

Applied Microbiology and Biotechnology

Supplementary Material

Characterization of a novel GH30 non-specific endoxylanase *AcXyn30B* from *Acetivibrio clariflavus*

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GH30_3_AAO78418.1

GH30_3_AAO78418.1

GH30_1_AAA35880.1

GH30_2_ABX45137.1

GH30_9_LcGUS30

GH30_11_ShGbha

GH30_5_BAC84995.1

GH30_4_PbFucA

GH30_HHU50367.1

GH30_WP_114299953.1

GH30_NLM60409.1

GH30_AcXyn30B

GH30_NLL05159.1

GH30_7_TcXyn30A

GH30_7_TcXyn30B

GH30_7_TcXyn30C

GH30_7_TrXynVI

GH30_6_WP_028726386.1

GH30_10_AcXbh30A

GH30_8_EcXyn30A

GH30_8_CaXyn30A

GH30_8_BsXyn30C

GH30_8_CpXyn30A

GH30_8_CpXyn30A

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1 .....minmrnialtflg.....c
1 mefsspsreecpkplsrvsimagsltgllllqavswasgARPCIPKSGFYSSVV.....C
1 .....MKKFQHEF.....
1 .....ml.spphllsssva.....ta
1 .....mrsivlpsla.....
1 .....mkrilpslff.....
1 .....mkrqyvlpvmvivgl.....
1 .....mkkgkililpvil.....a
1 .....mkirsklslillil.....t
1 .....mlkkrklvlsvvl.....s
1 .....mvmkkklalsvm.....s
1 .....mrhpi.....il.....
1 .....mvfskv.....ava.....
1 .....mwslksnsta.....
1 .....mialvp.....fv.....
1 .....mktrrnlrtlglf.....ac
1 .....mrktvslttvaallis.....
1 .....mng.....nvs.lwvrchlha
1 .....mniklrtl.islvafsmtc
1 .....miprikkticvllvcftm.l
1 .....mfknmkktkiskvlvssiims
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GH30_8_CpXyn30A

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15 ftilaACSNSDDAEKPVVPVPTGDVAIYTTTSSSLTRDLTRDAV.NFSPKDNLAPTTITLN
56 VCNATYCDSDFD...PPTFPAL.GTFSRYESTRS..GRMELSMGPIQANHTGTGLLLTLQ
1 .....MTTWIATTQD.....LADRT.SDIESTNATTAADLQL
9 .....TQRRTPLAT.PDYQSTSAAAVKIIID
16 lsils.....gilapqh.vta..ATVS
11 .....lalfsqrarabTTLTID
11 .....lcmgisagsntKVTIN
17 lvvlvg.....favvpqtnaVTYNASVN
15 vimts.....fisvnpnvsaaAYSVSVD
15 lvm.t.....cllnvqsgaANYNVSVD
15 mvl.t.....lvnfpktnaaSYTASVD
15 iil.s.....fmifpktdaASYTASVD
9 .avlg.....rasaWSYSQTLSANIQVN
10 .asal.....s.....lgayaidaQINVD
11 .lava.....l.....ngivalgQTITVN
9 .iiag.....a.....atlsvaQTITVN
15 lflllgsCTDNEFA.....DQKAGSSIATKAVANTDVVLH
17 mlil.....v.....lptnllyaASTVTVD
16 alfv.....satagsfsvyadTVKID
20 lpfv.....gt.gssvkaASNDATIN
20 svml.....gp.gatevlaASDVTVN
21 alfm.....vs.apagvsaASDVTVN
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GH30_8_CpXyn30A

```
74 PAEQYQTMDFGAAITGSTCYNLLLM..KPADRHAFLTETFSDK.D.....GFGFS
110 PEQKFQKVKGFGGAMTDAAALNIAL..SP.PAQNLLLSYFSE.E.....GIGYN
34 DGNEYQALRGFGGCFNELGWLPLQNV...SEAERDQIIKELFSP.D.....EMNFT
42 PDRHQPLWLGGAATIDSAAYLLWSV..MSAEQRRALLTELFDP.D.....QGGFS
35 .SEVAQTFLGIGGSG..AWWPTDLYLYP.DEVKKNLSA.LLFSE.D.....GMGIS
28 PTSNWGTWEGWGVSL..AWWAKAFGN.....RDDLAS.VFFSRNNQAVNGQTLPLGLGFN
29 PSKTYQTMRSFGASD..CWTAEYVAQYFSDTQRQNAARWLFSSSEVDA...QGNPLGIALS
39 VNTTYQTLLEGFGASI..AWYQSYLTA...HPNREEVIDLLFKD.....LGID
37 INTSYQTLLEGFGAAI..AWSNESLTE...HPNRAGLYKTLFFD.....SGLD
36 INTKYQTLLEGFGAAI..AWYNNWVTG...HPNKNELYNVLFYD.....LGLD
36 VNTTYQTLLEGFGAAI..AWYNDYLT...HPNKNDLYRILFYD.....SGLD
36 INTTYQTLLEGFGAAI..AWYNDYLT...HPNKNDLYKILFYD.....SGLD
31 ALQRYQEMIGFGCSGAFGWACQQFPPTGLTPENQEEVTKILFDENI.....G.GLS
28 LQARYQSVDFGFGCSQAFQRAEDIFGKYGLSPKNQSYVLDLMYSEER.....GAGFT
29 PSTTYQTIDGFGFSEAFGFGAPIASA...SASIQTQVTNYLFSTTT.....GAGLT
27 VGSKFQQIDGFGFSQAFGRAREFQSA...NATAQKQALDILFSTST.....GAGFS
46 WDKEEQNIDGFGVAQ..AGWADYLYA...HRKRDTVLDLMF..GKD.....GLHLN
36 WDTTYQTIDGFGVSEAFHQSNNIARL...GETKQNEIYDILFSTTD.....GAGFS
37 ANVNYQIIQGGFGGMSGVGWI.....NDLTTEQINTAYGSGVG.....QIGLS
40 VAAKHQTIIRGFGASS..AWC.....GALSDTCMDTLY...K.....NAGLD
40 VSAEKQVIRGFGGMNHPAWA.....GDLTAAQRETAFGNGQN.....QLGFS
41 LGSTKQEIIRGFGASS..AWC.....GTISDYVMNSLY...G.....DLGYS
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GH30_8_CaXyn30A
GH30_8_BsXyn30C
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GH30_8_CpXyn30A

β2
122 YIRISIGSDFSS...LSEYTCDDT...KGIEFNAL...
157 IIRVPMASCDFFS...IRTYTYADT...PDDFQLHNFSL...
81 FNRAPVGANDFA...DHWYSYDEV...DGDYGMHFVS...
90 SVRVPLGSCDFQ...SQDFYTYDDVPYGEHDQKLEQFSIGTGQPGAPDA
80 SYRYNVGGGGVNV...VTENPTRAIBTFYVSPGVYVNW...
79 IVRYNAGACSNNSYDGMTVMVSPNIKPSRQMDGFWDWASSDPSSSSWNW...
84 QWRVNLGAGSSTQGANNSI...DDETRRADCYLES DGQTYNW...
81 IYRFRNQYNRDQGF...
79 ILRLRNQYRNSDDF...
78 ILRLRNQYRNNNNF...
78 ILRLRNQYRNSNNE...
78 ILRLRNQYRNSNNE...
81 IVRNDIGSSPGST...ILPTCPATPAGPFNYQW...
79 ILRNGIGSSNSSTSNLMNS...IEPFSPGSPSPSTPNYTW...
77 ILRNRITAAGSGS...IEPNAPSGPNAQPTYT...
75 IIRNRIGSGGAGDS...IEPNNGSPSPAAPNYVW...
93 ILRGEV...YSHYWKKEGDT...SFYLDEEIDMPLDDPFFDIDYSADGNEAA
84 IFRSIL...GDGWTGNADDG...PNKTMQPAEDVWDW...
79 IMRVRI...DPSSKWN...
76 ILRVRI...APNEGWNRGD...
82 ILRIHV...DENRNNWYK...
77 ILRLRIEEGTGDWKTGN...
β2

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GH30_8_CaXyn30A
GH30_8_BsXyn30C
GH30_8_CpXyn30A
GH30_8_CpXyn30A

α2 β3
151 ..QSEEKDYILPILKEILAINPSI...KVIAAPWTCPKWMKVKS LTRDTPLD SWINGQL..
189 ..PEEDTKLKIPLIHRALQLAQRV...SLIASPWTSPWLKTNGAVN...GKGS LK
113 ..EHDEQ.TLIPYIHRALQAEWQPNM...QLFSSPWSPPTWMKRPKAYN...YGR L V
136 ..TKDL.KHIVPV LQEILAINPAV...KVIASPW SAPAMKNTGHLT...HGGH LRFGEFT
113 ..SADA..SGVYFMLEAAAYGV...SITAFVNSAPAPLTSGGASC...NGSFV
129 ..NVDANQRAMLQKAKANGAN...IFELFSNSPMWMCNNHNP...GSGSSDNLQ
123 ..NHCPGQQWFNMQAKTYGVN...DFLLFTNSPLIYYTTNGLAN...NKSNAAGSNL G
95AEDTETIIRMVASLGRPI...KMLLSSWTPPADLQNGVLN...GGTLI
93AYPDTEIVKLARCFNPNL...KILLCSWTPPADIKENGVLN...GGTLI
92AYDDAEIVKMAKILNPNL...KVFLASWSPPENLKR DGMN...GGTLK
92GYDDMEIVKYGKIMNPNL...KVLSSWSPPSDLKRN GVMN...GGTLA
92AYDDKEIVAYGKIMNPNL...KVLCSWSPPEDLKKDGMN...GGTLA
111 ...DGSDSCQFNLTALKYNPEL...YVYANAWSAPGCMKT VGTEN...DGGQIC
115 ...DHYNSGQFPLSQARARGLP...YIYADAWSAPGYMKT NQDEN...WSGF L C
106 ...DGNDAQGVVWSKQARAKGVK...YIYADAWSAPAFMKTINDVA...NGGY L C
106 ...DNNDSGQLWFTQQAVSYGVK...TIYADAWGAPGFMKTS GSDS...SPGY L C
139 EE..MAQRKGQLWINGRQV KALYDV...KFVYSVWSPPAYMKSNGSDS...KGN L
116 ...NESNDQIPMIRAIQSKYGV...QILYTVWSPPAWMKTN GSV...GGS L
94QLPSARQAVSLGA...KIMATPWSPPAYMKSNNSL...NGGRL
92YRA...WADELNSNAKKVVRAGG.IVFATPWTTPPASMKTNTTT...GANKGSL
97EVETAKSAVKHGA...IVFASPWNPPSDMVETFN RRG...DTSAKRL
95FSK...WSPELANAKKASAKGA.IVFASPWNPPASMQENFSKSG...DSSAQRL
α2 β3

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GH30_8_BsXyn30C
GH30_8_CpXyn30A
GH30_8_CpXyn30A

α3 β4
205NPDYYQDYATYFVKWIQ...AFKAEGIDIYAVTPQNEPLNRG...NSAS L Y
238 G.....QPGDIYHQTWARYFVKFLDAYAEHKLQFWAVTAENEPSPAGLL.SGYPFQCLG
159 Q.....TPENLKAYAKYFVKYIQ...AYAHEGITVNQLHVQNEVFADQ...KFP S A L
188 G.NGYTE.ENRFEIYAYQYFIRYIE...AYQKLGIPYIGLTIQNEPNSAA...HWPAMI
157 ..NG.....TGLEYGTFVADVIS...HWR AEGVNITRVSPMNEPDNNFGPSPCSQEGME
177 SW.....NYQNHA VYLADIAQ.HAQQSWRIQFQSV EAFNEPSSSWNTAEGTQEGCH
173 AA.....YYDDFSEYLAECVK...HFTEKGYPITYIDPVNEPAPFNWTD...QGEGTP
138 KKNG....QFAYDEFATYWYDSL.V...ELGNNGIYPDYISIQNEPDYEND...GWETCV
136 KQNG....AFVYDRFADYWKSLN...AYAAKGIVPDYISIQNEPDYQSS...DWETCI
135 KENG....SFVYDKFADYWYNSLI...AYKEKGIVPDYISIQNEPDYEDQ...NWETCI
135 KENG....SFVYEFKADYWYNSLV...AYRAKGIIPDYISIQNEPEYESG...DWETCI
135 KENG....SFVYDKFADYWYNSLV...AYKEKGIVPDYISIQNEVEYENA...DWETCI
158 GVRGTNC.TYDWRQAYADYLVQYVK...FYQAEGIDISLLGAWNNEP DFNPV...TYESME
161 GIEGETCPSGDWRQAYADYLVQYVK...FYAESGVPTHLGFLNEPQEVV...SYASMG
152 GTTGCTCSSGDWRQAYANYLVQYIK...DYANEGITIDFVGWLNNEPDYXP...NYDSML
152 GTTGHS CSSGDWRQAYANFLVQYVK...YAAAGYNITHLGFLNEPDYQT...TYSQMQ
186KSAYYQEYADYLSAFCD...AYGSVGLKPYAISPA NEPEYAA...SWSSCL
161RTDKYQAYATYLAEHK.NYKSKFGIEITHIGIQNEPNLET...SYSSCR
132LPANYSAYTSHLLDFSK...YMQTNGAPLYAISIQNEPDWKP...DYESCE
138KPSSYAAAYALKTFFVK...YMSDNGAPLYALSLQNEPDWAP...DYDACT
138KYNKYAAYA QHLNDFVT...FMKNNGVNLYAISVQNEPDYAH...EW..TW
142RYDKYTEYAQYLNAYVK...YMKDNGVDLYAISVQNEPDYAQ...DW..TW
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 GH30_8_CpXyn30A

250 M..EWEEQRDFVKTALGPQMKAAGLS.....TKIYAFDHN.....NYDNIES
 290 F..TPEHQRFIARDLGPTLANSTHNN....VRLMLDDQR.....LLLPWH
 205 W..DSEALKVFIRDYLGPAFDEAGLDTDIWLGTNLGPEDMAWTGGGYGMTL.NNYNRFVD
 239 W..TVPQLADFGYRRLPALNHSFPD.....TKLYLLDDSFHALT.....KPITA
 206 V...DPNQRAEVVEGLYEALTNNNLTDV...VGIIVDESSSLSRAT.....SEYSTWLP
 227 F...DVSTMATVIGYLNTELSSRGLS.....SFVASSDENTYDLAI.....STWQGFNS
 219 W...QNAEVSKLVRELDKSLTSRNL.....TQILIPAEASSWDRLYQQCSDYNGRASNQI
 187 FKPVESSSYPGYGKALDAVYRRRLQGLSRT..PKILGPETAGI.....GSNLVQ
 185 FYPTETSNYPGYDKALDAVYSKIQTLPSM..PKIIAAEATGIGTSMI.....GNNAAQ
 184 FKPTEDQNPYSYGKALDAVYNKIKSLPDM..PKILAPEAAGI.....GFNTVQ
 184 FRETETSEYPSYAKALDAVYEKIKDLDPDM..PKILGVESAGI.....MNNTVQ
 184 FRETETSDYPGYGKALDIVYNKIKDLDPDM..PKILGVESAGI.....MNNTVQ
 211 S..DGFOAKDFLE..ILYPTVKKAFPN.....LDVSCCDAT.....GARQER
 214 S..NGTQAAEFVK..ILGQTLEREGID.....IELTCCDGV.....GWSEQE
 205 IT..SGTQAASFIP..TLYNTIKSAGLS.....TGIACCDPF.....GWSDAV
 205 ISSNAQEAISFIP..ILSSTVKAAGLN.....TKLTCCDAT.....GWTTSQ
 231 WLPGTTTLGRFIVNMMGPTFASKQPD.....VKIIFGENAQWGTG.....ILGFIM
 207 W...SPEELRIFMRDYLVPTEFDKENIT....AKVVFENMSF.....NEQYAI
 177 W...SGDEFKSYLSKQSGSKFGS.....LKVIVAESLGF.....NPALTD
 183 W...TAQQFHDFLKQYGASLSST.....TKIIMPESLGF.....NPMASD
 181 W...TPQEILRFMRGYNAGSIN.....ARVIAPESEFQY.....LKNLSD
 185 W...TPQEMLNFMKNNAGSIN.....CRVMAPESEFQF.....LKNMSD

α4 β5 α5

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 GH30_8_CaXyn30A
 GH30_8_BsXyn30C
 GH30_8_CpXyn30A
 GH30_8_CpXyn30A

291 QK.NYPGKIYEDAAASQ.YLAGAAYHNYGGN.....REELLNIHQAYPEKELLFTET
 331 AKVVLT...DPEAAK...YVHGIAVHWYLDFLA....PAKATLGETHRLFPNTMLFASFA
 262 NI.....LFDDGARK...YIKGIAYQWAGQ.....NCIARTHESWPEIELIQS
 282 EV.....TPEQAA...AFDGLAVHTYSGP.....YDNLYHANRAYPNWSTIMTER
 254 QVQD.....KVSALCHHTYDFPTDASY...LSYIDDVHTNYPGVDTWMS
 273 STRN.....IVKRINVHGYQDGGG.....RRDTLYSLASQAGKRLWNS
 271 EAFWNPAHTDTYIGDVAHLAKIAAGHSYWTFTNDDLQNIQSQVATKAAEYGLETAQT
 233 EYARNLNMNQ.....LYGVAHHLYNGGDPNSPDSFVSAMQGIAGAFQDKPLFQTE
 236 QYFNKIDFSK.....IYGLAHHLYNGGDPNNPDSFNSVFKAIAAAAYPGKPIFQTE
 230 NYTNMMDLSK.....VYGLAHHLYNGGDPNSPDSFNSIFKTLAETYPDKPLFQTE
 230 NYARYMDLSK.....VYGLAHHLYNGGDPNPDSFNDIFRSLARDFADKPLFMT
 230 NYAKYMDLSK.....VYGLAHHLYNGGDANNPDSFNSNFESLARDFADKPLFMT
 249 NILYEYQQAG.....GEHFFDVATWHNYQS..SPER.....PFNVV.....GKPNIMTE
 252 AMIPGLQVVGPDGKSAEDYLSVVTGHGYSS..APTFF.....PLSTK.....RRTWLT
 244 TWTAQLASAG.....ATQYLARITSHWYAS..KGT.....PINTS.....LRVWET
 245 TYTTNLVNAG.....STQYLSVITSHSYSS..DATS.....PLSQTS.....LPKWNTE
 276 GSKNYVRDILNLNTRITNYPPIAAGHGYVD..PVTKKDPAIEPFTKAES...KNVPVWLT
 248 NSLNDPIAVKRV.....DIVGAHNYGS..SYI.....PFTTTS...KGKGWMT
 213 PVLKDSASKYV.....SITGGHLYGT..TPKP.....YPLAQN.....GKQLWMT
 220 PTLNDPTTAQYV.....SITGGHLYGS..PIRD....YPLARNK...GKDWMT
 216 PILNDPQALANM.....DILGTHLYGT..QVSQFP...YPLFKQKG...AGKDLWMT
 220 PILNDATALDNM.....DVLGCHFYGT..SVNNMA...YPLYQQKS...AGKELWMT

α5 β6 α6 β7

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 GH30_8_CaXyn30A
 GH30_8_BsXyn30C
 GH30_8_CpXyn30A
 GH30_8_CpXyn30A

341 S.....IGTWNSEGRDLSKRLMEDMEVALGTINNWC.....KGVIVWNMLDND
 381 CVGSKFWEQSVRLGSDWRGMQ.....YSHSIITNLLY.HVVGWTDWNLALNPE
 304 EC.....GTGDNSS.....YAEYIFHLINHYFRNGATAYTYWNMILDDQ
 324 RC.....MMTDTPEE.....AAHIMFGIIGNWLHVHNGLSMITLWNLALDER
 297 CCSLGEAN.....GTGRGWSG..GYDPTITNALMFSGMVQLQSFVLAGEPHYDFWTLVSN
 313 GDS DASGK.....SMYQNLLLDFTWLHPTAWVYQQAIDGAG
 331 SMLDAEPST...EAGFPASYD...AATYMDIALYMGKLIYSDIVYANNTSWSYWTAMAQEK
 284DQGTPTTTALLMHHSLSVEEGVNAFYFFWDLIWNENS
 287DQGTPTTTTQLIHNSLSVEEGVSSYFFWDLIWDNS
 281DYGTPTTTAQLIYNSLVEIEGVSAFYFFWDLIWNENS
 281DYGTPTTTAELIHNSLSLVVEGVSGYFFWDLIWNENS
 281DYGTPTTTAELIHNSLSLVVEGVSGYFFWDLIWNENQ
 292 ADGSGPWNT.....TWDVSGQLAEGLQWALYMHNAFTNSDTSGYNHWACAGGGA
 299 TDLSGAFTP.....YTFFADGGGAGEGMTWANHIQTAFVNANVSFAFIYWIGAENST
 286 ADLDDAFTT.....TWYSSGAANEGLTWANLIWQGVVEADLSAFLYWIGAQSNS
 288 .GPSTPFVK.....TWYSNGGTNEGFTWANKIAVAMVNAQLSAYLFWEGFEIQQ
 333 SDPHNS.....YSTSIEDGLNWAVKFHRYLCEANVSSIIWAGALPDS
 290 SDMNG.....NDTTINDGLRWAKEIHDFTITEGNAWFYWGWACFKT
 255 YVDSKQ.....SANNWTSIAIEVGTELNASMVS..NYSAYVWWYIRRSY.
 262 YLEGN.....DPGTCVKLAKEIHDGMTIGNMNAIVYWWISGDQ.
 262 YYPNSDT.....NSADRWPEALDVSQHIHNAWVEGDFQAYVWWYIRRSY.
 266 YFDD.....TTGNIMMSKEIHDMSMTGNMNAIYIWITWPN.

α7 β8

GH30_3_AA078418.1

GH30_3_AA078418.1

GH30_1_AAA35880.1

GH30_2_ABX45137.1

GH30_9_LcGUS30

GH30_11_ShGbhA

GH30_5_BAC84995.1

GH30_4_PbFucA

GH30_HHU50367.1

GH30_WP_114299953.1

GH30_NLM60409.1

GH30_AcXyn30B

GH30_NLL05159.1

GH30_7_TcXyn30A

GH30_7_TcXyn30B

GH30_7_TcXyn30C

GH30_7_TrXynVI

GH30_6_WP_028726386.1

GH30_10_AcXbh30A

GH30_8_EcXyn30A

GH30_8_CaXyn30A

GH30_8_BsXyn30C

GH30_8_CpXyn30A

GH30_8_CpXyn30A

385 RGPNREG.....GCQTCYGAVDINNS.....DYKTIIRNSHYIIAHLSS
428 GGPNNVVR.....NFVDSPIIVDITKD.....TFYKQPMFYHLGHFSK
344 DSTWGW.....WQNSLFTITAD.....KHEVRRNPEYYVMRHFSS
365 GLPNAAD.....STGREGVVTID.....HTTGKVRNLEYFMLRNFGQ
351 GCTPGDNSTCDPTVPNAEGWTDGVIYYDAN.....YATNGNYELYLTKHFWTYKHFNG
349 W.....GLIVGDND.....NLTSSASTKYFVLAQLTR
386 WSQKNRF.....YLLRLIDADDTDGGESYGDIRKGGTIEDSKSLWLVLGNYSR
318 QR.....PFIQLENPW.....DQSSWSDDKGYIITEFFHFVKHYSK
321 QR.....PMVIVEPPE.....NQNGWSNPQGGYKTDYFYSSIQHYAK
315 QR.....PLVFIENPF.....TPNSWTTQKGYILSDFYITIQQYAK
315 QR.....PLVAIEVPR.....NPEQWTTKEGYIISDFYIIMQQFAK
315 QR.....PLVFIIEKPR.....NPNEWTTKEGYIISDFYIPIQQYAK
341 DN.....VLISITG.....NSYEVSSRLWAFASYFR
349 TNS.....GMINLIN.....DEVIPSKRFWSMASFSK
335 NAA.....GLVTLNG.....STVQASGTLWAFAMFSR
336 SQSG.....SHLIDAL.....DRQATPSGIFWAFAMWSR
376 GTNE.....GLIYIAK.....NRTDYETGKRYETFGNFTR
332 YNGE.....GLIQMDL.....NSKTYKVAKRRLTYIGQFSR
296GLL.....TEDGKVSCKRGYVMSQYAR
300NGLYN.....TRTNETYKKTYYVMGQFSK
306GPM.....KEDGTISKRGYNMAHFSK
304GLA.....TSSGTIYKRAYVLGQFAK

1

1

α8

GH30_3_AA078418.1

GH30_3_AA078418.1

GH30_1_AAA35880.1

GH30_2_ABX45137.1

GH30_9_LcGUS30

GH30_11_ShGbhA

GH30_5_BAC84995.1

GH30_4_PbFucA

GH30_HHU50367.1

GH30_WP_114299953.1

GH30_NLM60409.1

GH30_AcXyn30B

GH30_NLL05159.1

GH30_7_TcXyn30A

GH30_7_TcXyn30B

GH30_7_TcXyn30C

GH30_7_TrXynVI

GH30_6_WP_028726386.1

GH30_10_AcXbh30A

GH30_8_EcXyn30A

GH30_8_CaXyn30A

GH30_8_BsXyn30C

GH30_8_CpXyn30A

GH30_8_CpXyn30A

425 VVKPGAVRIATTGY...TDNGITCSAFENT.DGTYAFVLI...NNEKSKKITVSDGQ...
465 FIPEGSQRVGLVAS...QKNLDLDAVALMHP.DGSADVVL...NRSSKDVPLTIKDPV...
379 YVRPGAKVLGTTGH...FNSMAIAFRNP.DGTVVVVAQ...NALDEERPFEFADPDAAE...
403 DVSVGATVIGSTNYTRDGYTGGLGSVAFVLT.AGDIAAHL...NPTAQPIQAAVTINGNA...
404 FVKPGSQRNIVTGDDA...SNTTLVVS...SDSYHVLAM...PATDPVNATLTFFDVPVCA...
377 HIRQGMQILTTDPDV...NTAVAYDAG.SQKLVI...TANWG.SAQITITFDLTRARTA...
433 FIRPGYQRINLEASNLG...INGLMGTAYLSPNDQDMVCVFV...MNGYTRRNLSFDDIGYSI...
354 FTDPGYQRVAADCS...VDDIKISAFVSPDHKSLTVL...NGHNTADVALDNGYAVN...
357 FTEPGYSRVKAESS...GSNVSVTAFTSPGKDKLTLV...NKASSESTISLNLNGYTAD...
351 FTDPGFKRVEANCN...SNDVKVTA...FVSPDESKLTMIF...INTASANTVSLDNLGFSFN...
351 FTDPGYKRVDASVS...SNDIKVS...AFVSPDKSKLTMIF...INKGYSEHSVALDLKGYSSN...
351 FTDPGYKRVDVSLN...ASDVKAS...AFVSPDQSKLTMIL...INKASSENTVALDNLNGYSSN...
367 FARPGSVRIEATSS...VENYVVSAYENK.NGTVSI...PVINAAHFPEYVITIDQLKAR...
376 FVRPNAQRVKATSS...DASVTVS...AFENT.NGVVAIQV...INNGTSAASLTIDLGKTHKE...
366 FIRPDVAVRISTSGS...PSNVNVGAFKNA.DGSIVV...VAINNNG.NSETISLSGITA...
366 YIRPGASRVATSGS...LSNVII...GAFQNT.DRSIVV...VFTNSGTSQAQARSVFSGFTF...
406 YIPVGSRRIRISAEYN...TDQGYMV...SGYKN.GNSFTA...VAINTNDTEKTLDLLNNTQT...
362 FIRPGWQRIEATKN...PVS...NVYVTA...YKDKPTGKFAIV...AINNGWSKQSI...TYTLKGFSPA...
317 FVRPGALRIQATEN...PQSNVH...LTAYKNT.DGKMVI...VAVNTNDSQDQMSLNSINANVT...
323 FIGNGYSRVDAATNS...PQSNVY...VSAYTG.NNKVVI...VAINNGQTYPVNQSFVNQSTVS...
327 FVRPGYVRIDATKN...PNANVY...VSAYKG.DNKVVI...VAINKSNVTGVNQNFVLQNGSAS...
325 FIRPGYKRVDATAT...PNTNVY...VSAYTG.DNKAVI...VAINTGTAAVSQKFNFQNGSAS...

β2X

β3X

β4X

β5X

GH30_3_AA078418.1

GH30_3_AA078418.1

GH30_1_AAA35880.1

GH30_2_ABX45137.1

GH30_9_LcGUS30

GH30_11_ShGbhA

GH30_5_BAC84995.1

GH30_4_PbFucA

GH30_HHU50367.1

GH30_WP_114299953.1

GH30_NLM60409.1

GH30_AcXyn30B

GH30_NLL05159.1

GH30_7_TcXyn30A

GH30_7_TcXyn30B

GH30_7_TcXyn30C

GH30_7_TrXynVI

GH30_6_WP_028726386.1

GH30_10_AcXbh30A

GH30_8_EcXyn30A

GH30_8_CaXyn30A

GH30_8_BsXyn30C

GH30_8_CpXyn30A

GH30_8_CpXyn30A

476RHFAVDVPGKSVTSYRWAKSK.....
517GFLETISPGYSIHTYLWHRQ.....
431RGFKVTLAPRSFNTFVLD.....
461NWQLVTVPPYGTVT...LHKSDAPLNTTNVP
457 T...TAVRTSADE...DFATVDPATEATINGTWVLALAAATSLTTFTEFGAC.....
427 GSNGATVPRWSTQTGGGDQYRSYTDTKINNGKFSASFSSGQVQTFEVSQVVLQ.....
490SHIDTYTTTSE...TQNLEHAS...YAADETFNVAKGTVTTVVLSISPTAGIEHLT...
409 D...S.VYRTVP...NGSERFALIGSLGAGHTLSMPAQSI...VTVVNVSS.....
412 T...S.VYRTVP...SGTAERFAHLGSLQ.GNTVTMPAQSVVTVVVALGFP.....
406 K...S.VI.YRTL...NGNEKFA...PAGSLT.GNTVTLPARSIVTVVVALGFD.....
406 K...S.II.YRTL...EGNERF...KKVGS...LF.GNTVTLPARSVVTVVVALGVD.....
406 K...S.VV.YRTL...NGTEGF...KKVGS...LS.GNTVTLPAQSVVTVVVALGVS.....
421KRVSTFLTDN...SHNVTLMDQSELHGSVLKATVP...PRAVQVFWLE.....
430 V...KKVVPWVTSN...DYDLEEMSEIDVKHNSFLASVPARSLTSFVTECE.....
413SKVSAYYMD...AVSSPSTFSATLNGGTGVSGLPARSMVTFVITTGSSGSA.....
419TSAAAYVTDN...SHTFSTTSAGLSGGAITVSVPSKGVVTVKLT.....
460GPIVGH...LTDA...THKWATLDTIQPVNNTYVVVTLPAKSVVTVFTGNVE.....
418SVTPYTTSS...TQNLK...GSDITVNNSSFSFELAPNSITTFVGD...TES.....
372KFEKYSTSA.SLNVEYGGSSQVDSSGKATVWLNPLSVTTFVSK.....
377NVSSWVSSG.TLNMAKTNSNISAAANGRFNASLPAQSVTTFVAD.....
381SVSRWITSS.SSNLQP.GTNLTVSGNHFWAHLPAQSVTTFVVR.....
379SVVSYVTDS.SRNMAA.GANIAVTNGSFTAQLPAQSITTFVGNTAPVVVEPIDAF

β6X

β7XA

β7XB

β8X

β9X

Fig. S1. Multiple sequence alignment of catalytic domain of *AcXyn30B* and the members from each GH30 subfamily. Four proteins most similar to *AcXyn30B* according to BlastP are also included and marked by a vertical bar on the left. Secondary structure elements of GH30_3 endo- β -1,6-glucanase BT3312 (from group 1) are shown on top (green) while secondary structure elements of GH30_8_*CpXyn30A* (from group 2) are depicted on bottom (blue). Catalytic acid/base and catalytic nucleophile (in *AcXyn30B* Glu171 and Glu279, respectively) are highlighted in magenta. The differences in the arrangement of the β ₉-domain relative to the (β/α) ₈ barrel in the group 1 (subfamilies 1, 2, 3, 9) and the group 2 (subfamilies 4, 5, 6, 7, 8, 10, 11) are highlighted in yellow. While the first three β -strands of the β ₉-domain precede the (β/α) ₈ barrel in group 1 (top four sequences), just one β -strand of the β ₉-domain is located in front of the (β/α) ₈ barrel in group 2 (other sequences). The prokaryotic arginine, which is responsible for glucuronoxylan specificity of the typical GH30_8 members, and the corresponding residues in other sequences are green highlighted. A different arginine residue, which often plays a similar role in eukaryotic GH30_7 glucuronoxylanases, is highlighted in grey. The shorter β 2- α 2 regions in *AcXyn30B* and GH30_8 sequences are marked in cyan and the shorter α 7 helix in *AcXyn30B* and related enzymes is depicted in orange. The blue region shows a longer β 8- α 8 segment in *AcXyn30B* and related enzymes.

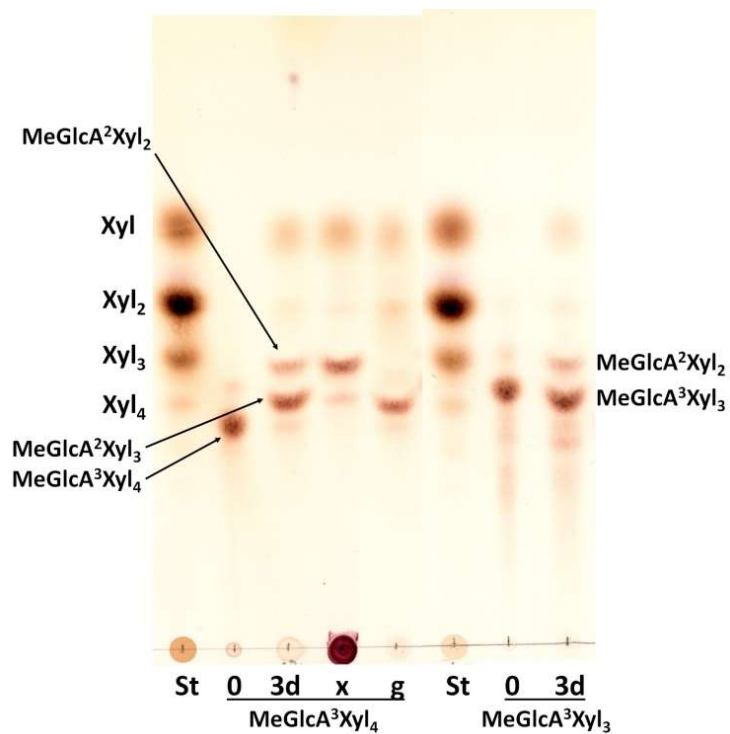


Fig. S2. TLC analysis of the hydrolysis products released from acidic XOs MeGlcA³Xyl₄ and MeGlcA³Xyl₃ by *AcXyn30B* after 3 days, and after subsequent addition of GH3 β -xylosidase (x) or GH67 α -glucuronidase (g). St – standards of linear XOs.

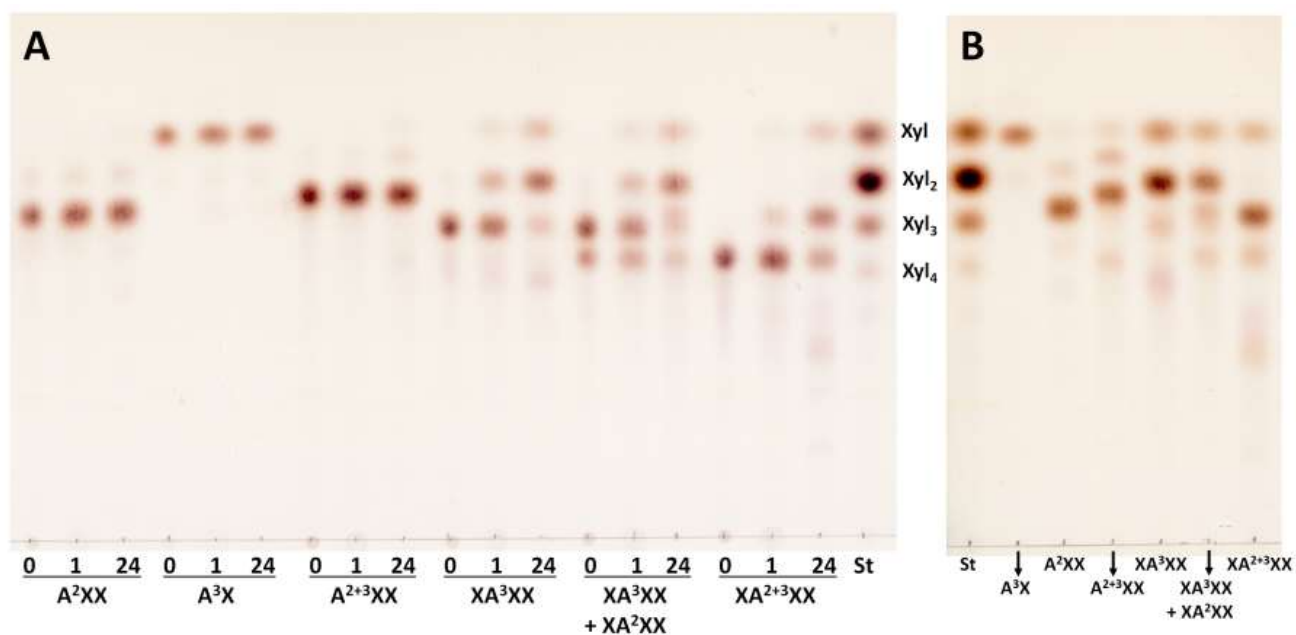


Fig. S3. TLC analysis of the products formed from arabinoxylooligosaccharides by *AcXyn30B* after 0 h, 1 h, and 24 h (**A**) and after 3 days (**B**). St – standards of linear XOs.

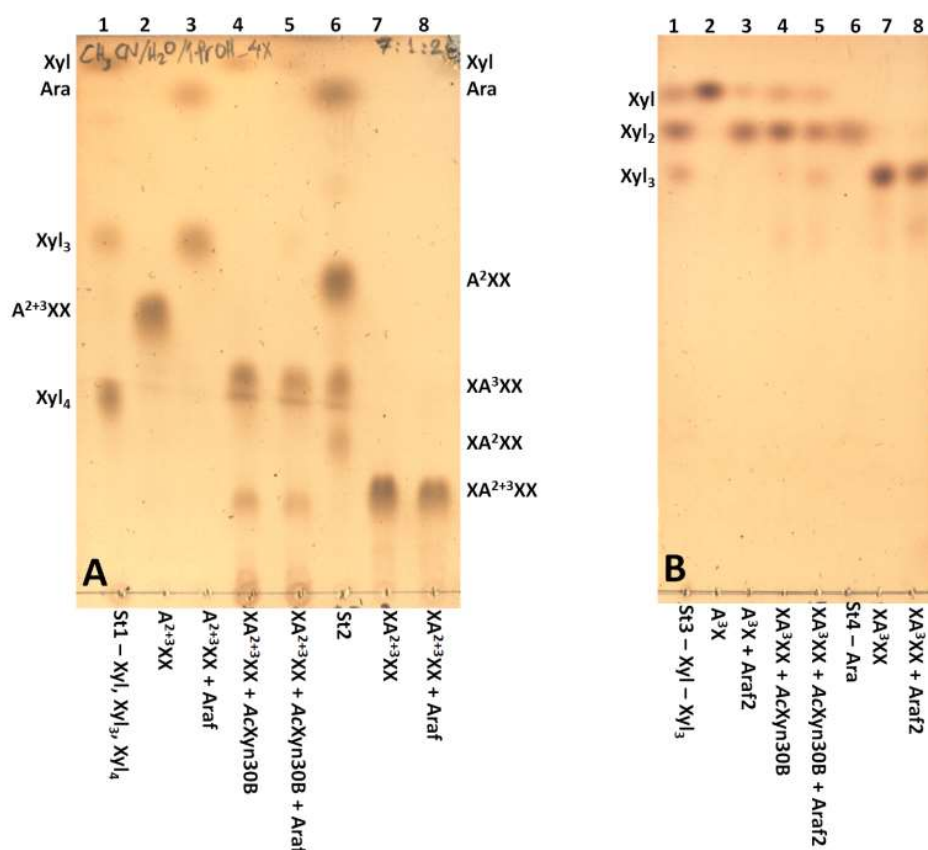


Fig. S4. Elucidation of the structure of products released by *AcXyn30B* from arabinoxylooligosaccharides (Ara-XOs) $XA^{2+3}XX$ (A) and XA^3XX (B). In panel A GH51 α -arabinofuranosidase *CjAraf51A* from *Cellvibrio japonicus* (Araf, ACE86344.1, Megazyme) was used. This α -arabinofuranosidase is able to release both Ara residues linked to the non-reducing end Xylp residue of $A^{2+3}XX$ (lane 2), thus yielding Ara and Xyl₃ as final products (lane 3). In contrast, the enzyme does not attack $XA^{2+3}XX$ (lanes 7, 8) because the substitution is at the internal Xylp residue. From $XA^{2+3}XX$ *AcXyn30B* forms xylose and an Ara-XO by one Xyl shorter than the substrate (lane 4). An addition of Araf to the hydrolysate didn't cause any change in the structure of Ara-XO formed by *AcXyn30B* (lane 5) which means that this Ara-XO doesn't have doubly arabinosylated Xylp residue at the non-reducing end but is internally disubstituted. *AcXyn30B* thus release Xyl from the reducing end of $XA^{2+3}XX$ producing $XA^{2+3}X$. St – standards of linear XOs Xyl, Xyl₃ and Xyl₄ (lane 1), St2 – standards of Ara, A^2XX , XA^3XX , XA^2XX (lane 6). The reactions were performed overnight at 35 °C using 0.1 % substrates and 0.01 mg/ml arabinofuranosidase (final concentration) in 50 mM sodium phosphate buffer, pH 6.0. The TLC plate was developed 4 times in the solvent system acetonitril/water/1-propanol 7:1:2 (v/v) and visualized using orcinol reagent (0.5% orcinol in 5% sulphuric acid in ethanol) and a heating at 105 °C.

In panel B a GH62 α -arabinofuranosidase from *Aspergillus nidulans* (Araf2, EAA59562.1, Megazyme) was used. This α -arabinofuranosidase rapidly converts A^3X (lane 2) which is 3-arabinosylated at the non-reducing end terminal Xylp residue, yielding Ara and Xyl₂ (lane 3). However, within the same period of time the enzyme does not attack XA^3XX , i.e. internally 3-arabinosylated XO (lanes 7, 8). From XA^3XX *AcXyn30B* releases xylose and an Ara-XO by one Xyl shorter (lane 4) that is essentially resistant to the Araf2 (lane 5). Therefore, the Ara-XO generated from XA^3XX by *AcXyn30B* carries internal 3-arabinosylated Xylp residue. i.e

it has the structure XA^3X , and xylose released from the substrate XA^3XX originates from the reducing end. St3 – standards of linear XOs – Xyl, Xyl₂, Xyl₃ (lane 1), St4 – standard of Ara (lane 6). The reactions were performed for 1.5 h at 35 °C using 0.1 % substrates and 0.01 mg/ml arabinofuranosidase (final concentration) in 50 mM sodium phosphate buffer, pH 6.0. TLC plate was developed twice in 1-propanol/ethanol/water 7:1:2 (v/v) and visualized using orcinol reagent as mentioned above.