

## Supplementary materials

### **Identification of an amyloamylase from the halophilic archaeon *Haloquadratum walsbyi* by functional metagenomics: structural and functional insights.**

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**Table S1. Amino acids of conserved regions in amyloamylases of *T. thermophilus* and *H. walsbyi*.**

	<i>T. thermophilus</i> (O87172)	<i>H. walsbyi</i> (MZ422727)
ACTIVE SITE		
Catalytic residues	D293 E340 D395	D345 E392 D445
<i>i)</i> Four conserved amino acids for substrate binding	Y59 D213 R291 H394	Y61 D266 R343 H444
<i>ii)</i> Other conserved amino acids of the active site	F217 W258 H294 L342 N464	Y270 W310 H346 L394 N508
<i>iii)</i> Conserved amino acids forming part of the cleft around the active center	S57 P58 D341 G343 T393 P466	S59 P60 D393 G395 T443 P510
<i>iv)</i> 250s LOOP	P247 P248 D249 Y250 F251 S252 E253 T254 G255 W302	P300 P301 T302 D303 -- S304 D305 D306 G307 Y354
SUBSTRATE BINDING SITE SECONDARY	Y54 Y101 Y465	H56 R107 E509



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P15977_Escherichia-coli          -----DDEQMAAFRQFVAEQGDSLFWQAQFDAL
Q6R608_Solanum-tuberosum        -----TILNSKSFQEFFSENQEWLKPAAFCFL
Q030T7_Lactococcus-lactis      -----KYQKTKSYQQFYKQNEEWLKYACFSYL
A6YM39_Borrelia-burgdorferi    -----RASAEVRSFEKFKKSSYWLLDFASVAF
B5RKZ2_Borrelia-duttonii      -----RATIDEMYAFEFKFKSAAYWLLDFSSVAF
A1QYW1_Borrelia-turicatae      -----RATIDEIHAFEFKFKSAAYWLLDFSSVAF
O87172_Thermus-thermophilus    -----KASPEEREAFAAFREAEAWLEDYALFMAL
UBZ54001_Haloquadratum-walsbyi_MdS VNSGVDGDTVPEVTVTLGESVNLSDQTPADSQIEKDAREFKSFYQRENHWLTDYALFMAL
                                     .   :   *   *   :   *   :
                                     α4           α5

P15977_Escherichia-coli          HAQQVKEDEMRRGW---PAWPEMYQNVDSPEVRQFCEEHRDDVDFYLWLQWLAYSQFAAC
Q6R608_Solanum-tuberosum        RNFFFETSERSQWGRF--SEFSKEKL----EKLVSKESLHYEVVSFYFYIYQFHLHLQLSEA
Q030T7_Lactococcus-lactis      RDINKSANFLAWGKN--ANYDKNLF---DKLKKET---SQLDLYIFVQYLLHSQLTEA
A6YM39_Borrelia-burgdorferi    KEYFFK---ESKNAFNVLFDGRGILKNEKDLFKLRNLSKEIKVQVGLQYFFFSQFQAL
B5RKZ2_Borrelia-duttonii      REYYCRF---QDPQTFNLLFSKEILKRDPKALMRLRETINVEINIQQVLQYFFFSQFKAL
A1QYW1_Borrelia-turicatae      KEYYSKF---QSSQAFNLLFSREILKRDAKALAKLRETFLEVEINIQQVLQYFFFSQFKAL
O87172_Thermus-thermophilus    KGAGHGL---PW----NRWPLPLRKREKALREAKSALAEVAFHAFTQWLFFRQWGAL
UBZ54001_Haloquadratum-walsbyi_MdS RTSYDG---AW----TDWPEPIRNRDPDALRDQYESLESVDLYHLIVQFIFDQQWQSL
                                     :           :           :           :           :           :
                                     α6           α7

P15977_Escherichia-coli          WEISQGYEMPIGLYRDLAVGVAEGGAETWCDRELYCLK-----ASVGAPPDILGPLGQN
Q6R608_Solanum-tuberosum        AEYAR--KKGVVLLKGDLPIGVDRNSVDTWVYPNLFRMN-----TSTGAPPDYFDKNGQN
Q030T7_Lactococcus-lactis      VDYCH--KLGIALKGDIAIGIAHDSVDAWTHPELPHLD-----KQAGAPPDVFAVNGQN
A6YM39_Borrelia-burgdorferi    KRYAN--DKGIELIMNVFFFIAYDSADVWAYQYFKLRFDASKDKIAGISPDYFLEQEQA
B5RKZ2_Borrelia-duttonii      KRYAS--NVGIKIISDMPIFMFYDSADVWAHQYFKLRFDASKDKVGTGMPSGNLSYKKYL
A1QYW1_Borrelia-turicatae      KRYAN--NAGIKIVSDIPIFVSYDSADVWAHQYFKLRFDASKDKVGTGMPDCLFSSKYL
O87172_Thermus-thermophilus    KAEAE--ALGIRIIGDMPIFVAEDSAEVWAHPEFWHLDEEGRPTVVAGVPPDYFSETGQR
UBZ54001_Haloquadratum-walsbyi_MdS QTVAA--NRGVTLVGDLPIYVALDSADVWATPEIFQLTDTNEPAVVAGVPPTD--SDDGQR
                                     .   :   :   :   :   :   :   :   :   :   :   :   :   :
                                     α9           α10          β8           β9

P15977_Escherichia-coli          WGLPPMDPHIITARAYEPFIELLRANMQNGALRIDHVM SMLRLWIPYGETADQ-----
Q6R608_Solanum-tuberosum        WGFPTYNWEEMSKDNYGWRARLTQMGKYFTAYRIDHILGFFRIWELPEHAMTGLCGKFR
Q030T7_Lactococcus-lactis      WGFPTYNWEKMAEDGYDWKKRLTAMSNYFDAYRLDHLGFFRIWQMPENSVRGLGQFS
A6YM39_Borrelia-burgdorferi    WDSAAYSWNVLKFKFYEWAKRIGVLRKYADIIKIDHFRGFSVSTWEVSAGESYAF----
B5RKZ2_Borrelia-duttonii      WGNAAYNWKALRKDDYVWWINRIGFMHKYVDIIKFDYFRGFVSTWEISEREFSVYSS---
A1QYW1_Borrelia-turicatae      WGNAAYNWKALRKDDYVWWINRIDFLRKYVDIVRIDYFRGFVSTWEVSAEESLLF----
O87172_Thermus-thermophilus    WGNPLYRWDVLEREGFSFWIRRLEKALELFHLVRIDHFRGFEAYWEIPASCPTAV----
UBZ54001_Haloquadratum-walsbyi_MdS WGNPVYDWAYLRNTGYNWLLDRLDRLFDLVDVTRIDHFKGDFSYYAIPIDADNPA----
                                     *   :   :   :   :   :   :   :   :   :   :   :   :
                                     β10          α11

P15977_Escherichia-coli          -----GAY-----VHY--PV-----DDL
Q6R608_Solanum-tuberosum        PSIPISQEELESE----GLWDFNRLTHPYIGQDLLQEKFGASW-TIIASTFLNEYQKGF
Q030T7_Lactococcus-lactis      PALALSAAEEIENNYGIPFRQWGIERFLLPFIKDWVIDEVFGRDNRDWIQTFLDYIGDSN
A6YM39_Borrelia-burgdorferi    -----NGLW-----VKS--PG-----RDF
B5RKZ2_Borrelia-duttonii      -----SGQW-----VKC--PG-----KDF
A1QYW1_Borrelia-turicatae      -----GGQW-----VKC--PG-----KDF
O87172_Thermus-thermophilus    -----EGRW-----VKA--PG-----EKL
UBZ54001_Haloquadratum-walsbyi_MdS -----AGEW-----HSV--PG-----YDF
                                     :
                                     β11

P15977_Escherichia-coli          L SIL-----AL-----
Q6R608_Solanum-tuberosum        YEFKDECNTEKKIASALKSFLTSMFVSEEEKLRRKLPDLLQNALIKDPEDPRKFFYPRF
Q030T7_Lactococcus-lactis      YRFKAEFNNQKAIENT-----QMENWVREGLYKQLQENVI FLKDDENSEKYHPRF
A6YM39_Borrelia-burgdorferi    FNFILNEI-----K-----DLK-----
B5RKZ2_Borrelia-duttonii      FRNVLSKI-----N-----DLE-----
A1QYW1_Borrelia-turicatae      FKQILNEI-----N-----DLE-----
O87172_Thermus-thermophilus    PKIQEVF-----G-----EVP-----
UBZ54001_Haloquadratum-walsbyi_MdS FETVREHI-----G-----SLP-----

P15977_Escherichia-coli          -----ESKRHRMVGIEDLG
Q6R608_Solanum-tuberosum        NVEDTTSFMDLDQHSQNVLKRLLYDYYFHRQEGLRDWNFAKTLFVLLNSSDMLACGEDLG
Q030T7_Lactococcus-lactis      GLLSTISFREFGDDYKGRRLERLYNDYFYGRNYDFWKEKAYEKLPA LKNATKMLACGEDLG
A6YM39_Borrelia-burgdorferi    -----IWVEDFQ
B5RKZ2_Borrelia-duttonii      -----IWVEDLL
A1QYW1_Borrelia-turicatae      -----IWVEDLV
O87172_Thermus-thermophilus    -----VLAEDLG
UBZ54001_Haloquadratum-walsbyi_MdS -----FIIEDLG
                                     ** :

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          α12          β12          α13          β13          α14
P15977_Escherichia-coli      TVPVEIVGKLRSSGVSYKVLVYFENDHEK-TFRAPKAYPEQ--SMAVAATHDLPTLRGYW
Q6R608_Solanum-tuberosum    LIPSCVHPVMQELGLVGLRIQRMPEPDV-EFGIPSQNYM--TVCAPSCHDCSTLRAWW
Q030T7_Lactococcus-lactis   MVPDNVDPVMYHLDLILRLIIERMPADERF-VSS-LSEVPYL--SVVTTSSHDTSPLRRAW
A6YM39_Borrelia-burgdorferi NDLEDVSRLLRDFNFNPGMKIMNLAFFDSSNQNLPHNYIKN--CIVYTGSGDNDTIREFI
B5RKZ2_Borrelia-duttonii    EDRGDAFRLRDYFNFPETRIMQFAFDSDKNLCLPHNYIKN--CVVYTSTNESNTIRQFI
A1QYW1_Borrelia-turicatae   KDRGDTFRLRDYFGFPGTKMMQCAFDFDSANIYLPNHNYIRN--CVVYTGTHESNTIRGFI
O87172_Thermus-thermophilus VITPEVEALRRDFGLPGMKVLQFAFDGGMENPFLPHNYPAHGRVVVVTGTHDNDTTLGWY
UBZ54001_Haloquadratum-walsbyi_MdS FIDQALHDLREYDFDPMRVPHYADWCREGDMYQPMHYPPQ--SVAYSSTHTDITIVGY
          ..          :          :          :          :
          α15          :          :          :          :          α16
P15977_Escherichia-coli      ECGDLTLGKTLGLYPDEVVLRGLYQDRELAKQGLLDALHKYGCLPKRAGHKASLMSMTP
Q6R608_Solanum-tuberosum    EDEEERHRFF-----QAVMGSDLEPPDQCTPE-----
Q030T7_Lactococcus-lactis   EENHDLTQRY-----NEVMGWYGEAPNYASVE-----
A6YM39_Borrelia-burgdorferi NSLDDLHKKI-----FDYLN-----TNENF-----
B5RKZ2_Borrelia-duttonii    NSVDDEHREYI-----FNYFN-----TNEDF-----
A1QYW1_Borrelia-turicatae   NSVDNEHKKYI-----FDYFN-----TSENA-----
O87172_Thermus-thermophilus RTATPHEKAFM-----ARYLADWGITF-REEE-----
UBZ54001_Haloquadratum-walsbyi_MdS DLSLSQSQRDCL-----HYNLG-----VDGSE-----
          :
          β14          α17          β15
P15977_Escherichia-coli      LNRGLQRYIADSNALLGLQPEDWLDMAE-----PVNIPGTSYQKNWRRKLSAT
Q6R608_Solanum-tuberosum    IVHFVLRQHVEAPSMWSIFPLQDLLALKEDYTRPAVEETINDPTNPKHY--WRYRVHVT
Q030T7_Lactococcus-lactis   IIQEIIRKRLNSNAMMVLIPIDQDLAMSEHFRKENAKSEQINIPADPYHY--WNYRLHCN
A6YM39_Borrelia-burgdorferi VVWDMIRSAMGSVSDNVIVPMQDYINLGDKFS-----ANIPKSTLDN--CIFRLLES
B5RKZ2_Borrelia-duttonii    VVWDMIRGAMSSVADNVIVSMEDYLDHVVHD-----KNVSGSMLND----FRILSS
A1QYW1_Borrelia-turicatae   IVWDMIRGAMASVANSVILIPMKDYLDLVADFS-----MNISDAMLNN----FRILSG
O87172_Thermus-thermophilus VPWALMHLGMSVARLAVYPVQDVLLALGSEAR-----MNYGPRPSGN--WAWRLLP
UBZ54001_Haloquadratum-walsbyi_MdS INWSIIDAVWRSDAKIGLTTLQDVLGLDISHAR-----FNEPGTASGN--WEWRCTDS
          :          :          :          :          :
          α18
P15977_Escherichia-coli      LESMFADDGVNKKLLKDLDRRRRAAAKKK-----
Q6R608_Solanum-tuberosum    MESLLNDKDLTKTIKDLVRGSGRFYPQKDLSESGQAKLQLGSEHPSQTQKYGLSNK
Q030T7_Lactococcus-lactis   LQALIENQEWTFDFLKNFKIKESKRAY-----
A6YM39_Borrelia-burgdorferi D----LDATLSQNISFLTRLYGRALE-----
B5RKZ2_Borrelia-duttonii    D----LSTDLSRKKISNITKLYGRI-----
A1QYW1_Borrelia-turicatae   D----LSDDLKISKISDITKLYGRT-----
O87172_Thermus-thermophilus E----LSPHEGARLRMAEATERL-----
UBZ54001_Haloquadratum-walsbyi_MdS G----LDEDLARRLAGLTLEHVVD-----
          .          :          :

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**Figure S1. Clustal alignment of Hw-A and GH77 representative sequences.** Secondary structural elements highlighted in the *T. thermophilus* sequence were according to the 3D structure described by Przylas et al. [1]. In particular, green regions refer to the  $(\beta/\alpha)_8$  barrel (subdomain A); the other coloured regions correspond to subdomains B2 (yellow), B1 (orange) and B3 (cyan). The three catalytic amino acids are reported in red. The non conserved amino acids in the sequences from borreliae discussed in the Introduction are shown in blue. The carbohydrate binding domains of *E. coli* [2] and potato [3] are highlighted in red.

1. Przylas, I.; Tomoo, K.; Terada, Y.; Takaha, T.; Fujii, K.; Saenger, W.; Sträter, N. Crystal Structure of Amylomaltase from *Thermus Aquaticus*, a Glycosyltransferase Catalysing the Production of Large Cyclic Glucans. *Journal of Molecular Biology* **2000**, *296*, 873–886, doi:10.1006/jmbi.1999.3503.
2. Mareček, F.; Möller, M.S.; Svensson, B.; Janeček, Š. A Putative Novel Starch-Binding Domain Revealed by in Silico Analysis of the N-Terminal Domain in Bacterial Amylomaltases from the Family GH77. *3 Biotech* **2021**, *11*, 229, doi:10.1007/s13205-021-02787-8.
3. Lloyd, J.R.; Blennow, A.; Burhenne, K.; Kossmann, J. Repression of a Novel Isoform of Disproportionating Enzyme (StDPE2) in Potato Leads to Inhibition of Starch Degradation in Leaves But Not Tubers Stored at Low Temperature. *Plant Physiology* **2004**, *134*, 1347–1354, doi:10.1104/pp.103.038026.

## Figure S2

```
AUV80673.1 -----
QCC51341.1 -----
QSG15659.1 -----
QSG11910.1 -----
QSG08987.1 -----
QSG05483.1 -----
QGN07616.1 MHSIGPEWREEIDDSTKTQYPAGQDRRADEQGPQRDQKRADRPLARHVDEFGRVCVEETVH
QSG11921.1 -----
QSG08998.1 -----
QSG05496.1 -----
CCQ33055.1 -----
QSG02731.1 -----
AUX09035.1 -----
QFU81686.1 -----
AWB27107.1 -----
QLC34969.1 -----
ACV11232.1 -----
QPV62520.1 -----
QLH84085.1 -----
QLH79093.1 -----
QAU11732.1 -----
QAY20111.1 -----
QWC20591.1 -----
QUO46499.1 -----
QKG93724.1 -----
QKY17977.1 -----
ACM57093.1 -----
AZQ14643.1 -----
ATW89261.1 -----
ABQ75827.1 -----
QZX99028.1 -----
QZP38174.1 -----
QZY03180.1 -----
AUG48211.1 -----
AEM58055.1 -----
AHB66799.1 -----
AJF25101.1 -----
AAV46825.1 -----MDTIYMFAYP-----RRRVLT
QCP91534.1 -----
QUJ72368.1 -----
UBZ54001.1 -----
CCC40696.1 -----
CAJ52690.1 -----
QCJ48434.1 -----
AHZ21176.1 -----
AFK19479.1 -----
AFK19479.2 -----
QCQ75940.1 -----
AKU07735.1 -----
QOS11852.1 -----
QIB78738.1 -----
ADE05003.1 -----
ADE05003.2 -----
QCC48543.1 -----
ADQ67101.1 -----
QIQ76396.1 -----
QIB74651.1 -----
AIF04697.1 -----
AIF05955.1 -----
QEE14682.1 -----
ADL19304.1 -----
AMD30778.1 -----
AKT33963.1 -----
ABL88479.1 -----
ACB39188.1 -----
AEA11843.1 -----
ABO08194.1 -----
ABP49821.1 -----
AFA40300.1 -----
AAL63326.1 -----
NP_559144.1 -----
AET33957.1 -----
ABD40312.1 -----
QXO95450.1 -----
QVV87979.1 -----
```

CCJ37196.1 -----  
SAI88308.1 -----  
CVK34217.1 -----  
BBL68280.1 -----  
ABN57193.1 -----  
SAI88240.1 -----  
CVK34142.1 -----  
CCJ37132.1 -----  
BBL68006.1 -----  
ABN56987.1 -----

AUV80673.1 -----MTLDRQSGVFC  
QCC51341.1 -----MCYFQVRAAASREIRMDFDRQSGVFL  
QSG15659.1 -----MDFDRQSGVFL  
QSG11910.1 -----MEFDRQSGVFL  
QSG08987.1 -----MEFDRQSGVFL  
QSG05483.1 -----MEFDRQSGVFL  
QGN07616.1 -----QLREKRGHATTSQPGHLD---AWPGHSRPGVSRRESDLSRECRQPTGSMAFEKQSGVFL  
QSG11921.1 -----MSFERQSGVFL  
QSG08998.1 -----MSFERQSGVFL  
QSG05496.1 -----MSFERQSGVFL  
CCQ33055.1 -----MSFERQSGVFL  
QSG02731.1 -----MFA  
AUX09035.1 -----MTDFDRRSGVFC  
QFU81686.1 -----MAAFDRRSGVFC  
AWB27107.1 -----MALDRESGIFL  
QLC34969.1 -----MNFERTSGVFC  
ACV11232.1 -----MSFDRQSGVFL  
QPV62520.1 -----MQQRRSGVFL  
QLH84085.1 -----MQQERRSGVFL  
QLH79093.1 -----MQQERRSGVFL  
QAU11732.1 -----MRFDRSDGVFC  
QAY20111.1 -----MRFDRSDGVFC  
QWC20591.1 -----MRFDRSDGVFC  
QUO46499.1 -----MRFDRSDAVFC  
QKG93724.1 -----MRFDRSDAVFC  
QKY17977.1 -----MRFDRSAGVFC  
ACM57093.1 -----MRLDRSGVFC  
AZQ14643.1 -----MRLDRSGVFC  
ATW89261.1 -----MDFDRQSGVFL  
ABQ75827.1 -----MQFERQSGVFL  
QZX99028.1 -----MRFDRRSGVLL  
QZP38174.1 -----MRFDRRSGVLL  
QZY03180.1 -----MRFDRRSGVLL  
AUG48211.1 -----MQFERQSGVFL  
AEM58055.1 -----MFL  
AHB66799.1 -----MQFERQSGVFL  
AJF25101.1 -----MQFERQSGVFL  
AAV46825.1 -----RIRRRLLTGRSSPLALPRAAHFVRAPR---EKPRPLKCLSGQVRMQFERQSGVFL  
QCP91534.1 -----MQFERQSGVFL  
QUJ72368.1 -----MQFERQSGVFL  
UBZ54001.1 -----MQFDRQAGVFA  
CCC40696.1 -----MQFDRQAGVFA  
CAJ52690.1 -----MQFDRQAGVFA  
QCJ48434.1 -----MRFDRQSGVFL  
AHZ21176.1 -----MRFDRQSGVFL  
AFK19479.1 -----MGLATESVMRFSRQSGVFM  
AFK19479.2 -----MRFDRQSGVFL  
QCQ75940.1 -----MGLATESVMRFSRQSGVFM  
AKU07735.1 -----MRLSRQSGVFM  
QOS11852.1 -----MRLSRQSGVFM  
QIB78738.1 -----MRLSRQSGVFM  
ADE05003.1 -----MGMRLSRQSGVFM  
ADE05003.2 -----MRLSRQSGVFM  
QCC48543.1 -----MRFDRRSGVFL  
ADQ67101.1 -----MRFDRQSGVFA  
QIQ76396.1 -----MRFDRQSGVFA  
QIB74651.1 -----MRFDRQSGVFA  
AIF04697.1 -----MAVEGTSKHYGSNGRRPFMRQAGVLC  
AIF05955.1 -----MAVEGTSKHYGSNGRRPFMRQAGVLC  
QEE14682.1 -----MELPRSAGILL  
ADL19304.1 -----MERGSGLLL  
AMD30778.1 -----MERGSGLLL  
AKT33963.1 -----MLRGFGVLL  
ABL88479.1 -----MLRGFGVLL  
ACB39188.1 -----MLRGFGVLL  
AEA11843.1 -----MLRGAGILL

ABO08194.1 -----MLRGAGVLL  
ABP49821.1 -----MLRGAGVLL  
AFA40300.1 -----MLRGAGVLL  
AAL63326.1 -----MLRGAGVLL  
NP\_559144.1 -----MLRGAGVLL  
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ABD40312.1 -----MKTRGSGVLL  
QXO95450.1 -----MKTRGCGTLL  
QVV87979.1 -----MKTRGCGVLL  
CCJ37196.1 -----MNRRGSGVLL  
SAI88308.1 -----MNRRGSGVLL  
CVK34217.1 -----MNRRGSGVLL  
BBL68280.1 -----MNRRGSGVLL  
ABN57193.1 -----MNRRGSGVLL  
SAI88240.1 -----MIHTRGSGVLL  
CVK34142.1 -----MIHTRGSGVLL  
CCJ37132.1 -----MIHTRGSGVLL  
BBL68006.1 -----MIQTRGSGVLL  
ABN56987.1 -----MIQTRGSGVLL

:

AUV80673.1 HVASLPGPQGIGSLGAPARRFVDFLARADQSLWQFCPIGPTTVDYGNPSPYSALSAVAGNP  
QCC51341.1 HVASLPGPDGIGTLGEPAEFFVDFLAESGQSLWQFCPLGPTIPIHDNSPYQSYSAFAGNP  
QSG15659.1 HVASLPGPDGIGTLGDPKTFVDFLADSGQSLWQFCPLGPTIPIHDNSPYQSYSAFAGNP  
QSG11910.1 HIASLPGPDGIGTLGDPKTFVDFLADSGQSLWQFCPLGPTIPIHDNSPYQSYSAFAGNP  
QSG08987.1 HIASLPGPDGIGTLGDPKTFVDFLADSGQSLWQFCPLGPTIPIHDNSPYQSYSAFAGNP  
QSG05483.1 HIASLPGPDGIGTLGDPKTFVDFLADSGQSLWQFCPLGPTIPIHDNSPYQSYSAFAGNP  
QGN07616.1 HVSSSLPGPDGIGTLGAPAEFFVDFLATAEQSLWQTCPIGPTDPPVGNPSPYSAYSARAGNP  
QSG11921.1 HVSSSLPGPDGIGTLGEPAEFFVEYLETAEQSLWQTCPIGPTDPPRQGNPSPYSAYSARAGNP  
QSG08998.1 HVSSSLPGPDGIGTLGEPAEFFVEYLETAEQSLWQTCPIGPTDPPRQGNPSPYSAYSARAGNP  
QSG05496.1 HVSSSLPGPDGIGTLGEPAEFFVEYLETAEQSLWQTCPIGPTDPPRQGNPSPYSAYSARAGNP  
CCQ33055.1 HVSSSLPGPDGIGTLGQPAEFFVDFLVEDQSLWQTCPIGPTDAGQGNPSPYSAYSARAGNP  
QSG02731.1 HPTSLPSSHGIGSLGQPSREFVDFLAEADQSLWQFCPLGPTISSIHGNSPYQAYSFAIEP  
AUX09035.1 HPTALPGPGGIGTLGEPARGFLDRITEAGQSLWQFCPLGPTVGIHGNSPYQTCSAFAIDP  
QFU81686.1 HPTALPGPHGIGSLGAPARSFVETIAAAGQSLWQFCPLGPTVGIHGNSPYQSSSFFALSP  
AWB27107.1 HLSSSLPGPHGIGDLGAT-TAVLDWLDRADQSVWQVCPINPTQGVHGHSPYASVSTFAGNP  
QLC34969.1 HLTSPLPGPHGIGDLGAGARAFVDFLADQSLWQFCPLGPTGVHGHSPYSSPSTFAGNP  
ACV11232.1 HLTSPLSPHGIGDLGAGARAFVDFLADQSLWQFCPVPTPRGVHGHSPYASPSAFAGNP  
QPV62520.1 HLTSPLPGPHGIGDLGAGARAFVDFLADQSLWQFCPVGPTSDAYGHSPYSSSFAFAGNP  
QLH84085.1 HLTSPLPGPHGIGDLGAGAREFLDFLDRADQSLWQFCPVGPTSAAYGHSPYSSSFAFAGNP  
QLH79093.1 HLTSPLPGPHGIGDLGAGAREFLDFLDRADQSLWQFCPVGPTSAAYGHSPYSSSFAFAGNP  
QAU11732.1 HVTSPLPGAYGIGDLGGGAREFLSFLGDADVDHWQICPIGPTLSVAGESPYQSPSAFAGNP  
QAY20111.1 HVTSPLPGAYGIGDLGEGAREFLSFLGDADVDHWQICPIGPTLSVAGESPYQSPSAFAGNP  
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QZP38174.1 HPTALPGPHGIGDLGAGARSFLDFLDRADQSIWQVCPVPLGPTVGIHGNSPYQTFSGFAGNP  
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 ABN56987.1 HITSLSPSYVIGDFGPAAYRFVDEALERGRQHYWQILPLNPTETAYAHSPYSPSAFAANI  
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 QSG15659.1 LFIDLDELVERGWLEELD-----RP-----DFDDGNVEYGPVREYKEAR  
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 QSG05483.1 LFLDLDLVERGWLEELD-----RP-----DFDDANVEYGPVREFKEAR  
 QGN07616.1 LFIDLDELVERGWLEELD-----RP-----DFDDREVEYDRVDAFKQEA  
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 QSG08998.1 LFIELDALVEEGWLDLDP-----RP-----DFDDREVEYERVMFAFKREA  
 QSG05496.1 LFIELDALVEEGWLDLDP-----RP-----DFDDREVEYERVMFAFKREA  
 CCQ33055.1 LFIELDPLVEAGWLDLDP-----RP-----DFDDREVEYGRVRFKQGA  
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 AUX09035.1 LLIDLEALNERGLLSGNRVETER-----DDA-----RLSDDRVYDAVRKYRPL  
 QFU81686.1 LLIDLEALNERGLLTDALDP-----ELDH-----ELTDDRVAYDAVRSFKTSR  
 AWB27107.1 LFDVLDLDRVGRWLSADALAD-----AP-----R--TDRVDYDRVVPFTEDC  
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 ACV11232.1 LLVDLTDLDRVGRWLEDEETLEN-----PP-----G-DPRVTQYDVTDFKRER  
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 ATW89261.1 LLISLDRVDDGWLTDEDLQP-----VP-----DFDEHSVDYEAAGDYKRNQ  
 ABQ75827.1 LLVSLDRLSEQAYFSLNDSI-----DA-----PDRHEVEYDAVREFKTA  
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 QZP38174.1 LLIDLVDLAERGLIDEIDVDA---GGDGAGV-----DFSLHEVRYDAVESFKLDR  
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 CCC40696.1 LLISLDDLVAEGYLTSEDLEP-----VP-----DFSPHEVYDTRVREYKHDC  
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 QIB78738.1 LLIDLTDLVARGWLTADLEP-----VP-----DFDPHAVDYERVGEYKRSR  
 ADE05003.1 LLIDLTDLVARGWLTADLEP-----VP-----DFDPLAVDYERVGEYKRSR  
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 QIQ76396.1 LLVLDLQREDGYLTDDDDLEP-----VP-----AFSEHEIEYDRVAEYKREQ  
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 AIF04697.1 RFRDPAMHY-----RLDYE-----  
 AIF05955.1 RFRDPAMHY-----RLDYE-----  
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 ADL19304.1 IMVSPQLLANEGLLADELAS-----LP-----RGEPNRVDYGLAYEHRMRL  
 AMD30778.1 LMVSPQLLAEGLLTKEEVAS-----IP-----QGPRGRVDYPLAYQHRRL  
 AKT33963.1 PLSLDLDMKRDGLIDQVPQC-----QPTDKADYEAAWQIKRRL  
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 CVK34217.1 WLISPEQMVIDGLLAPEDIRD-----PP-----GFPEDRVYRAVMDYKNSL  
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 BBL68006.1 LLISPEMLIREGYLRKSELEP-----AP-----GFPDGRAAYEAAVWYHERL  
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AUV80673.1 LRAHDFEER-----A  
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 QSG08998.1 LRDAFEGFSDV-----A  
 QSG05496.1 LRDAFEGFSDV-----A  
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ACB39188.1 LEEAFEGRLGW-----  
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CVK34217.1 FDRAFERFRER-----  
BBL68280.1 YDKAFERFKQQ-----  
ABN57193.1 FDTAFERFKGR-----  
SAI88240.1 FSIAYQRFLLHS-----  
CVK34142.1 FSIAYQRFLLHS-----  
CCJ37132.1 FSIAYQRFLLHS-----  
BBL68006.1 FAAAYERFRYS-----  
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QAY20111.1 TKEDRAALGAFRERE-PWLADYALFRALSDARPEDVWTEWPAPLRTDPEALAEAREEHA  
QWC20591.1 TDADRAAVDEFRERE-PWLPDYALFRALSDARPEDVWTEWPEPLRTDPEALASAREEHA  
QUO46499.1 SEDDRAAAVEAFREHE-PWLADYALFRALSDARPEDVWTDWPAVLRTDPEALADAREEHA  
QKG93724.1 TEADRAALDEFRERE-PWLADYALFRALSDARPEDVWTDWPAVLRTDPEALAEAREDEHA  
QKY17977.1 GEEAEADLDAFRERE-PWLADYALFRALSEARDEDTWVEWPEPLRTDPEALAEARERHA  
ACM57093.1 AGKAHSEFADFREHE-PWLDDYALFRAMSGERDDTWVEWPDPLRTREPEALDEARERHA  
AZQ14643.1 AGDAAPDFDAFCERE-SWLDDYALFRALSARDEATWVDWPDPLRTREPEALADARERHA  
ATW89261.1 TDDDYAALDEFRD-EESWDDYALFRALKQKHDGVAVVDWPEPIRTRDPDALSEAREELS  
ABQ75827.1 ---PTESFGRFCESQSAWLDDYALFMSLHDALE-GAWTEWPEPIRTHESAAYEHYRNELE



QAY20111.1 AEVRFRAFVQWTFDRQWHELRAVAADDEGVSI VGDVPIYVALDSADVWASPEAFRLDEG NR  
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QKG93724.1 REIRFRFVQWTFDRQWRDLRAVAADDEGVSI VGDVPIYVALDSADVWASPEAFRLDEG NR  
QKY17977.1 TEVRFRFVQWTFDRQWRDMRAVAADDEGVSI VGDVPIYVALDSADVWANPEAFRLDES NR  
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CAJ52690.1 SEVIYHLIIQFIFDQWQSLQAAATDRDITLVG DLPYIYVALDSADVWATPDVFLTKDNE  
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QIQ76396.1 DEVSYRKAQVFDQWRDLKAYANDHGVKLVGD LPYIYVALDSADVWAAPEAFDLTEENE  
QIB74651.1 DEVSYRKAQVFDQWRDLKAYANDHGVKLVGD LPYIYVALDSADVWAAPEAFDLTEENE  
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AIF05955.1 GSMGKWMEEQFIKIDWSQLRNTAECEGVS LFGDLFFFVAHDSADVWANQHFLKLEASGA  
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AMD30778.1 SRVELELFTQYEFFSQWSSLRAHARRLGI RIIGDPIYVSYDSADVWSNPRIFKLRDLR  
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ACB39188.1 RRADFYRYVQVFWQWLELRRHVNNLGIFI IGDLPIYPAHDSADVWDGRRYFKLAPDGA  
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ABO08194.1 DRVEFYAFAQVFWQWRDLKRYVNSLGI FFLIGDLPIYVGPDSADVWAHRELKFLVQDGR  
ABP49821.1 ELVELYKFAQVFWQWRDLKRYVNSLGI FFLIGDLPIYVSLDSADVWRHRRYFKLITEDGA  
AFA40300.1 ELVELYKFAQVFWQWRDLKRYVNSLGI FFLIGDLPIYVSLDSADVWRHRRYFKLITEDGA  
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AET33957.1 RLVELYKFAQVFWQWRDLKRYVNSLGI FFLIGDLPIYVSLDSADVWRHRRYFKLITEDGK  
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BBL68280.1 DRILREKFLQYVFDQRQWTLKNHCRSRHLQV IGDLPVYLYTYSVDLWANPDLFKLDEQKK  
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SNI88240.1 DGVRRKEKFLQYLAARQWSALHRHCTDLG IQVIGDPIYVAYDSVDVWQNPGFIFKLEDDL  
CVK34142.1 DVVRKEKFLQYLAARQWSALHRHCTDLG IQVIGDPIYVAYDSVDVWQNPGFIFKLEDDL  
CCJ37132.1 DEVRRKEKFLQYLAARQWSALHRHCTDLG IQVIGDPIYVAYDSVDVWQNPGFIFKLEDDL  
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ABN56987.1 DRIRKEKFLQYIAARQWSALRRYCTDHGIQV IGDPIYVAYDSVDVWQNPGFIFKLEDDL

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AUV80673.1 PTVAGVPPDAPDEVFSAQRWGMPLFDWEALERTGF AWWRSRLERLFAQCDIVRIDHFRGL  
QCC51341.1 PKYVSGVPPDE - FSDDGQMWGTPVYDWDALDERDY GWWWKRFERLLQRVDIFRIDHFKGF  
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QSG08998.1 PIYVSGVPPDD - FSDTGQLWGTTPVYDWTETLADREYDWWIQRFAGLLDRFDVFRIDHFKGF  
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QSG11910.1 ESYWEIPADADTAREGEWVVGPHHEEVFYAVRDQ---LGDLP--IVVEDLGEITEEMDRI  
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QSG05483.1 ESYWEIPADADTAREGEWVVGPHHEEVFYAVRDQ---LGDLP--IVVEDLGEITEEMDRI  
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AZQ14643.1 VKYWAIPADADDPAGEWCDGPGRALFFETVERE---LQAP--FIAEDLGFEEPPAMNEL  
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ABN56987.1 ADYIEVVPAGDATAERGTWVDGPGEDFFEALARK --- HPCFP -- IVAEDLGANTPAVQAL  
: : \* \* \* .: . \*::\* .  
  
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QCC51341.1 RDTLGYPGMVVAADFADWC - DGDSRYHPANYDANSVAYTSTHDTDTVVGWYDA - LDEQDRE  
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AWB27107.1 RDRFEFFPGMRVPHYADWC - AERHRKPKAGYPERSVAITSTHDTNTARGYVES - LDDRQRS  
QLC34969.1 RDHLGFPGMRVPEYADWC - AEHHRKPKPSYPRDCVAYTSTHDTDTAVGYYES - LGERQRD  
ACV11232.1 RDRFEFFPGMRVPHYADWC - REGHRYKPTVYDPDHCYGTSTHDTDTAVGYEYK - LSAEQRD  
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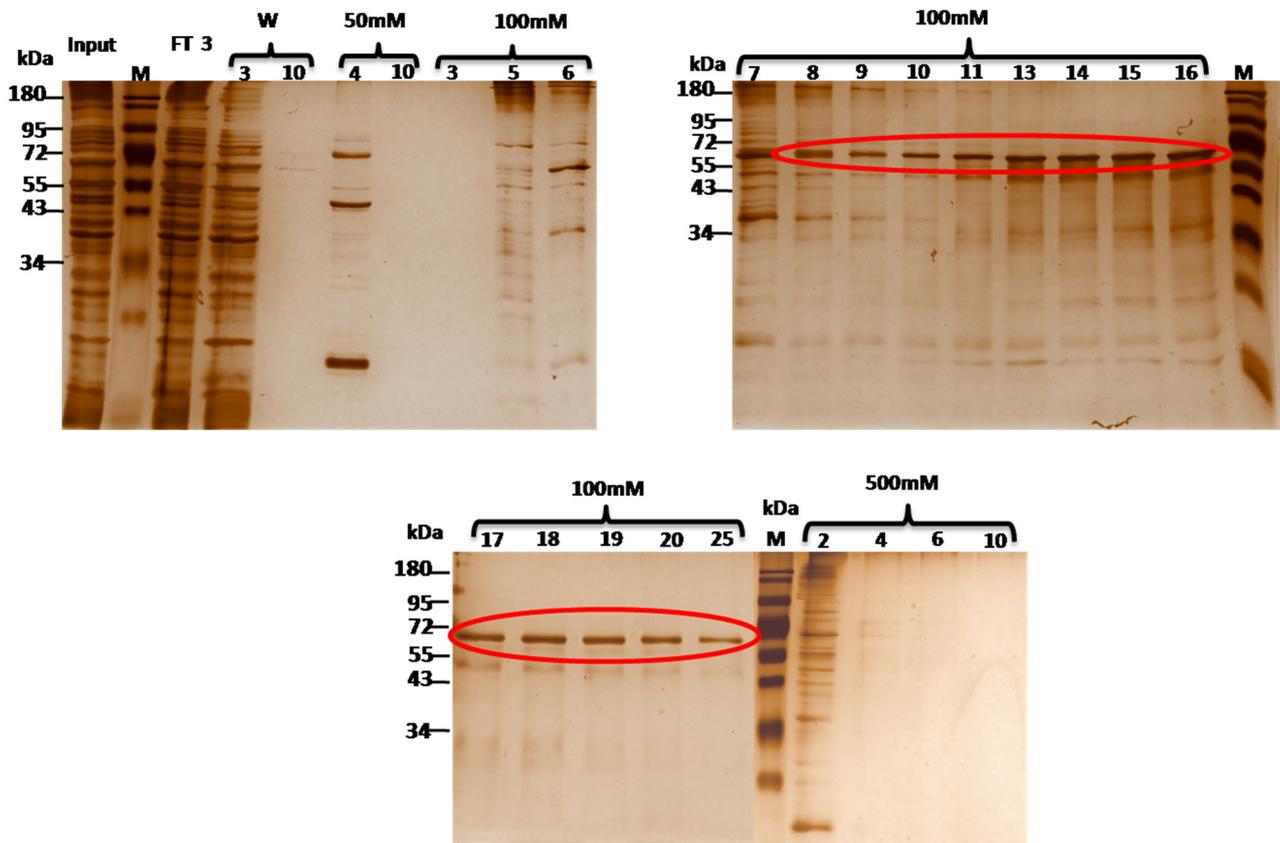
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QEE14682.1      VKIDNDVK-NRAKFLKKITKLYGRKVII---
ADL19304.1      LESFDGLR-RAARLRDMTRAYGR-----
AMD30778.1      LDSLEGLW-RTASRLRDMTRSYGR-----
AKT33963.1      LHAQPP-P-TYARLRRLAKLYGR-----
ABL88479.1      LAKDPP-I-NFAVRLRRLAKLYGR-----
ACB39188.1      MAGDPP-R-AVAARLRRLARIYGR-----
AEA11843.1      MTELPG-R-ALARRIRRLARLYGR-----
ABO08194.1      LAEQPP-R-ALLRKLREVTRLYGR-----
ABP49821.1      LAKMPN-A-AVRRRLRKLTRYGR-----
AFA40300.1      LAKMPD-A-AVLRRLRKLTRYGR-----
AAL63326.1      LGAQPE-P-RVWKRLRDVTRTYGR-----
NP_559144.1     LGAQPE-P-RVWKRLRDVTRTYGR-----
AET33957.1      MAKQPE-P-RQWRLRSLARLYGR-----
ABD40312.1      LQERMISE-ELSRRLSELVKIYGR-----
QXO95450.1      MQERMITE-DLAYYIRTLTTYGR-----
QVV87979.1      MQERMITE-DLAYYIRTLTTYGR-----
CCJ37196.1      LRREQMRE-QAMQEFSEVTGLYGRK-----
SAI88308.1      LRREQMRE-QAMQEFSEVTGLYGRK-----
CVK34217.1      LRREQMRE-QAMQEFSEVTGLYGRK-----
BBL68280.1      LRPDQIGE-ESMRELAEVTGIYGRG-----
ABN57193.1      LRPDQAGE-ESMREFAEVTGIYGRG-----
SAI88240.1      MTPAEFAG-APFDRLRRLTELCGRA-----
CVK34142.1      MTPAEFAG-APFDRLRRLTELCGRA-----
CCJ37132.1      MTPREFAD-APFDRLGRFTELCGRA-----
BBL68006.1      MTPPEFAG-APFEWLRELTGTGR-----
ABN56987.1      MTPPEFAG-APFEWLRELTGTGR-----
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**Figure S2. Clustal alignment of Hw-A and 83 archaeal amyloamylases present in the CAZy GH77 family. Hw-A is reported in red.**

Figure S3



**Figure S3.** SDS-PAGE on 15% polyacrylamide gel of the fractions obtained from IMAC chromatography. Input: Soluble fraction of sonicated induced expression cells; M: Marker Thermo Scientific Page Ruler Prestained Protein Ladder; FT: flow-through; W: wash buffer; 50mM: fraction eluted with 50mM imidazole elution buffer; 100mM: fraction eluted with 100mM imidazole elution buffer; 500mM: fraction eluted with 500mM imidazole elution buffer. The recombinant protein was circled in red. Protein electrophoretic bands were revealed by silver staining.