

## Supplementary Materials

### **A novel thermostable GH 3 $\beta$ -glucosidase from *Talaromyce leycettanus* with broad substrate specificity and significant of soybean isoflavone glycosides-hydrolyzing capability**

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Table S1: Primers used in this study.

Reactions	Names	Sequences(5'→3') <sup>a</sup>
The core region PCR	DP-F	GGCCGCAAYTGGGARGGNTT
	DP-R	GTCACCAGGCATNGHCATRTC
The upstream TAIL-PCR	SF1	TGAAGGGTCTTGTCGTCAATGTTGGAGCTAAT
	SF2	GGCAATAACACCGACATCCTGAATACCCTTG
The downstream TAIL-PCR	SR1	TCACGAATTGTACCTGTGGCCCTTTGCGGAT
	SR2	GCTACACGATGAACAAGCTGCTCAAAGGTGA

<sup>a</sup> The letters R, Y, N, and H represent A/G, C/T, A/T/C/G, and A/T/C, respectively.

Figure S1: Multiple sequence alignment of Bgl3B with BGLI, 5FJI and Bgl3A. Identical and similar amino acids are indicated by black and gray boxes, respectively. The catalytic residues, nucleophile Asp261 and acid/base Glu490, are also indicated.

Bgl3B.	.....KDLAVCPFFYPSFWALGNGEWEPHSRAVEFVSGTLLEKVNLTGCGWNGEFCVGNIGSIPRLGF.WGFCQDSDPLGGR	79
BGLI.	MRLGWLELAVAAAATVSAKDDLAYSPFFYPSFWMGNGEWADYRRAVDFVSQLTLLEKVNLTGCGWNGEFCVGNIGSIPRLGF.RGICQDSDPLGGR	99
5FJI	.....QELAFSPFFYPSFWALCGEWADHRRRAVEIVSQMTLEKVNLTGCGWNGEFCVGNIGSIPRLGF.RGICQDSDPLGGR	80
Bgl3A.	.....MLAEQIFLSVLAARVTVQAYGFGSGGWDPAVYGRKAAINKINQTEKVGIVTGRWNGGFCVGNIGSIPRLGF.PSICQDSDPLGGR	85
Bgl3B.	DITVNSAFFPAGNVVATWDDNLAIVRGRAMGEPHRDGVQVGLGPAAGPLGRPEGRWEGFSDPDLIGQLMPEITIGQDAGVITACARHTIENDEEH	179
BGLI.	FADIVSAFFPAGNVVATWDDNLAIVRGRAMGEPHRGVDVQVGLGPAAGPLGRPEGRWEGFSDPDLIGQLMPEITIGQDAGVITACARHTIENDEEH	199
5FJI	FSVINSAFFPAGNVVATWDDNLAIVRGRAMGEPFNDGVNIIILGPRAGPLGRPEGRWEGFSDPDLIGQLMPEITIGQDAGVITACARHTIENDEEH	180
Bgl3A.	EANPVTAFFPAGNVAATWDDSNINRGRAMGEPKAGLGVNIVGLGPAAGPLGRPEGRWEGFSDPDLIGQLMPEITIGQDAGVITACARHTIENDEEH	185
Bgl3B.	FRQVGEACQGYNYITCAISSNDDTTHELYWPFPAVAVRACVGSVCSYNGVNSVGCSSNYIINLLKCEINFGFVMSDVAARHSGVGLAAGLDM	279
BGLI.	FRQVGEAVGQYGFDTESVSSNDDTTHELYWPFPAVAVRACVGSVCSYNGVNSVGCSSNYIINLLKCEINFGFVMSDVAARHSGVGLAAGLDM	299
5FJI	FRQVGEACQGYNYITCAISSNDDTTHELYWPFPAVAVRACVGSVCSYNGVNSVGCSSNYIINLLKCEINFGFVMSDVAARHSGVGLAAGLDM	280
Bgl3A.	NR.....ETISSNDDTTHELYWPFPAVAVRACVGSVCSYNGVNSVGCSSNYIINLLKCEINFGFVMSDVAARHSGVGLAAGLDM	272
Bgl3B.	MPG...DITINCEBYWGNLITVWNGIIEFRDDMAVRINSAFYVGVG.DHVRTEPFFSWITDEYGYEHAAVNQG.YIKVNRVVRVSNRFDITRQVGS	377
BGLI.	MPG...DIAFGCKRSEWGNLITVWNGIIEFRDDMAVRIMAAAFYVGVG.DRYQVPEVFFSWIRDEYGYEHALVGN.NVKNVKVVRADPADIRQIGS	397
5FJI	MPG...LISDDICLSEWGNLITVWNGIIEFRDDMAVRIMTYFYVGVG.DRLRIFPFFSWIRDEYGYEHAASVSEGAWTKVNVFVVRQSESQLIREIGA	379
Bgl3A.	MPGSEFNNPFGSIMGNEEAVANGSVHQSRLDDVTRILASWHVGVGIEGYEYVAFESWNGK.....ANVITVGRERVVRAR	355
Bgl3B.	SSVLLKNDNLPPIPTGKRELVGINGDAGEANGVNGCSDRGCDDGLTAMWGSGTAFPPYLITPECAICREVISNG.SEVFAITDNGALDQMSVASQ	476
BGLI.	ASVLLKNDNLPPIPTGKRELVGINGDAGEANGVNGCSDRGCDDGLTAMWGSGTAFPPYLITPECAICREVISNG.SAVITDNGALDQMEQVASQ	496
5FJI	ASVLLKNTNLPPIPTGKRELVGIVGIDAGEANPVGANGCSDRGCDDGLTAMWGSGTAFPPYLITPECAICREVISNG.SNVFAITDNGALSQMDVASQ	477
Bgl3A.	DSIVLLKNDNLPPIPTGKRELVGINGDAGEANGVNGCSDRGCDDGLTAMWGSGTAFPPYLITPECAICREVISNG.SNVFAITDNGALSQMDVASQ	452
Bgl3B.	ASVSIYFVNDVSGEGSNVVGNGGDRNLDLIRKGEVIVKVAANCNMIVVVEIVGVPVLEIDWYDNPVVAIHWGLPGQESGNSLVDVLYGRVPSGK	576
BGLI.	ASVSIYFVNDVSGEGSNVVGNGGDRNLDLIRKGEVIVKVAANCNMIVVVEIVGVPVLEIDWYDNPVVAIHWGLPGQESGNSLVDVLYGRVPSGK	596
5FJI	SSVSIYFVNDVSGEGSNVVGNGGDRNLDLIRKGEVIVKVAANCNMIVVVEIVGVPVLEIDWYDNPVVAIHWGLPGQESGNSLVDVLYGRVPSGK	577
Bgl3A.	AGTAVYFVNDVSGEGSNVVGNGGDRNLDLIRKGNELVQVAANANKMIVVVEIVGVPVLEITLACPNVAIHWGLPGQESGNSLVDVLYGSTESGK	552
Bgl3B.	LPFTNGKSFDSVGSWVITFPNNGNDAPQIDFSEGVFIDYRFRDKNNTPEYEFEGLSYTTFRYSNLQITPLN...APKVTASGKIDPAESFGQPGS.AS	673
BGLI.	LPFTNGKTRRESVGAPELLTKPNNGKGAQDDFREGVFDYRFRDKNNTPEYEFEGLSYTTFRYSNLIWQPLN...ARPVTASGSKRAAPTFFGNISTDYA	694
5FJI	LPFTNGKTRRESVGAPELLTEPNNGKGAQDDFREGVFDYRFRDKNNTPEYEFEGLSYTTFRYSNLIWQALNSSSSAVVETSGEKKPAPITYEIGS.AA	676
Bgl3A.	LPFTNGKTRRESVGAPELLTEPNNGKGAQDDFREGVFDYRFRDKNNTPEYEFEGLSYTTFRYSNLIWQALNSSSSAVVETSGEKKPAPITYEIGS.AA	619
Bgl3B.	QYVFERILNRIYEIYYPWLNSTNLRSSGDPDYGMKASAVTEGATIGSAQELLPASGPGGNEGLDELFRVSAITINNGVNGEVEVQLYVSLGG..P	771
BGLI.	DVLYPEDIHRVLIYIYPWLNSTNLPKSSGDPDYGMKAEVIEGATIGSPQELIPAGGPGGNEGLDEMYRVSATINNGVNGEVEVQLYVSLGG..P	792
5FJI	DVLYPEGLKRITKFIYYPWLNSTNLRSSGDPDYGMKAEVIEGATIGSPQELIPAGGPGGNEGLDLYVVSATINNGVNGEVEVQLYVSLGG..P	774
Bgl3A.	.....EGAAEGP.....IVPQGVKREFTIIVGTIVTVQNSGRVAGVEVAQLYIGIEFSAP	669
Bgl3B.	NEEKVLLRNFDRINLEPGQSVETDILRRDLSDWDVAQDQVWISKYPTIVVGGSSSRRLPLQATLPCV	840
BGLI.	DEEKVLLRNFDRINLEPGQSVETDILRRDLSDWDVAPQVWVTKYPTIVVGGSSSRRLHLQALPLPY	861
5FJI	NBERVLLRNFDRINLEPGQSVETDILRRDLAWDVDAQVWVTKYPTIVVGGSSSRRLPLRPLRNV	843
Bgl3A.	SEPKQLRGEORLHLRPGQREGATFELRRDLSDWDVCCQVWVPSGTRKVVGGSSSRDIREQGSRI.	737

Figure S2: The homology modeled structure of Bgl3B with the crystal structure of  $\beta$ -glucosidase from *A. fumigatus* (5FJI) as the template. The N-terminal catalytic domain (TIM-barrel), the C-terminal catalytic domain ( $\alpha/\beta$  sandwich), and the fibronectin type III domain (Fn3) are indicated in green, yellow and blue, respectively.

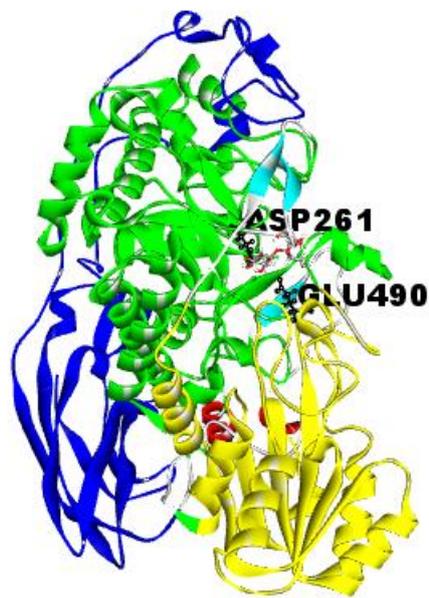


Figure S3: HPLC chromatograms of isoflavones. The purple line indicates the isoflavones in soybean flour extract; the red line indicates soybean flour extract hydrolyzed with 0.05 U of Bgl3B at 37 °C for 10 min; the black line indicates soybean flour extract hydrolyzed with 0.05 U of commercial  $\beta$ -glucosidase G4511 at 37 °C for 10 min.

