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Supplementary Material

Role of the non-conserved amino acid Asparagine 285 in the glycone-binding pocket of *Neosartorya fischeri* β-glucosidase

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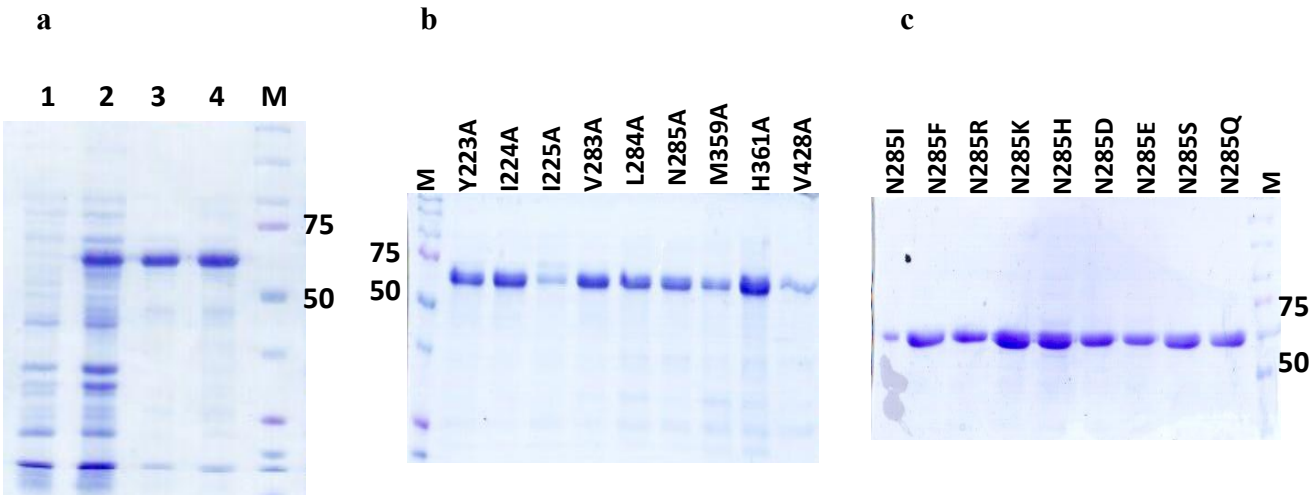
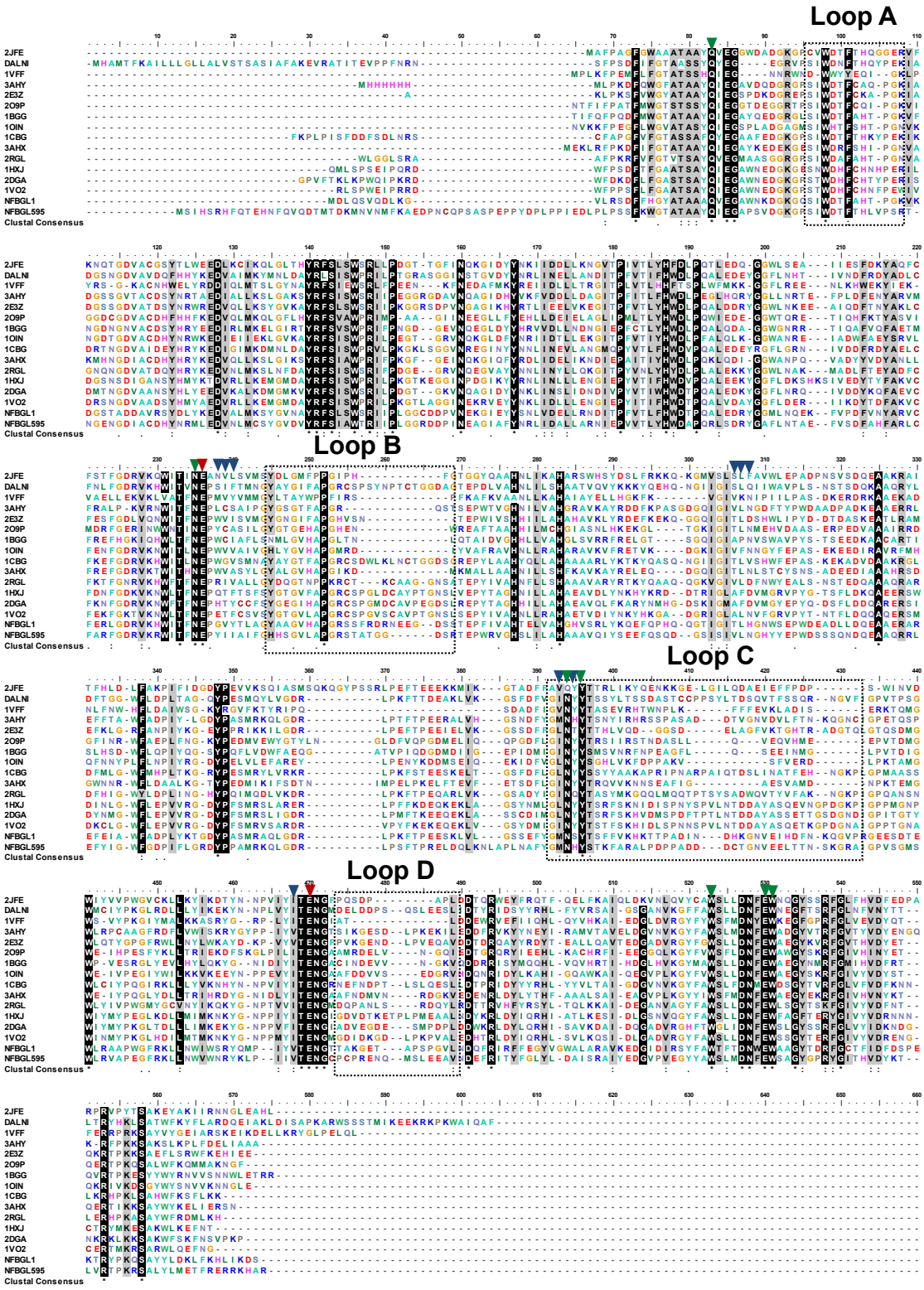
Supplementary Fig. S1



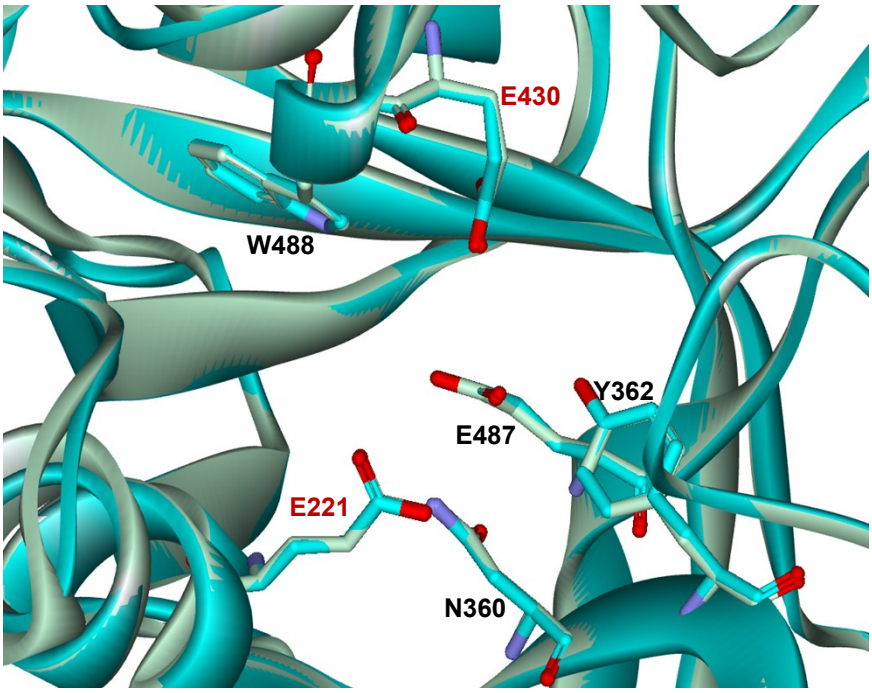
Supplementary Fig. S1 Analysis of protein expression for NfBGL595 and its mutants. **(a)** Purification and determination of the subunit molecular mass by SDS-PAGE. Lane 1 shows the soluble fraction of *E. coli* expressing pET28a; lane 2 shows the soluble fraction expressing pET28a-bgl1; lane 3 and 4 show the purified NfBGL1; and lane M is the marker. **(b)** SDS-PAGE analysis of the NfBGL595 mutants. Lane M contains the protein marker and lanes Y223A, I224A, I225A, V283A, L284A, N285A, M359A, H361A, and V428A correspond to the purified mutant enzymes with a molecular weight of ~60 kDa. **(c)** SDS-PAGE analysis of the N285 mutants. Lane M contains the protein marker and lanes N285I, N285F, N285R, N285K, N285H, N285D, N285E, N285S, and N285Q correspond to the purified mutant enzymes with a molecular weight of ~60 kDa.

Supplementary Fig. S2a



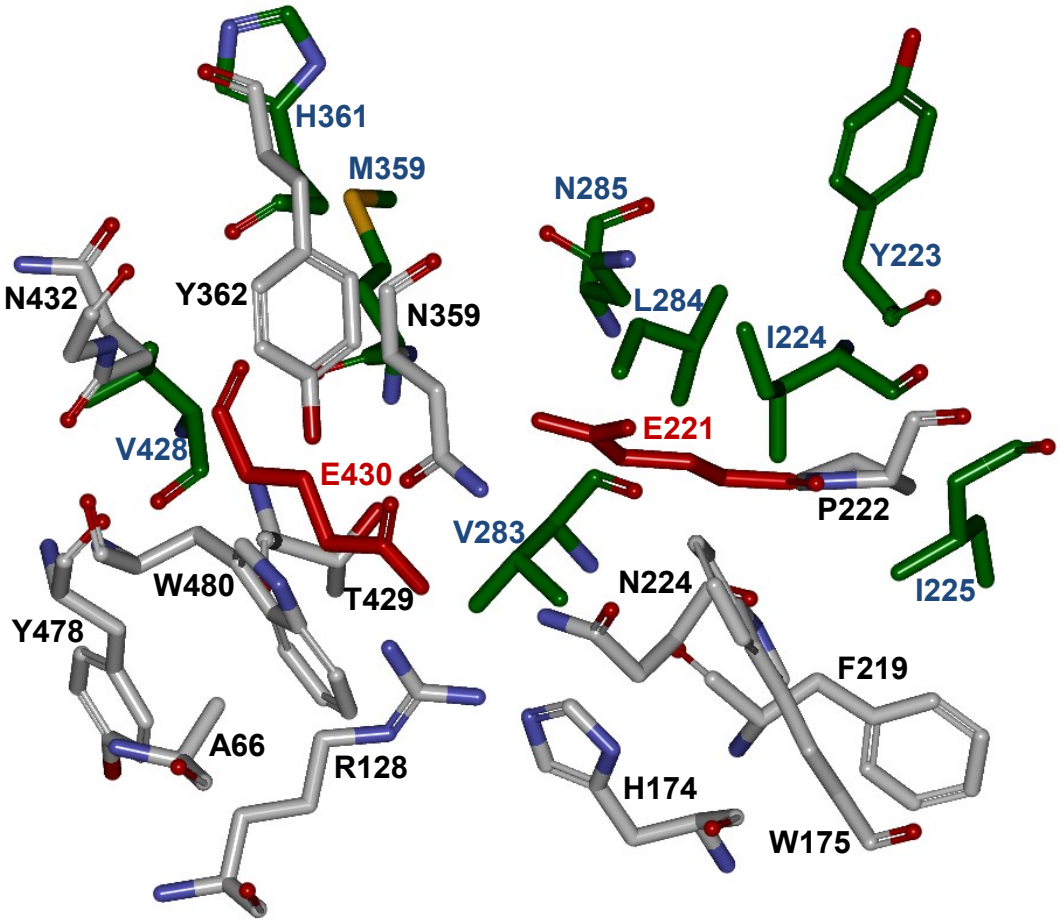
Supplementary Fig. S2a Sequence alignment of BGL from various sources. The glycone binding residues, active site residues, and non-conserved residues are indicated by green, red, and blue inverted triangles, respectively. The major loops A, B, C, and D are boxed with dotted lines. The codes 2JFE, DALNI, 1VFF, 3AHY, 2E3Z, 2O9P, 1BGG, 1OIN, 1CGB, 3AHX, 2RGL, 1HXJ, 2DGA, 1VO2, NfBGL1, and NfBGL595 correspond to β-glucosidases from *Homo sapiens*, *Dalbergia nigrescens*, *Pyrococcus horikoshii*, *Trichoderma reesei*, *Phanerochaete chrysosporium*, *Paenibacillus polymyxa* BGLB*, Paenibacillus Polymyxa* BGLA, *Thermotoga Maritima*, *Trifolium repens, Clostridium cellulovorans, Oryza sativa, Zea mays, Triticum aestivum, Sorghum bicolor, N. fischeri* BGL1, and *N. fischeri* BGL595, respectively. DALNI, NfBGL1, and NfBGL595 protein accession numbers are A3RF67, XM_001263203.1, and XM_001258595.1, respectively. Other codes represent the PDB codes.

Supplementary Fig. S2b



Supplementary Fig. S2b Molecular modeling of the active site pocket of NfBGL595 (cyan). Active site residues were superimposed on the template TrBGL2 (gray). Based on the superimposition, the active site residues and conserved residues of NfBGL595 (E221, N360, Y362, E430, E487, and W488) and those of TrBGL2 (E165, N296, Y298, E367, E424, and W425) have a similar orientation and position.

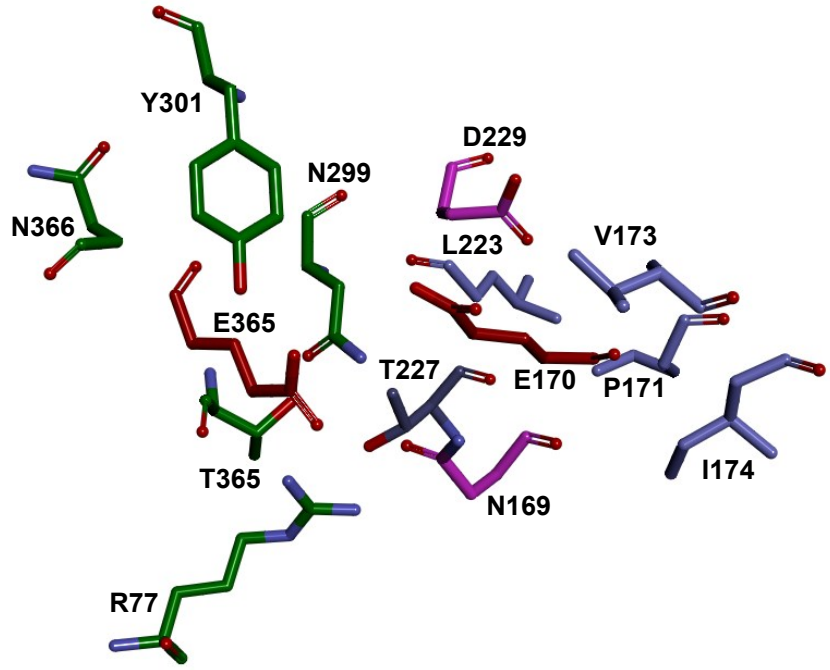
Supplementary Fig. S3



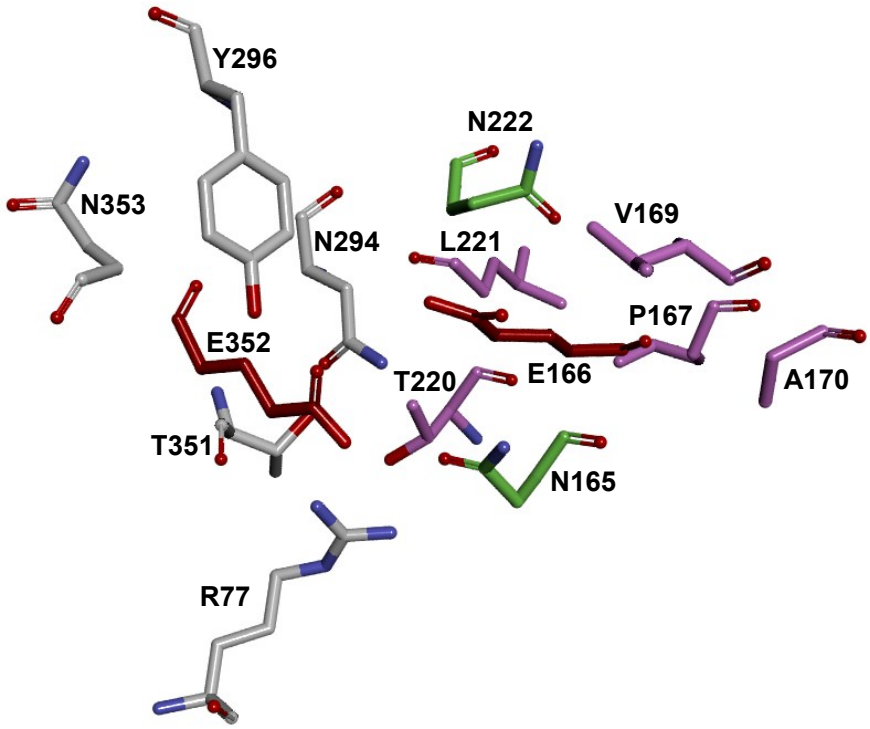
Supplementary Fig. S3 Residues in the substrate-binding pocket of NfBGL595 from *N. fischeri*. Conserved residues (grey) and non-conserved residues (green) around the catalytic amino acids (red) are shown.

Supplementary Fig. S4

a



b



Supplementary Fig. S4 Distribution of the polar and non-polar residues in the substrate-binding pocket of BGL from *P. chrysosporium* (2E3Z) and *T. reesei* (3AHY). The catalytic nucleophile residue E365 of 2E3Z is surrounded by the polar residues R77, N299, Y301, and T365, and in 3AHY, the nucleophile E352 is surrounded by R77, N294, Y296, and T351. Whereas the general acid/base residue E170 of 2E3Z is dominated by the non-polar residues L223, V173, P171, and I174, the acid/base residue E166 of 3AHY is dominated by L221, V169, P167, and A170. *P. chrysosporium* (2E3Z) and *T. reesei* (3AHY) have D229 and N222 residue, respectively, at the equivalent position of N285 from NfBGL595. The catalytic residues are shown in red.

Supplementary Table S1 List of oligonucleotide primers used in the study. The mutation site is bold faced underlined.

Primer name	Sequence (5' to 3')
Y223AForward	ATTACGTTTAACGAGCCT**GCG**ATCATCGCGATC
Y223A Reverse	AGGCTCGTTAAACGTAATCCATCTCTTGAC
I224AForward	GTTTAACGAGCCTTAT**GCG**ATCGCGATC
I224AReverse	ATAAGGCTCGTTAAACGTAATCCATCTC
I225AForward	AACGAGCCTTATATC**GCG**GCGATCTTTG
I225A Reverse	GATATAAGGCTCGTTAAACGTAATCCA
V283AForward	ATGGGAGCATCTCAATC**GCG**CTGAATG
V283AReverse	CGATTGAGATGCTCCCATCCTGCGA
L284AForward	GAGCATCTCAATCGTG**GCG**AATGGGCAC
L284A Reverse	CACGATTGAGATGCTCCCATCCTGCGA
N285A Forward	ATCTCAATCGTGCTG**GCG**GGGCACTATTAC
N285A Reverse	CAGCACGATTGAGATGCTCCCATCCTG
M359A Forward	AACGCCTTCTACGGA**GCA**AACCACTACTCC
M359A Reverse	GGGCGAGTTGCGGAAGATGCCTTACTTG
H361A Forward	CCTTCTACGGAATGAAC**GCG**TACTCCACCAA
H361A Reverse	GTTCATTCCGTAGAAGGCGTTGAGCGGGGC
V428A Forward	GTACAAGCTGCCTATCATT**GCG**ACAGAGAATGG
V428A Reverse	CAATGATAGGCAGCTTGTACCGATTCCACA
N285 Reverse	CAGCACGATTGAGATGCTCCCATCCTGCGA
N285HForward	AGCATCTCAATCGTGCTG**CAC**GGGCACTATTAC
N285F Forward	AGCATCTCAATCGTGCTG**CAC**GGGCACTATTAC
N285I Forward	CATCTCAATCGTGCTG**ATT**GGGCACTATTA
N285IReverse	TCAGCACGATTGAGATGCTCCCATCCTGCG
N285DForward	CATCTCAATCGTGCTG**GAT**GGGCACTATT
N285S Forward	GCATCTCAATCGTGCTGA**GCG**GGCACTA
N285S Reverse	TCAGCACGATTGAGATGCTCCCATCCTG
N285E Forward	ATCTCAATCGTGCTG**GAA**GGGCACTATT
N285R Forward	ATCTCAATCGTGCTG**CGT**GGGCACTATT
N285K Forward	CTCAATCGTGCTGAA**AGG**GCACTATTAC
N285Q Forward	AGCATCTCAATCGTGCTG**CAG**GGGCACTATTAC

N285 Reverse acts as a common primer for N285H Forward, N285F Forward, N285D Forward, N285E Forward, N285R Forward, N285K Forward, and N285Q Forward primers.

Supplementary Table S2 Properties of BGLs active toward flavones from various sources.

BGL Source	Flavone substrates	K_m (mM)	k_{cat}/K_m (mM^{-1} s^{-1})	Reference
Thermotoga neapolitana (recombinant)	Quercetin-3-glucoside Quercetin-3,4-glucosides	0.06 ± 0.03 0.13 ± 0.06	0.055 0.122	(Khan et al. 2011)
Aspergillus oryzae RIB40	Daidzein Genistein Glycitein	ND	ND	(Kaya et al. 2008)
Aspergillus oryzae CBS 12559 *(native)*	Quercetin Naringin	-	-	(Riou et al. 1998)
Saccharomyces cerevisiae (recombinant)	NAR 7-glucoside Eriodictyol 7-*O*-*β*-glucoside Lu 4-gluc Genistin Daidzein	+ + + + +	-	(Schmidt et al. 2011)
Penicillium decumbens (native) GI/GII	Daidzein-7-glcoside Quercetin-3-rhamoside Kaempferol-3-glcoside Quercetin-3-glcoside Apigenin-7-glcoside Naringenin-7-glcoside	0.97/0.65 ND/ND ND/ND ND/0.06 0.54/0.41 0.67/0.45	0.335/1.65 ND/ND ND/ND ND/0.01 0.30/1.5 0.28/1.39	(Mamma et al. 2004)
Neosartorya fischeri (recombinant)	Quercetin 3-β-D-glcoside Hesperetin 7-Rhamnoglucoside Genistein-7-glucose Apigenin-7-glucose Kaempferol-7-glucose	0.65 ± 0.08 1.06 ± 0.19 0.075 ± 0.014 0.030 ± 0.005 0.17 ± 0.02	759 9.42 1670 747 98.8	This study

Reference

Kaya M, Ito J, Kotaka A, Matsumura K, Bando H, Sahara H, Ogino C, Shibasaki S, Kuroda K, Ueda M, Kondo A, Hata Y (2008) Isoflavone aglycones production from isoflavone glycosides by display of beta-glucosidase from Aspergillus oryzae on yeast cell surface. Appl Microbiol Biotechnol 79(1):51-60.

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Mamma D, Hatzinkdaou DG, Christakopoulos. P (2004) Biochemical and catalytic properties of two intracellular beta-glucosidases from the fungus Penicillium decumbens active on flavonoid glucosides. J Mol Catal 27:183-190.

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