## **Supporting information for**

Unique active-site and subsite features in the arabinogalactan-degrading GH43 exo-β-1,3-galactanase from *Phanerochaete chrysosporium* 

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Fig. S1. HPLC analysis of hydrolysis products formed from Gal2 (A) and Gal3 (B) by *Pc*1,3Gal43A\_WT and its mutants.

Chromatograms of incubation mixtures of 0.263 mM Gal2 (A) and 0.266 mM Gal3 (B) with WT and each mutant. The enzyme (20 nM) was incubated with each substrate in 20 mM sodium acetate, pH 5.0, for 30 min at 30 °C. The reaction mixtures were subjected to HPLC. The amount of released Gal was calculated based on the peak area.



Fig. S2. 2Fo-Fc omit maps (1.0 sigma) of Gal3 at the CBM ligand binding site. A–D show each chain of E208A. The left side is the non-reducing end and the right side is the reducing end.



Fig. S3. Catalytic residue fluctuation of PcCel45A. Ensemble refinement was performed for Pc1,3Gal43A with PcCel45A D114N complexed with cellopentaose (PDB ID: 3X2K). In total, 86 states were calculated, representing all catalytic residues and ligands (two molecules of cellopentaose). Histograms of the dihedral angles of the three residues involved in the catalytic reaction are shown in the diagram.















Fig. S4. Histograms of the dihedral angles of each conformation obtained by ensemble refinement of amino acid residues involved in catalysis and substrate recognition. A–H indicate Glu102, Asp158, Glu(Gln)208, Trp229, Gln263, Tyr355, Arg388, and Tyr438, respectively.

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Phanerochaete_chrysosporlum_BAD96241.1 Phanerochaete_chrysosporlum_BAD98241.1 Fusarium_oxysporum_BAC80558.1 Irpex_lacteus_BAH29957.1 Bacteroides_thetaiotaomicron_AA078788.1 Clostridium_thermocellum_ABN51896.1 consensus>70	21 21 21 281 32	TT TT T. AGN TIQAHGAG TIQVSTYWFGEDKSHSALF.KAVSCYT SLDIVSGATMTDT.AGN TIQAHGAG TLQVGSTYYMFGEDKSHSALF.KAVSCYT SLDIVSGATMTATNTGEHVQAHGAG TLKVGSTYYMFGEDKT DGTYF.QNVNCYS QKEKGIASGKVMRDT.SGNVIQAHGAG TLKVGSTYYMFGEDKTENSALF.HAVSCYT QKEKGIASGKVMRDT.DGNVINAHGGG TLFHEGKYYMFGEHRPASGFVTEKGINCYS GNVQSFNVAAAEGVIVNGTQFRDT.SGNVINAHGGGLLFHEGKYYMFGEHRPASGFVTEKGINCYS 
Phanerochaete_chrysosporium_BAD98241.1 Phanerochaete_chrysosporium_BAD98241.1 Fusarium_oxysporum_BAG80558.1 Irpex_lacteus_BAH29957.1 Bacteroides_thetaiotaomicron_AA078788.1 Clostridium_thermocellum_ABN51896.1 consensus>70	74 74 74 337 95	β4     ττ     ττ     β5     β6     ττ     β7       SSDLVNWSRQNDALSPIAGTMISTSNVVERPKVIFNQKNSEYVMWFHSDSSNVGAMVGVAT     TT     TT     TT     TT     7       STNUVEWTYRGALLSRTSEAG.DLGPNRIVERPKVIFNQKNSEYVMWFHSDSSNVGAMVGVAT     STDUNWTRQSNALSPVANTMISSNNIVERPKVIFNKKNQEYVMWFHSDSSNVGAMVGVAT     SNVGAMVGVAT       STDUNWTRQSNALSPVANTMISSNNIVERPKVIFNKKNQEYVMWFHSDSSNVGAMVGVAT     STDUNWTRQSNALSPVANTMISSNNIVERPKVIFNKKNQEYVMWFHSDSSNVGAMVGVAT       STDUNWTRQSNALSPVANT
Phanerochaete_chrysosporium_BAD98241.1 Phanerochaete_chrysosporium_BAD98241.1 Fusarium_cyxsporum_BAG80558.1 Irpex_lacteus_BAH29957.1 Bacteroides_thetaiotaomicron_AA078788.1 Clostridium_thermocellum_ABN51896.1 consensus>70	136 137 136 403 157	TT <sup>β8</sup> <sup>2,2</sup> <sup>11</sup> <sup>1</sup> <sup>6</sup> <sup>6</sup> <sup>6</sup> <sup>6</sup> <sup>6</sup> <sup>1</sup>
Phanerochaete_chrysosporium_BAD98241.1 Phanerochaete_chrysosporium_BAD98241.1 Fusarium_oxysporum_BAG80558.1 Irpex_lacteus_BAH29957.1 Bacteroides_thetaiotaomicron_AA078788.1 Clostridium_thermocellum_ABN51896.1 consensus>70	156 157 156 469 188	β9     TT     β10     β11     TT     β12     TT     β13       SRDESTEODDSAOTATLEYASDNNONFKISKLDANYVNVTAO.VSVNNGATLEAPGIVENNEEVF     TT
Phanerochaete_chrysosporium_BAD98241.1 Phanerochaete_chrysosporium_BAD98241.1 Fusarium_coxysporum_BAG80558.1 Irpex_lacteus_BAH29957.1 Bacteroides_thetaiotaomicron_AA078788.1 Clostridium_thermocellum_ABN51896.1 consensus>70	221 218 221 533 252	B14       TT       B15       TT       B16       D22020       B17       TT       B18         LIASHTSCWAPNPNKWFSASSLAPWSAQQD.       TAPSATRWYSQNAFDLFL.       GS.NAIYMGDR         IFGSHLTGWNANDNIXSYAKSLSQPWSNWTE.       FAPVGSKVSVIQOPL.       GNAFDLFL.       GS.NAIYMGDR         LIASHTSGWAPNPNKFFSASSLSGPWSNWTE.       FAPVGSKVSVIQOPL.       GNAFDLFL.       GNAFNATVGPR         LIASHTSGWAPNPNKFFSASSLSGPWSNUTE.       FAPVGSKVSVIDOPL.       GNAFDLFL.       GN.NAIYMGDR         LIASHTSGWAPNPNKFFSASSLSGPWSVQD.       ITTASTRWYSQNAFDLFL.       GN.NAIYMGDR         LITSGCTGWPNQAXAXISALASUNGUVN.       LOKST.       GWZSQPTILEPLPKGS.       GPFFMADM         LITSGCTGWPNQAXXXISALASGWSQUVN.       LOKST.       GWZSQPTILEPLPKGS.       GPFFMADM         LISSGW.PN.       Axs\$.g.WS#       TSQ%.Plg.n%mgDr       G.
Phanerochaete_chrysosporium_BAD98241.1 Phanerochaete_chrysosporium_BAD98241.1 Fusarium_oxysporum_BAC80558.1 Irpex_lacteus_BAH29957.1 Bacteroides_thetaiotaomicron_AA078788.1 Clostridium_thermocellum_ABN51896.1 consensus>70	281 279 281 597 314	TT 0.0.0 FIFT F20 F21 F22 F23 H4 F24 WRPSLLGSGRVINUTDDF.SSGAPQIVHADVWSVNVQAGTYGVASGTSVEAENGORGGSST WVSTNLAASTYVNDGLKV.DGTKVTLSWYDSWSPNLSKCTWSTXASGTSVEAENGORGGSST WRPSLLGSGRVINUTDF.SSGAPQIVHADVWSVNVQAGTYGTXASGTSVEAENGORGGSST WRPKSLADSRVINUTDF.SSGAPQIVHADVWSVNVGAGTYTVAGGTTYEAEKGTLGGSSK WRPKSLADSRVINUTDF.SSGAPQIVHADVWSVNVGAGTYS
Phanerochaete_chrysosporium_BAD98241.1 Phanerochaete_chrysosporium_BAD98241.1 Fusarium_oxysporum_BAG80558.1 Irpex_lacteus_BAH29957.1 Bacteroides_thetaiotaomicron_AA078788.1 Clostridium_thermocellum_ABN51896.1 consensus>70	341 339 341 624 380	β25       n5       β26       TT       β27         TISCSEFSGKAVGY       IGHG.GTVTINNVQ       SNG         LLSNSGFSGSAVGY       IGHG.GTVTINNVQ       SSG         LLSNSGFSGSAVGY       IGHG.GTVTINNVQ       GNG         VLDGSVDNAAQIVQWTDNGSLSQQWYLVDVGGSVKIVNVKSGRALDVKDESKEDGGVLIQYTSNG       G
Phanerochaete_chrysosporium_BAD98241.1 Phanerochaete_chrysosporium_BAD98241.1 Fusarium_oxysporum_BAG80558.1 Irpex_lacteus_BAH29957.1 Bacteroides_thetaiotaomicron_AA078788.1 Clostridium_thermocellum_ABN51896.1 consensus>70	372 372 372 627 446	β28     β29     TT     β30       G. SHWVALYFANGDSTYRNVTVSVNG.     GPSVLVDQPDSGGGNVVISVPVKININS       G. JATINVKVENGDNGSRYATVNVNG.     ESQKLAFLSTSHLSQTGLSRGF.FDLKE       G. JHWVALYFANGDSTYRNVTVSVNG.     GSSVLVDQPDSGGGVVISVPVKVNLNN      RWNFDX.
Phanerochaete_chrysosporium_BAD98241.1 Phanerochaete_chrysosporium_BAD98241.1 Fusarium_oxysporum_BAG80558.1 Irpex_lacteus_BAH29957.1 Bacteroides_thetaiotaomicron_AA078788.1 Clostridium_thermocellum_ABN51896.1 consensus>70	425 424 425 509	$ \begin{array}{c} & \beta 31 \\ \hline G & E N S I T F G S G O S N Y A A D L D K I I V Y X \\ G & S D M T T I S N G D . G W G P D V D A L M P A A X \\ G & A N S I T F G S G O S N Y A A D L D K I I V Y X \\$

Figure S5. Sequence alignment of GH43\_sub24 galactanases of two basidiomycetes, one mold, and two bacteria. The alignment was built by using MUSCLE on MEGAX: Molecular Evolutionary Genetics Analysis (53, 54), and the figure was generated with ESPrint 3.0 (http://espript.ibcp.fr; 55). The rate of conservation of the GH43\_sub24 region (up to approximately the sixth row in this alignment) is high, although C-terminal region is less well conserved because of the differences of the CBM.



Fig. S6. Comparison of galactan conformation.

Comparison of Gal3 conformations between catalytic-site-bound Gal3 of

*Pc*1,3Gal43A\_E208Q (white) and the "ideal" conformation of Gal3 (green). The ideal model was built by using web tool "SWEET"

(http://www.glycosciences.de/modeling/sweet2/doc/index.php#; 56, 57)