAAGGGGTACTATGCTGGTACAA
H112Y/F113Y_r	ATAGTACCCCTTTTTACCGTCAATCACGAAG
L375H_f	GTATGGTTCAACTAGTCACTGTAGCTCC
L375H_r	TGACTAGTTGAACCATACTCTTCGAGGA
A408P_f	GAGTACTGGACAGTCTCCGCATGACGAGT
A408P_r	CGGAGACTGTCCAGTACTCAATGTATCAC
A99K/S100N_f	GGCTCTCTTCCTAGTAAGAATGGCACTAGCT
A99K/S100N_r	ATTCTTACTAGGAAGAGAGCCGGCAGTAG
T102L_f	CCTAGTGCTAGTGGCCTCAGCTTCGTG
T102L_r	GAGGCCACTAGCACTAGGAAGAGAGCC
V105T_f	AGTGGCACTAGCTTCACCATTGACGGT
V105T_r	GGTGAAGCTAGTGCCACTAGCACTAGG
Y108H_f	GACGGCAAGACCGCCCACTATGCAGG
Y108H_r	GGGCGGTCTTGCCGTCAATGGTAAAC
Y108H/Y109F_f	GACGGCAAGACCGCCCACTTTGCAGGCACC
Y108H/Y109F_r	AAGTGGGCGGTCTTGCCGTCAATGGTAAAC
H371L_f	ATGGATCCACCAATCTCTGCTCTTCC
H371L_r	AGATTGGTGGATCCATATTCTTCCAG
P406A_f	AGCACGGGCCAGTCGGCAAATGACGGGT
P406A_r	TGCCGACTGGCCCGTGCTTAAGGTATCA

^a Restriction sites are underlined.

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3WH9_A
T1Man5A
PMan5A

VANYGQCGGGNYSGPTTCNPGWMSQCYLNPYYSCQLPATQTTTLTTSTKPT...STSTTTT
.ANWGGCGGGENWNGDDTTCNPGWYCSYLNPNWYSQCVPGSGSSSSSTTLSTVVSQSTSSIR

3WH9_A

β1 β2 β3 β4 η1 α1

1 10 20 30 40

3WH9_A
TlMan5A
PMan5A

.....SFASSGLQFTIDGEGTYFAGTNSYWIQFLFDNADVDVLVMCHLK
RTSTSTTTSTGGSSSTSIPISSGNLKFITIDGKTAYFAGTNSYWLPLFLNADVDVLVMSHLQ
ISATSTLAASASTTAGSLPSASGTSFVIDGKKGHFAGTNSYWLPLFLNADVDVLVMSHLQ

3WH9_A 50 60 70 80 90 100
 3WH9_A S SGLKILRVWGPNFNDVTSQPSGGTVVYQLHQDGKSTINTGADGLQRLDYVVS SAECDIKL
 T1Man5A S SGLKILRVWGPNFNDVTSQPSGGTVVYQLHQDGKSTINTGANGLQRLDYVVS SAEADIKL
 PMan5A S SGLKILRVWGPNFNDVTSQPSGGTVVYQLHQDGKSTINTGSDGLQRLDYVVS SAEANGIKL

3WH9_A

170 TT α6 β11 η2 220 TT

3WH9_A LANEPRCPSGCDTSVLYNWFKENTKFIKGLADRMVVICDGEGLNIDSDGSYPYCFSEGL
TlMan5A LANEPRCHGCGDTSVIYNWVSTSTAYIKSLPNNRMVVICDGEGLMGLTTCSDGSYPYQYLEG
EMan5A LANEPRCKGCGDTSVIYNWASTSTQYIKSLPGRMVVICDGEGLGLSVDSGDSYPYCFSEGL

3WH9_A	A
T1Man5A	.
PMan5A	A

Fig. S1. Multiple sequence alignment of *PMan5A* and *TlMan5A* (GenBank: KJ607175) with the secondary structure elements of *ManBK* (PDB ID: 3WH9) as the template

N3

95 113

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PMan5A      ..TTSAT..STLAASASTTAGS..LPSASGTSFVIDGK.KGHFAGTNSYWLPPFL.TNNADVD
TlMan5A     ..TRTST..STTSTQGGSSST..SIPSKNGLKFTIDGK.TAYYAGTNTYWLPPFL.TNNADVD
3WH9        PKASP.....APSTSSSAASTSFFASTSGLQFTIDGE.TGYFAGTNSYWIGFLL.TDNADVD
3WFL        ...YAMG..IPTSGTH.NKRDTPPGTNGLDFTIDGT.AGYFAGSNAYWLAFL.TNNDVD
Man5XZ3     ...RTTT..TTTSTTSVPSSSTFPPSASGLNFTIDGV.TDYFAGSNSYWISML.TNDADVD
1QNO        .....M..GFLPQAQGGGAAASAKVSGTRFVIDGK.TGYFAGTNSYWIGFLL.TNNRDVD
3ZIZ        PKASP.....APSTSSSAASTSFFASTSGLQFTIDGE.TGYFAGTNSYWIGFLL.TDDSDVD
ABC59553.1  PKASP.....APSTSSSAASTSFFASTSGLQFTIDGE.TGYFAGTNSYWIGFLL.TDNADVD
ADZ99027.1  PKASP.....APSTSSSAASTSFFASTSGLQFTIDGE.TGYFAGTNSYWIGFLL.TDNADVD
XP_001262744.1 .....LSAKKFASASDGLQFTIDGK.TGYFAGSNSYWIGFLL.TNNADVD
AAA67426.1  PRT.....PNHNAATTAFPPSTSGLHFTIDGK.TGYFAGTNSYWIGFLL.TNNADVD
Q00012.1    PRT.....PNHNAATTAFPPSTSGLHFTIDGK.TGYFAGTNSYWIGFLL.TNNADVD
ACH56965.1  PSTTTPPTPAYICTSRAPFASIDDVHPRLFNYNGTGAKYFAGTNAWWTSYL.MIDSVDN
CRG88507.1  ...YAMG..FPILNPYKRSNSTFPSTSGLQFTIDGT.AGYFAGSNAYWLGFLQ.TNNDVD
XP_002480621.1 ..TSPA.....GSSSSGSASSTFPSTSGTQFVIDGK.KGYFAGTNSYWLPPFL.TNNADVD
OOQ88079.1  ..TGESM..DRAVQDVHQAASIPSRDGLRFVIDGK.AGYFAGSNSYWIGFLL.KNSDND
CEJ54968.1  ...TSST..TSTTSTAVSTSTVFPTTNGLNFTIDGE.TGYFAGTNSYWIGFLL.TNDADVD
XP_001275153.1 ..AAPSS..TTSHTLPTGSGSFAKTDGLKFNIDGK.TKYFAGTNAYWLPPFL.TNNADVD
OTA64229.1  .....QAVDAASPAATGYPKVDGLLFTIDGE.TKYFAGTNSYWISFL.TNNADVD
GAO81348.1  ..TPTSS..TSTPPTNPTGSSSFAKTDGLKFNIDGK.TKYFAGTNSYWIGFLL.TKNDVD
AEY76082.1  PKASP.....APSTSSSAASTSFFASTSGLQFTIDGE.TGYFAGTNSYWIGFLL.TDNADVD
OCL08622.1  .....ST..TTSAAAIAVATGSIKASGTRFTIDGV.TKYFAGTNSYWIAFL.TNNADVD
XP_014532729.1 ...KTAT..TTKATTTSSASTGFPSTNGLDFTIDGK.TSYFAGSNSYWIGFLL.TNDNDVD
B8NVK8.2    PQASSST..VSPSPSPSATPGSFVTTSGLNFTVIDGK.TGYFAGSNSYWIGFQ.KNNDVD
GAQ05480.1  .....LSAKKFASASDGLQFTIDGK.TGYFAGSNSYWIGFLL.TNNADVD
KKK19644.1  PPMT.....TRTSGSGSFPTTSGVQFVIDGE.AGYFPGSNSYWIGFLL.TNDRDVD
KMK55820.1  .....PSAKTFASASGTQFSIDGK.TGYFAGSNSYWIGFLL.TNNADVD
ETW81009.1  ...TISSTPTPTSSSSAPAPSGFVKTSQKFTLNLS.TFTVVGCANSYWWGLTGLSTANMN
XP_013958821.1 ..TAAST..LAAVLQPVPRASSFVTVSGTQFNIDGK.VGYFAGTNCYWCFSFL.TNPADVD
XP_013949127.1 ..SAAST..LAAVVQFAPRASSFVTISGSHFDIDGE.VGYFAGTNCYWCFT.YNTAEVD
EFI94550.1  ...TLASLALALSVARGASAAAGFVSTNGTKFTLDGE.PYTVVGCANSYWWGLNGYSTDAMD
ABB88954.1  ...STSRRTTATSTTASAPSSSTGFVTTSGTEFRLNGA.KFTIRGCANSYWWGLMGYSTTDMN
KKP04059.1  ..TAAST..LAAVLQFVPRASSFVTISGQFNIDGK.VGYFAGTNCYWCFSFL.SNSADVD
KXH47903.1  ..NLASI..QALAVPEPRQASTFPPSASGTRFTIDGQ.TKYFAGSNSYWISFL.TNNADVD
KZT38981.1  STTSKSTTTSSSTSTSTPPASTGFVGVSGQKFTLNCA.TYTVAGTNAYWLA..QYSNADID
OBR11693.1  ..NLVAI..RCLAVPEPRQASAFPPSASGTRFTIDGQ.AGYFAGSNSYWISFL.TNDADVD
PBK74405.1  ...HTTSTTATSTTVPAPSPSGFVTTSGTEFRLNGA.KFTVFGANSYWWGLMGYSTSDMN
POS70172.1  HVDNKQPPPAYPIGQVKGS...NAKVSGRLFSIDGK.KGYFAGSNAYWLAHL.SNDDID
PON21667.1  ..AAAST..LAAVVQPTPRASSFVTISGSHFDIDGE.VGYFAGTNCYWCFT.YNTAEVD
EIW63496.1  ...TTAPSSPTTTAAPPPSSSTGFVKVSGQKFTVLNGE.TFPLVGCANSYWWGLMGFSTAQMN
EPQ57046.1  ...SIASGAALLASLGVQAATSFVKTSQKFTLNLS.PFTVVGCANSYWWGLMGLTTAQMN
OHF02723.1  ..NLASI..QCLAVSEPRQASTFPPSASGTRFTIDGQ.TKYFAGSNSYWISFL.TNNADVD
EXP81305.1  ..NLVSI..QALAVPEPRQASTFPPSASGTRFTIDGQ.TKYFAGSNSYWISFL.TNNADVD
Q4WS1.2    ..SVPSA..TTSKKPVPTGSSSFVKADGLKFNIDGE.TKYFAGTNAYWLPPFL.TNDADVD
AFC38441.1  ..SAVSS..TPLGNARPSGSSSFVKADGLKFNIDGV.AKYFAGTNAYWLPPFQ.TNNADVD
ACH58410.1  ..SVPSA..TTSKKPVPTGSSSFVKADGLKFNIDGE.TKYFAGTNAYWLPPFL.TNDADVD
KZL71980.1  ..NLAVI..RCLAVPEPRQTAFFPSASGTRFTIDGQ.TKYFAGSNSYWISFL.TNNADVD
Q99036.1    ..TAASA..LAAVLQPVPRASSFVTISGQFNIDGK.VGYFAGTNCYWCFSFL.TNNADVD
XP_012185003.1 ...IVERKHAARDATSTSAVTGYVKTSGQNFVLDGE.TFTVVGCANSYWWGLTGLTISEMD
AGW24296.1  ...PTKT..TTTTSGPISTSTGFAHTNGLNFTIDGE.TSYFAGSNSYWIGFLL.TNNADVD

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Loop 7

	368	375	396
PMan5A	SWGSSWITAHGQACKNAGKPCFLFEYVGS...	TSLCSSEAPWQTITAIS...	VAAADLFW
TlMan5A	SWGSTWISAHGQACVNAAGKPCFLFEYVGS...	T.NHCSSEAPWQSTALST..	NGIAADSF
3WH9	DWGNWITAHGAACKAAGKPCFLFEYVGV...	TSNHCSVEGSWQKTALST..	TGVGADLFW
3WFL	SFGSPWVTAHGAACAAAGKPCFLFEYVGV...	TSNHCSEEGWQQTALDT..	RIGIADSF
Man5XZ3	DWGNLWAIITHGAACATAGKPCFLFEYVGA...	PSDHCAIEVPWQITAVSS..	TGIAGDLFW
1QNO	TWNGWITQTHAAACLAAGKPCVFEYVGA...	QONPCTNEAPWQITSL..	TTRGMGGDMFW
3ZIZ	SFGPGWIKDHAAACRAAGKPCFLFEYVGY...	ESDRCNVQKGWQASRELSRD	GMGGDLFW
ABC59553.1	DWNGWISAHGAACKAAGKPCFLFEYVGV...	TSNHCSVESPWQQTALNT..	TGVSAADLFW
ADZ99027.1	DWNGWITAHGAACKAAGKPCFLFEYVGV...	TSNHCSVEGSWQKTALST..	TGVGADLFW
XP_001262744.1	EWNGWQAQSHGAACKAAGKPCFLFEYVGV...	TSNHCALETPWQKTSLNT..	TGVSGDLYW
AAA67426.1	DWNGWITAHAAACKAVGKPCFLFEYVGV...	TSNHCAVESPWQQTAGNA..	TGISGDLYW
Q00012.1	DWNGWITAHAAACKAVGKPCFLFEYVGV...	TSNHCAVESPWQQTAGNA..	TGISGDLYW
ACH56965.1	SWGSTWILQHNEVGAAHNKAVVLEFYGGPTPN	NHTAVEEPWQATVLKD..	TKLAMQDFW
CRG88507.1	SFGSPWVTAHGAACAAAGKPCFLFEYVGV...	YSDKCTVEGGWQKTALNT..	KGIADTFW
XP_002480621.1	SWGSSWLTAHGQACKNAGKPCFLFEYVGS...	T.SHCSEAPWQSTAVSS...	VGADLFW
OOQ88079.1	PWGNLWIEAHGAACVKAGKPCFLFEYVGS...	L.AHASSEVPWQQTALKT..	NGIAGDTFW
CEJ54968.1	TWGPWTIKAHGDAACVAAGKPCFLFEYVGY...	PSNHCSVEAPWQAAALDT..	KGIAGDLFW
XP_001275153.1	AWGNRWIKSHAKVCEAAGKPCFLFEYVGI...	KDDHCGDSLKWQKTSLTT..	TANSADLFW
OTA64229.1	DWNGWITQAHGKACETAGKPCFLFEYVGS...	TTDHCGIEGKWQSTALST..	KGLAADLFW
GAO81348.1	SWGNTWIKSHAKVCEAAGKPCFLFEYVGI...	KDNHCGDSLQWQKTSLAT..	TGNSADLFW
AEY76082.1	DWNGWITAHGAACKAAGKPCFLFEYVGV...	TSNHCSVEGSWQKTALST..	TGVGADLFW
OCL08622.1	DWGAAWIKNHAAACVAAGKPCFLFEYVGV...	ASSHCSVEGSWQSTSLST..	AGMAADLFW
XP_014532729.1	DWNGWVTSHGAACAAAGKPCFLFEYVGV...	TYDHCAIEAPWQKTALDT..	KGIADLFW
B8NVK8.2	DWNGWVTSHGAACKEAGKPCFLFEYVGV...	TSDHCAVEKPWQNTALNT..	TAISGDLYW
GAQ05480.1	EWNGWVQSHGAACKAAGKPCFLFEYVGV...	TSNHCALETPWQKTSLNT..	TGVSAADLYW
KKK19644.1	DWNGWVASHGAACEAAGKPCFLFEYVGV...	TSNHCAVETPWQTSALNT..	TAVSADLYW
KMK55820.1	EWGDLWVQSHGAACATAAGKPCFLFEYVGV...	TSDHCALETPWQKTSLNT..	TGLSGDLYW
ETW81009.1	AWGVQWIQDHATSQKTANKPVILEEFGV...	TSQQATYTAWYNAIIS...	GLTGDLIW
XP_013958821.1	PWGTITWQTHAAACLAANKPCVFEYVGA...	QONPCTNEAPWQSTSLALAAR	GMGGDMFW
XP_013949127.1	PWGSSWVQTHAQACVEAGKVICIMEYVGA...	VTDRCTNMVWPQNASM..	ASLGMGGDLFW
EFI94550.1	AWGTQWIADHATSQETVGPVILEEFGV...	TSNQAQEVYTAWLDEVVSS...	GLAGDLIW
ABB88954.1	GWGTQWIADHATSMTAAGKPVILEEFGV...	TTNQATVYGAWYQEVVSS...	GLTGALIW
KKP04059.1	PWGTITWQTHAAACVAAGKPCVFEYVGA...	QONPCTNEAPWQSTSL..	ATRGMGGMFW
KXH47903.1	TWSPGWIKSHGEACAKAGKPCFLFEYVGS...	LSDHCSIEGPWQQVSRTA..	PGLGADLFW
KZT38981.1	AWGVQWIIDHAASQKSANKPVVILEEFGV...	TNNQATIIYQSWLNTIVVSS...	GLTGEQIW
OBR11693.1	TWAPGWIKSHGEACAKAGKPCFLFEYVGS...	LTDHCAVEKAWQEVSRTA..	PGLGADLFW
PBK74405.1	GWGTQWIADHATSMKAAGKPVILEEFGV...	TTNQTTVYQAWYQEVVSS...	GLTGVLIW
POS70172.1	PWGNDWIVQHDEVGKKSGKPVILEEYVGA...	PFPGNHTPIYKPWQDIVVKS...	GVAADQVW
PON21667.1	PWGSSWVQTHAQACVEAGKVICIMEYVGA...	VVDRCCTNMIPWQNTSM..	TSPGMGGDLFW
EIW63496.1	GWGSQWIADHATSQKANKPVILEEFGV...	TDGQAATYTAWYNSVSS...	GLTGDLIW
EPQ57046.1	GFGVQWIQDHATSQKTYNKPVVILEEFGV...	TSNQTGTYAQWYSTILSS...	GLTGDLIW
OHF02723.1	TWSPGWIKSHGEACAKAGKPCFLFEYVGS...	LSDHCAVEGFWQEVSRTA..	PGLGADLFW
EXF81305.1	TWSPGWIKSHGEACAKASKPCFLFEYVGS...	LSDHCSIEGPWQQVSRTA..	PGLGADLFW
Q4WBS1.2	SWGNGWVTSHAKVCKAAGKPCFLFEYVGL...	KDDHCSASLTWQKTSVS...	SGMAADLFW
AFC38441.1	SWGNGWVTSHAKVCKAAGKPCVFEYVGI...	KNDHCSASLKWQKTALAT...	NAGDLIW
ACH58410.1	SWGNGWVTSHAKVCKAAGKPCFLFEYVGL...	KDDHCSASLTWQKTSVS...	SGMAADLFW
KZL71980.1	TWSPGWIKSHGEACAKAGKPCFLFEYVGS...	LTDHCAIERAWQEVSRTA..	PGLGADLFW
Q99036.1	TWNGWITQTHAAACLAAGKPCVFEYVGA...	QONPCTNEAPWQITSL..	TTRGMGGDMFW
XP_012185003.1	EWGVWVLENHATAQKAQNKPVVILEEFGI...	TSNKTETYEAWYSAIVSS...	GLTGDLIW
AGW24296.1	DWGNLWVKAHGDACAAAGKPCFLFEYVGY...	PSDHCTIEAAWQKTALNT..	KGVAADLYW

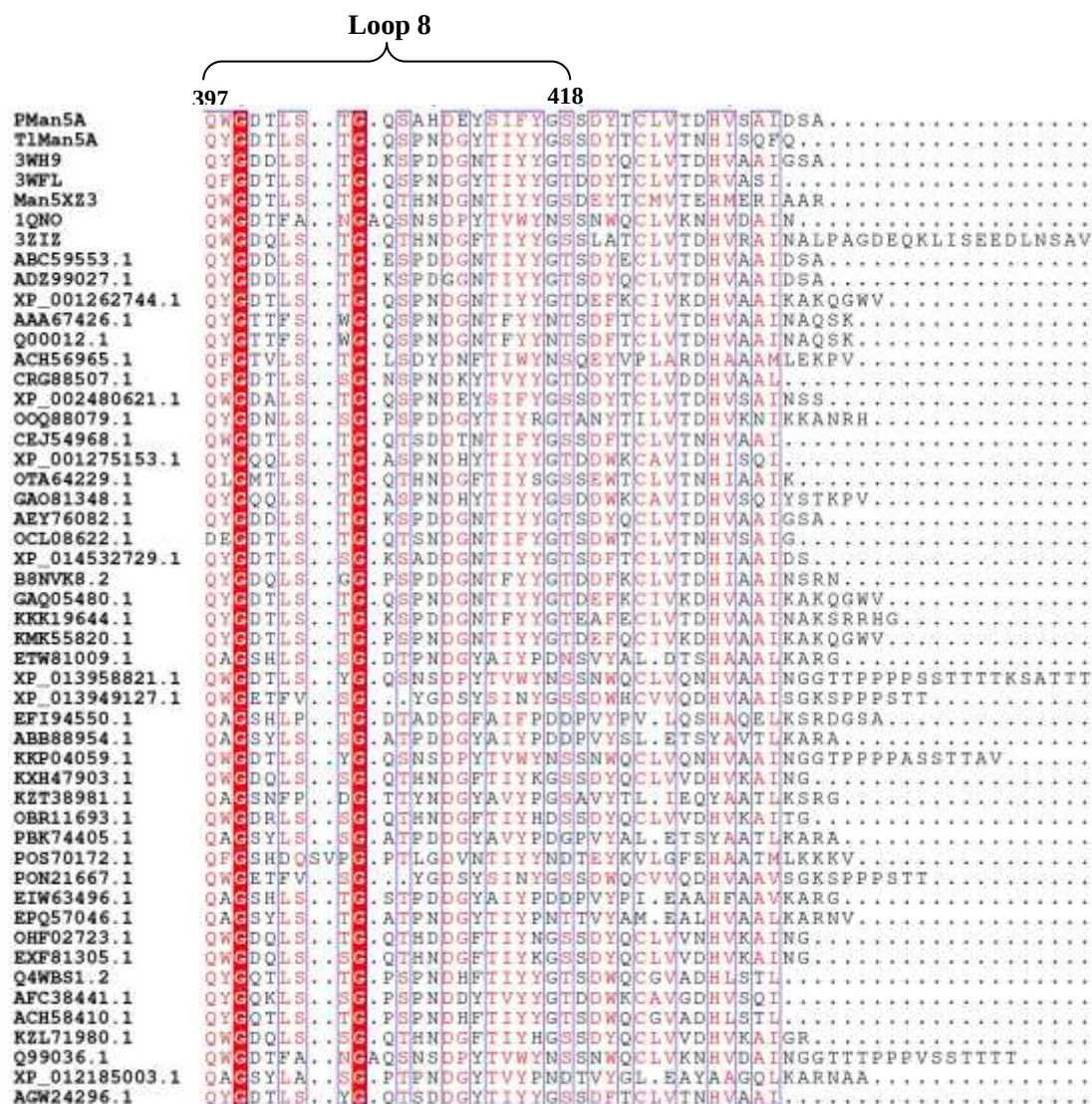


Fig.S2. Multiple sequence alignments of 50 fungal β -mannanase of GH5. Three modules of N3, Loop7 and 8 are labeled by braces.

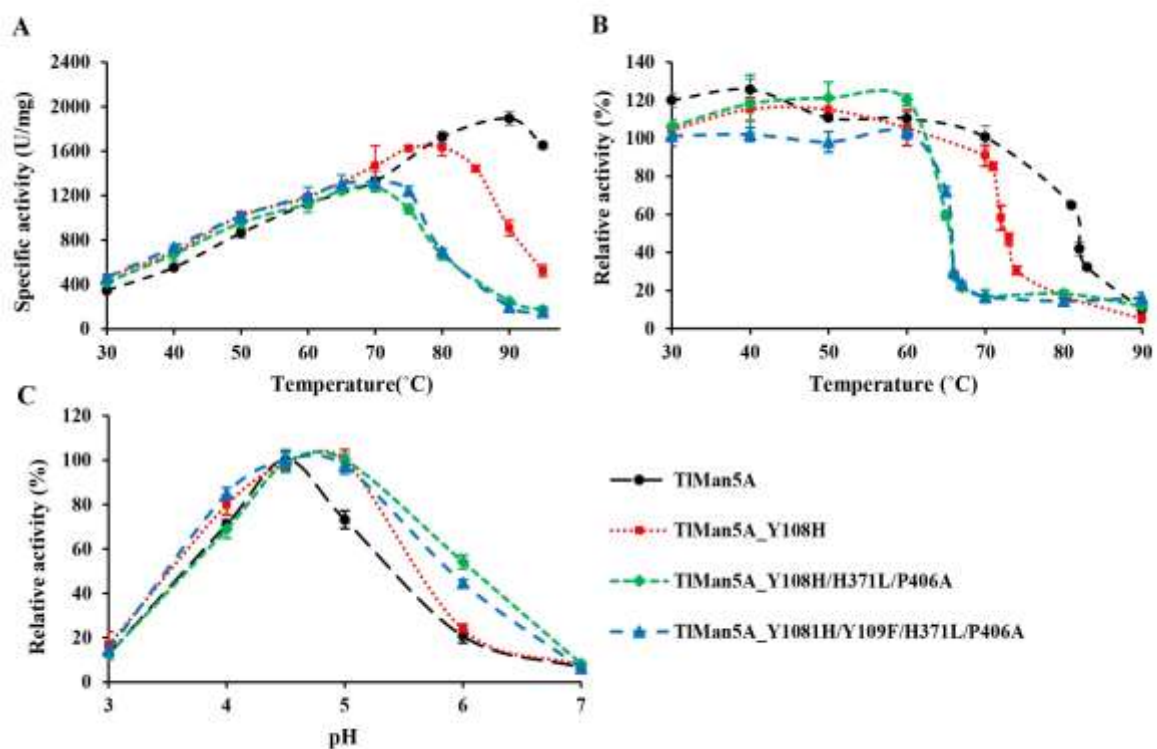


Fig.S3. Enzymatic properties of the wild type *TlMan5A* and their variants. (A) Temperature-activity profiles of *TlMan5A* and its variants tested at the optimal pH of each enzyme in the temperature range of 30–95 °C for 10 min. (B) Thermostability of *TlMan5A* and its variants investigated after 30-min incubation without substrate at indicated temperatures and pH 4.5. (C) pH-activity profiles of *TlMan5A* and its variants tested at the optimal temperature of each enzyme (70–90 °C) over the pH range of 3.0–7.0 for 10 min.