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The crystal structure of a hyperthermoactive exopolygalacturonase from Thermotoga maritima reveals a unique tetramer

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                        α1                    β1   η1      β2          α2              β3     β4    β5
Tma_ExoPG          ℓℓℓℓℓℓℓℓℓℓℓ          →   ℓℓℓℓ     →        ℓℓℓℓℓℓℓℓℓℓℓℓℓ      →     →     →   TT
Tma_ExoPG     1   MIMEELAKKIEEEILNHVREPQIPDREVNLLDFGARGDGRTDCSESFKRAIEELSKQGGGRLIVPEGVFLTGPIHLKSNI
Yen_ExoPG   130   VKAQYADGSLSVASKPITAKTSAKPQIVNVRDFGAIDDGKTLNTKAIQQAIDSCKPGC..RVEIPAGTYKSGALWLKSDM
Eca_EndoPG    1   .........SDSRTVSEPKTPSSCTTLKADSSTATSTIQKALNNCDQGKAVR..LSAGSTSVFLSGPLSLPSGV
Ani_EndoPGI  33   ...................STCTFIS...ASEASESI.....SSCSDVVLSS.IEVPAGE
Ani_EndoPGII  1   .........MHSFASLLAYGLVAGATFASASPIEARDSCTFTT...AAAAKAGK.....AKCSTILTLNN.IEVPAGT
Spu_EndoPGI   3   ...................ATCTVKS...VDDAKDI.....AGCSAVTLNG.FTVPAGN
Clu_EndoPG   24   ...................ASCTFTD...AAAAIKGK.....ASCTSIILNG.IVVPAGT
Aac_EndoPG    1   ...............ATTCTFSGSNGASSASKSK...TSCSTIVLSN.VAVPSGT
Fmo_EndoPG   25   ...................DPCSVTE...YSGLATAV.....SSCKNIVLNG.FQMPIGK

                    β6       β7    η2      β8      β9          β10     β11      β12          η3    η4
                                  ℓℓℓ            TT    →                    →      →      coil 4 - T3    ℓℓℓℓ   ℓℓℓℓ
Tma_ExoPG    81   ELHVKG.TIKFIPDPERYLPVVLTRFEGIELYNYSP....LVYALDCENVA....ITGSGVLDGSADNEHWWPWKGKRDF
Yen_ExoPG   208   TLNLQAGAILLGSENPDDYPAGYRLYPYSTIERPASLIN..AIDPNNSKPGTFRNIRITGSGVIDGNG..WLRAKTAEIT
Eca_EndoPG   64   SLLIDKGVTLRAVNNAKSFENAPSSCGVVDKNGKGCDAFITAVSTTNSGIYGPGTIDGQGVVDKKVSWWELAADAKV
Ani_EndoPGI  65   TLDLSD....AADGSTITFEGTTSFGYKEWKGP....LIRFGGKDLT...VTMADGAVIDGDGSRWWDSKGTNGG
Ani_EndoPGII 60   TLDLTG....LTSGTKVIFEGTTTFQYEEWAGP....LISMSGEHIT...VTGASGHLINCDGARWWDGKGTSG.
Spu_EndoPGI  34   TLVLN....PDKGATVTMAGDITFAKTTLDGP....LFTIDGTGIN...FVGAD.HIFDGNGALYWDGKGTNNG
Clu_EndoPG   56   TLDMTG....LKSGTTVTFQGKTTFGYKEWEGP....LISFSGTNIN...INGASGHSIDCQGSRWWDSKGSNNG
Aac_EndoPG   37   TLDLTK....LNDGTHVIFSGETTFGYKEWSGP....LISVSGSDLT...ITGASGHSINGDGSRWWDGEGGNGG
Fmo_EndoPG   57   QLDLSS.....LQNDSTVTFKGTTTFATTADNDFN.....PLVISGSNIT....ITGASGHVIDGNGQAYWDGKGSNSN

                       TT        α3           η5      TT                β13    β14    β15      β16
                              ℓℓℓℓℓℓℓℓℓℓℓℓ    ℓℓℓ                    →     →     →  coil 5 - T3  →
Tma_ExoPG   152   GWKEGLPNQQEDVKKLKEMAERGTPVEERVQFYGKG.........HYLRPSFVQFYRCRN.VLVEGVKTINSPMWCIHPVLS
Yen_ExoPG   284   DELGRSLPQYVASKNSKVHEDGILAKNQVEKAVSDGMDLKNAYGQRRSSLMTLRGVEN.VYLAGFTVRNPAFHGIMNLEN
Eca_EndoPG  144   KKLKQNTPRLIQINKSKN..FTLYNVSLINSPN................FHVVFSDGDG.FTAWKTTIKTPS.........
Ani_EndoPGI 129   ....KTKPKFMYIHDVED..STFKGINIKNTPV................QAISVQATN.VHLNDFTIDNSDGDDN.....
Ani_EndoPGII 123  ....KKKPKFFYAHGLDS..SSITGLNIKNTPL................MAFSVQ.AND.ITFTDVTINNADGDTQ.....
Spu_EndoPGI  96   ....THKPHPFLKIKGSG...TYKKFEVLNSPV................QAISVGPTDAHLTLDGDVTVDDFAGDTKN....
Clu_EndoPG  120   ....KTKPKFFYAHSLKS..SNIKGLNVLNTPV................QAFSINSATT.LGVYDVILDNSAGDSA....
Aac_EndoPG  101   ....KTKPKFFAAHSLTN..SVISGLKIVNSPV................QVFSVAGSDY.LTLKDITIDNSDGDDN....
Fmo_EndoPG  122   S...NQKPDHFIVVQKTTGNSKITNLNIQNWPV................HCFDITGSSQ.LTISGLILDNRAGDKPNAKSG

                    β17      β18    TT    β19      β20    β21    β22    coil 8 - T1      α4       β23    β24    β25
                                  coil 6 - T3                    coil 7 - T3                ℓℓℓℓℓℓℓ       →      →     →
Tma_ExoPG   222   ENVIIRN.IEISSTGPNNDGIDPESCKYMLIEKCRFDTGDDSVVIKSGRDADGRRIGVPSEYILVRDNLVISQASHGGLV
Yen_ExoPG   363   HNVVANGLIHQTYDANNGDGIEFGNSQNVMVFNNFFDTGDDCINFAAGTGEKAQEQEPMKGAWLFNN...YFRMGHGAIV
Eca_EndoPG  197   ..........TARNTDGIDPMSSKNITIAYSNIATGDDNVAIKAYKGRAET...RNISILHN...DFGTGHG.MS
Ani_EndoPGI 181   .......GGHNTDGFDISESTGVYISGATVKNQDDCIAINSGE.....SISFTGG...TCSGGHG.LS
Ani_EndoPGII 175  .......GGHNTDAFDVGNSVGVNIIKPWVHNQDDCLAVNSGE.....NIWFTGG...TCIGGHG.LS
Spu_EndoPGI 150   .......LGHNTDGFDVSAN.NMTIQNCIVKNQDDCIAINDGN.....NIRFENN...QCSGGHG.IS
Clu_EndoPG  173   .......GGHNTDGFDVGGSTGVYISGATVKNQDDCLAINSGI.....NITFTGG...TCSGGHG.LS
Aac_EndoPG  154   .......GGHNTDAFDIGTSTYMTISGATVYNQDDCVAVNSGE.....NIYFSGG...YCSGGHG.LS
Fmo_EndoPG  183   S.........LPAAHNTDGFDISSSDHVTLDNNHVYNQDDCVAVTSGT.......NIVVSNM...YCSGGHG.LS
                                 ▲                            ▲▲

                    ►    TT    β26      β27      β28        β29      β30      β31    η6   coil 11 - T1
                                 →       →         →          →        →        →  ℓℓℓ
Tma_ExoPG   301   IGSEMSG...GVRNVVARNNVYMNVERALRLKTN.SRRGGYMENIFFIDNVAVNVSEEVIRINIRYDNE.......EGEY
Yen_ExoPG   440   TGSHTG...AWIEDILAENNVMYLTDIGLRAKST.STIGGGGARNVTFRNNAMRDLAKQVMVMTLDYADSNANIDYPPAKI
Eca_EndoPG  255   IGSETMG....VYNVIVDDLKMNGTINGLRIKSD.KSAAGVVNGVRYSNVVMKNVAKP.IVIDTVYEKK.....EGSNV
Ani_EndoPGI 233   IGSVGGRDDNTVKNVTISDSTVSNSANGVRIKTI.YKETGDVSEITYSNIQLSGITDVIVIEQDYENG.....SPTGT
Ani_EndoPGII 227  IGSVGDRSNNVVKNVTIEHSTVSNSVRIKTI.SGATGSVSEITYSNIVMSGISDYGVVIQQDYEDG.....KPIGK
Spu_EndoPGI 201   IGSIATG..KHVSNVVIKGNTVTRSMYGVRIKAQRTATSASVSGVTYDANTISGIAKYGVLLSQSYP.....DDVGN
Clu_EndoPG  225   IGSVGGRSDNTVKNVTISNSVGVYISGATVKNQDDCLAINSGI.....SPTGT
Aac_EndoPG  206   IGSVGGRSDNTVKNVTFVDSTIINSDNGVRIKTN.IDITGSVSDVTYKDITLTSIAKYGIVVQQNY..G...DTSST
Fmo_EndoPG  238   IGSVGGKSDNVVMDGVQFLSSQVVNSQNGCRIKSN.SGATGTINNVTYQNIALTNISTYGVDVQQDYLNG.....GPIGK

                    ─         β32      β33      β34    TT      β35             β36            β37
                              →        →        →                →                →              →
Tma_ExoPG   370   LPVVRSVFVKNLKATGSKYAVRIEGLENDYVKDILISDTIIEGAKISVLLEFGQLGMENVIMNGSRFEKLYIEGKALLK.
Yen_ExoPG   516   PAQFYDFTLKNVTVDNSTGKNPSIEIKGDTANKAWHRLVHVNNVQLNNVTPTAISDLRDSEFNKVTFTELRGDTPWHFSE
Eca_EndoPG  323   P....DWSDITFKDVTSETKG...VVVLNGENAKKPIEVTMKNVKLTSDSTWQIKNVNVKK.............
Ani_EndoPGI 306   PSTGIPITDVTVDGVITGLEDDATQVYILCGDGSCS.DWTWSGVDLSGGKTSDKCENVP..SGASC.........
Ani_EndoPGII 300  PTNGVTIQDVKLESVTGSVDSGATEIYLLCGSGSCS.DWTWDDVKVTGGKKSTACKNFP..SVASC.........
Spu_EndoPGI 271   PGTGAPFSDVNFTGGATTIKVNNAATRVTVECGNCSGNWNWSGLTVTGGKAGTIKSDKAKITGGQYL.........
Clu_EndoPG  298   PTNGVPITGLTLSKITGSVASSGTNVYILCASGACS.NWKWSGVSVTGGKKSTKCSNIPSGSGAAC.........
Aac_EndoPG  277   PTTGVPITDFVLDNVHGSVVSSGTNILISCGSGSCS.DWTWTDVSVSGGKTSSKCTNVP..SGASC.........
Fmo_EndoPG  311   PTNGVKISNIKFIKVTGIVASSAQDWFILCGDGSCS.GFTFSGNAITGGGKTSSC.NYPTNTCPS.........

Tma_ExoPG
Tma_ExoPG
Yen_ExoPG   596   VKNVKVDGKPVAP
Eca_EndoPG        .............
Ani_EndoPGI       .............
Ani_EndoPGII      .............
Spu_EndoPGI       .............
Clu_EndoPG        .............
Aac_EndoPG        .............
Fmo_EndoPG        .............
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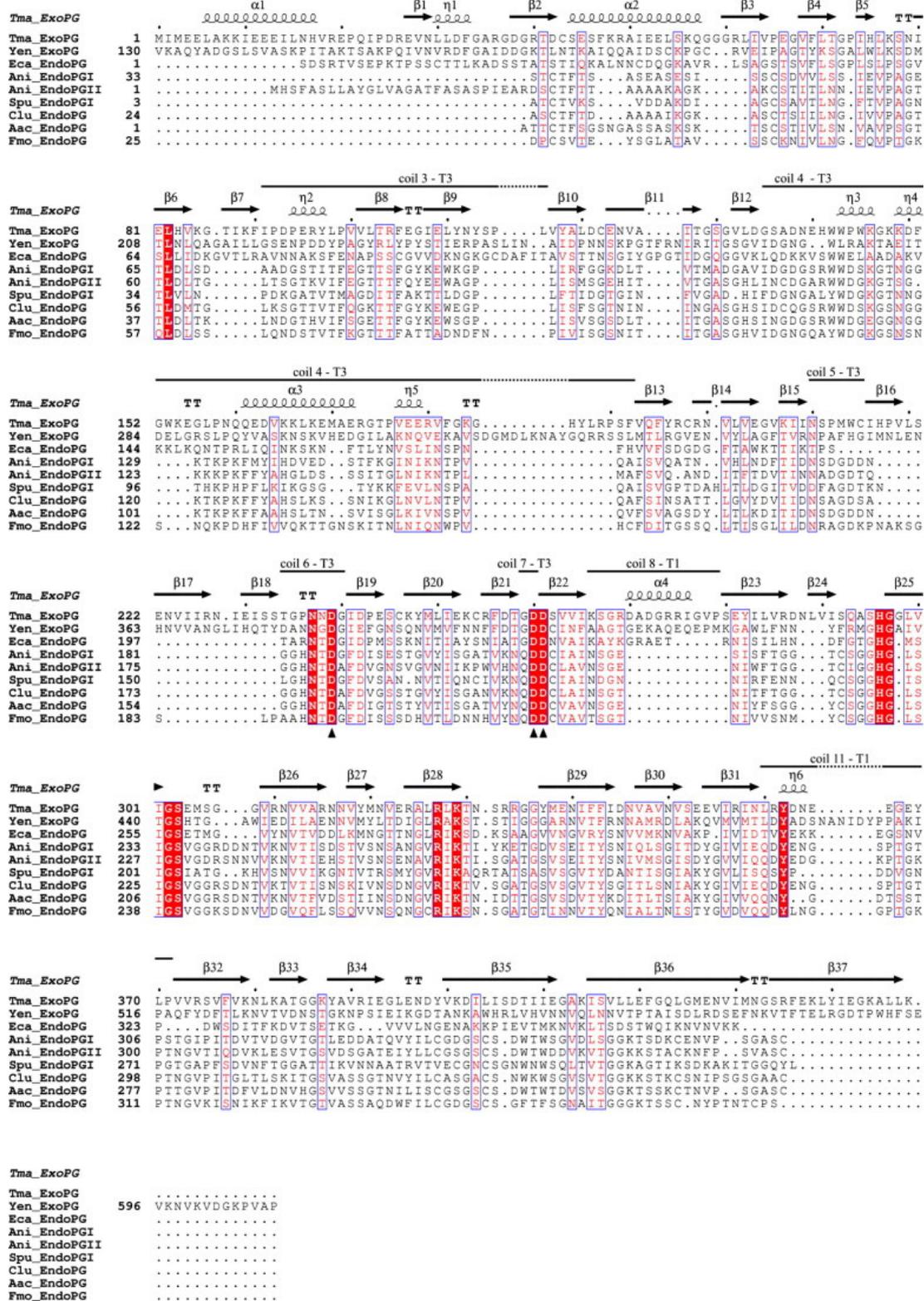
Fig. S1. Sequence alignment of polygalacturonases, figure prepared by ESPript. *Tma = Thermotoga maritima*, *Yen = Yersinia enterocolitica* (β-helical domain), *Eca = Erwinia carotovora*, *Ani = Aspergillus niger*, *Spu = Sterum purpureum*, *Clu = Colletotrichum lupine*, *Aac = Aspergillus aculeatus*, *Fmo = Fusarium monoliforme*. Fully conserved residues are boxed in red; the three catalytic aspartates are marked with ▲. Secondary structure elements are coded as follows: α = α-helix, β = β-strand, η = 310-helix, T = hydrogen bonded turn. Turns and loops lining the active site are indicated with their coil number and turn type.