

In-Depth Characterization of Debranching Type I Pullulanase from *Priestia koreensis* HL12 as Potential Biocatalyst for Starch Saccharification and Modification

Daran Prongjit ¹, Hataikarn Lekakarn ^{1,*}, Benjarat Bunternngsook ², Katesuda Aiewviriyasakul ², Wipawee Sritusnee ², Nattapol Arunrattanamook ² and Verawat Champreda ²

¹ Department of Biotechnology, Faculty of Science and Technology, Thammasat University, Rangsit Campus, Khlong Nueang, Khlong Luang 12120, Pathum Thani, Thailand

² Enzyme Technology Research Team, Biorefinery Technology and Bioproduct Research Group, National Center for Genetic Engineering and Biotechnology, 113 Thailand Science Park, Phahonyothin Road, Khlong Nueang, Khlong Luang 12120, Pathum Thani, Thailand

* Correspondence: hataikarn.lek@sci.tu.ac.th; Tel.: +66-2564-4441 (ext. 2452)

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1BF2  -----AINMSLGASYDAQANITFRVSSQATRIVLVLYSAGYGVQESATYTL-----SPAGSGVNAVTFPVSSIKAAGITGAVVYGYRAWGNPNWPNYASNWKGSQAGFVSDVDANGDRFNPNKLLDPYAEVVSQDP----- 129
4J7R  -----TAYGPALTGRPAPLGASIDATGAINFSVFSSAESVSLVLTADLNAGRATFEIPLDPYVNRGTGDVNHIMLF-----DLRDDLLGYRVEGVHQEE-----DKDYPGMRHDKRRVLDPYAVAVLNRRRWGQ 175
2WSK  -----MTQLAIGKPAPLGAHY--DGQGVNFTLFSAHAERVELCVFDANGQEH--RYDL-----PGHSGDINHGYLF-----DARPGRLRYGYRVHGPWQPA-----EGHRENPAKLLIDPCARQIDGFEKDNF 108
2VNC  -----TRDRPLRPGDPYPLGSNWIEDDDGVNFSLSFSENAEKVELLLYSLTNQKYPKEIIEV--KNKTGDIHHVFP-----GLRPGQLYAYRVYGPYKPE-----LGLRFNPKNVLIDPYAKAINGSVIWND 121
4AIO  DVTGLQLPGVLDDMFAYTG--PLGAVFSEDS--VSLHLNAPTAAQGVSVCFDGPAG--PA-LETVQ--LLEBSNGVNSVTGP-----REWENRYLYEVDVYH-----PTKAQVLKCLAGDPYARSLSANG----- 219
2FGZ  SATQVQTAGVLDDTYAAAAEALSYGAQLTDGG--VTRFVNAPTAAQGVSVCFDGPAG--PA-LETVQ--LLEBSNGVNSVTGP-----REWENRYLYEVDVYH-----PTKAQVLKCLAGDPYARSLSANG----- 388
2YOC  SATQVQTAGVLDDTYAAAAEALSYGAQLTDGG--VTRFVNAPTAAQGVSVCFDGPAG--PA-LETVQ--LLEBSNGVNSVTGP-----REWENRYLYEVDVYH-----PTKAQVLKCLAGDPYARSLSANG----- 376
3FAW  NVTTQSWEFKQQLYAYSG--NLGAVLNQDQGSKEASLSPSADSVTMIIYDKNDQNRVVTPL--VNNKGVMQTLID--TKLGKINYTGYYLYEIKR-----GKDKVKILDPYAKSLAEDSNT-- 231
2YA0  -----SWRLKDETSYDYG--KLGAADLKEEGKQVDLTLSPSADKSVVVYDKNDPKVVGTVLAL--EGERGTNKTQLDSTNKLGITDFTGYYYQYQIER-----CGKTVLALDPYAKSLAEDSNT-- 114
2WAN  --NILPRNVNLPRYDYSN--DLGNVYSKDA--TSFVNAPTASNVQLLLYNSEK--SITKQLEM--QSDNGTKKLQVS-----GNLENWYLYQVTV-----NGTQTAVDPYAKSLAEDSNT-- 403
2E8Y  --GAVIRTAAFDDFFYYDG--ELGAVYTADH--TVFKVWAPATSAVKLSHPNKS--GRTFQM--TLEKGVYAVTVT-----GDLHGVEYLFICN-----NSEWMTVDQYAKAVTVNG-- 189
3WDH  --GAVMRKAFDDLYAYDGN--DLGATYDPEK--TTFKVNAPTATDVLLKLIHPTTK--EETTYVM--TEKKGLWYTVYV-----DDVERFLYTYMTYV-----NFIWREAVDPYAKSVSVNG-- 195
HL12PUL--GAVIRTPFDQLFFYTGS--DLGVTLTKTE--TVFKLWAPSAATEAKVKLLHPETK--EEKLREM--MSEKGVWAWCEP-----KHLDGILYTYLVRI-----NGTWTEAVDPYARGLTANS-- 189

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Figure S1. Sequence alignment of CBM48 in HL12Pul and α -glucan debranching enzymes in GH13_11-14 subfamilies [3,9]. Two conserved tryptophans and a functionally important lysine based on [25] are highlighted in yellow and red, respectively. Alpha-glucan debranching sequences in sequence alignment include soamylase from *Chlamydomonas reinhardtii* (4J7R), glycogen debranching enzyme from *Escherichia coli* K-12 (2WSK), isoamylase from *Pseudomonas amyloclavata* (1BF2), glycogen operon protein GLGX from *Sulfolobus solfataricus* (2VNC), reticulocyte binding protein from *Streptococcus agalactiae* COH1 (3FAW), putative alkaline amylopullulanase from *Streptococcus pneumoniae* (2YA0), limit dextrinase from *Hordeum vulgare* (4AIO), alpha-dextrin endo-1,6-alpha-glucosidase from *Klebsiella pneumoniae* (2FGZ), pullulanase from *Klebsiella oxytoca* (2YOC), type I pullulanase from *Anoxybacillus* sp. LM18-11 (3WDH), pullulanase from *Bacillus acidopullulyticus* (2WAN), and AmyX protein from *Bacillus subtilis* (2E8Y).

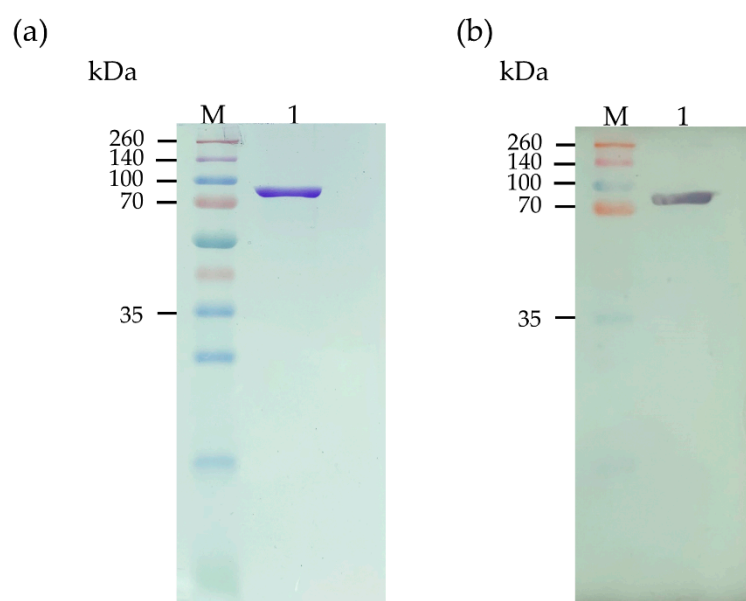


Figure S2. Investigation of recombinant intracellular HL12Pul produced by *E. coli* BL21(DE3). **(a)** Purified HL12Pul on SDS-PAGE analysis **(b)** Purified HL12Pul on Western blot analysis. Lane M: Prestained protein marker (Thermo Scientific). Lane 1: purified HL12Pul fraction.