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Supporting information for article:

Structural and functional investigation of a fungal member of carbohydrate esterase family 15 with potential specificity for rare xylans

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Table S1 Primers utilized in the generation of protein substitution variants. Introduced mutations are displayed in lower case.

<i>Lf</i> CE15C-N241A-r	AGTACCGgcCTTTTGGATTTCGTCAGCAATTC
<i>Lf</i> CE15C-N241A-f	CAAAAGgcCGGTACTAAAGTTGAGACAGCCC
<i>Lf</i> CE15C-G254E-r	GAATCTtCATTGACAATCTGGTGGGC
<i>Lf</i> CE15C-G254E-f	GTCAATGaAGATTCTTGGTTTTCCACAGACTTC
<i>Lf</i> CE15-Y300W-r	TGGTCCAAGccAGTCAATAGCGGTGTT
<i>Lf</i> CE15-Y300W-f	ATTGACTggCTTGGACCAACATCAAATTATCATTGTG

Table S2 nDSF measurements of *Lf*CE15C. Protein concentration was 1 mg/mL.

Protein	Ligand	Buffer	T _i in °C*	Initial fluorescence ratio
<i>Lf</i> CE15C	-	20 mM Tris pH 8.0	54.7±0.13 (5)	0.8498±0.0014
<i>Lf</i> CE15C	-	100 mM MES pH 6.0	59.9	0.7702
<i>Lf</i> CE15C	-	100 mM HEPES pH 7.5	55.2	0.8182
<i>Lf</i> CE15C	-	100 mM sodium citrate pH 5.0	48.7	0.7795
<i>Lf</i> CE15C	-	50 mM sodium acetate pH 5.5	60.2	0.7696
<i>Lf</i> CE15C	-	100 mM sodium acetate pH 4.5	54.4	0.7701
<i>Lf</i> CE15C-G254E	-	20 mM Tris pH 8.0	57.2	0.9591
<i>Lf</i> CE15C-G254E	-	100 mM sodium phosphate pH 6.5	59.6	0.9775
<i>Lf</i> CE15C	-	100 mM sodium phosphate pH 6.5	57.1±0.08 (3)	0.7861±0.0014
<i>Lf</i> CE15C	10 mM GlcA	20 mM Tris pH 8.0	54.8±0.18 (4)	0.8493±0.0011
<i>Lf</i> CE15C	20 mM GlcA	20 mM Tris pH 8.0	55.2±0.42 (4)	0.8463±0.0029
<i>Lf</i> CE15C	20 mM GlcA	100 mM sodium phosphate pH 6.5	57.2±0.04 (3)	0.7878±0.00022
<i>Lf</i> CE15C	10 mM BnzGlcA	20 mM Tris pH 8.0	55.9±0.67 (4)	0.8330±0.012
<i>Lf</i> CE15C	20 mM BnzGlcA	20 mM Tris pH 8.0	55.4±0.56 (4)	0.8529±0.0051
<i>Lf</i> CE15C	20 mM BnzGlcA	100 mM sodium phosphate pH 6.5	56.0±0.04 (3)	0.7835±0.0016
<i>Lf</i> CE15C	10 mM cellobiose	20 mM Tris pH 8.0	54.9±0.30 (4)	0.8480±0.0017
<i>Lf</i> CE15C	20 mM cellobiose	20 mM Tris pH 8.0	55.4±0.22 (4)	0.8452± 0.0021
<i>Lf</i> CE15C	20 mM cellobiose	100 mM sodium phosphate pH 6.5	57.4±0.07 (3)	0.7849±0.00032
<i>Lf</i> CE15C	10 mM xylobiose	20 mM Tris pH 8.0	54.9±0.16 (4)	0.8483±0.0012
<i>Lf</i> CE15C	20 mM xylobiose	20 mM Tris pH 8.0	55.4±0.28 (4)	0.8460±0.0024
<i>Lf</i> CE15C	20 mM xylobiose	100 mM sodium phosphate pH 6.5	57.2±0.08 (3)	0.7852±0.00031
<i>Lf</i> CE15C	10 mM xylotriose	20 mM Tris pH 8.0	54.8±0.25 (4)	0.8479±0.0017

<i>Lf</i> CE15C	20 mM xylotriase	20 mM Tris pH 8.0	55.3±0.25 (4)	0.8453±0.0026
<i>Lf</i> CE15C	20 mM xylotriase	100 mM sodium phosphate pH 6.5	57.3±0.12 (3)	0.7851±0.00032
<i>Lf</i> CE15C	10 mM xyloetraase	20 mM Tris pH 8.0	54.8±0.16 (4)	0.8499±0.0011
<i>Lf</i> CE15C	20 mM xyloetraase	20 mM Tris pH 8.0	55.2±0.25 (4)	0.8453 ±0.00090
<i>Lf</i> CE15C	20 mM xyloetraase	100 mM sodium phosphate pH 6.5	57.2±0.05 (3)	0.7856±0.0015
<i>Lf</i> CE15C	10 mM XUXXr	20 mM Tris pH 8.0	55.1±0.11 (4)	0.8481±0.00083
<i>Lf</i> CE15C	20 mM XUXXr	100 mM NaH ₂ PO ₄ pH 6.5	58.0±0.25 (3)	0.7799±0.0022

* ± std. dev. for repeated measurements. The number of measurements is indicated in parentheses.

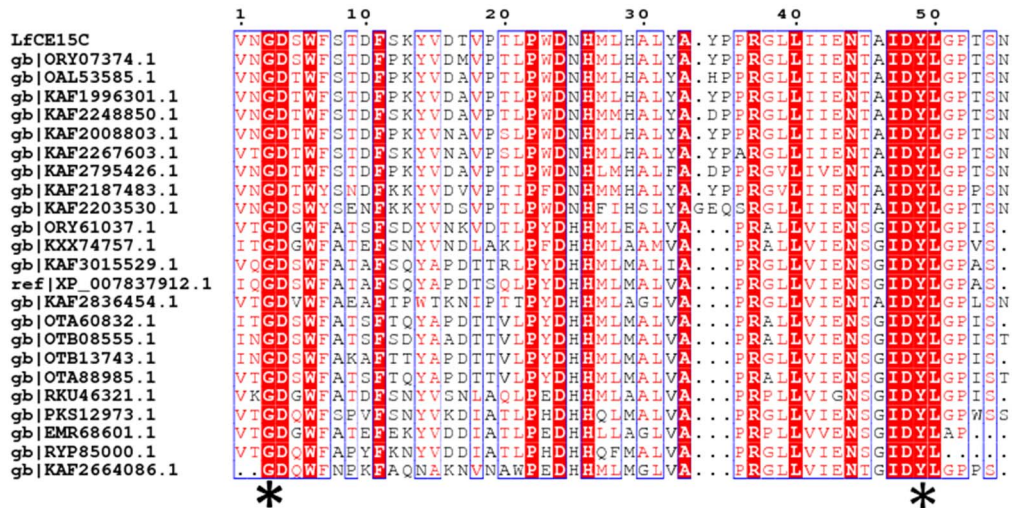


Figure S1 Alignment of found CE15 sequences containing G and Y at key positions in the region shown in Figure 6 (marked with an asterisk under the alignment. Genbank accession, species (habitat/isolation when known) are: ORY07374, *Clohesyomyces aquaticus* (submerged wood); OAL53585.1, *Pyrenochaeta* sp. DS3sAY3a (soil and plant debris); KAF1996301.1, *Amniculicola lignicola* CBS 123094 (submerged wood); KAF2248850.1, *Trematosphaeria pertusa* (submerged wood); KAF2008803.1, *Aaosphaeria arxii* CBS 175.79 (isolated from corn); KAF2267603.1, *Didymosphaeria enalia* (mangrove wood); KAF2795426.1, *Melanomma pulvis-pyrius* CBS 109.77 (debris of trees and shrubs); KAF2187483.1, *Zopfia rhizophila* CBS 207.26 (*Zopfia* root rot of asparagus); KAF2203530.1, *Delitschia confertaspora* ATCC 74209 (dung of rock hyrax); ORY61037.1, *Pseudomassariella vexata*; KXX74757.1, *Madurella mycetomatis* (human pathogen, mycetoma); KAF3015529.1, *Neopestalotiopsis* sp. 37M (fruit pathogen); XP_007837912.1, *Pestalotiopsis fici* W106-1 (plant endophyte); KAF2836454.1, *Patellaria atrata* CBS 101060 (canopy of floodplain forests); OTA60832.1, *Hypoxylon* sp. EC38 (*Neea floribunda* shrub); OTB08555.1, *Hypoxylon* sp. CI-4A (*Persea indica* endophyte); OTB13743.1, *Daldinia* sp. EC12 (*Myroxylon balsamum* endophyte); OTA88985.1, *Hypoxylon* sp. CO27-5 (mangrove); RKU46321.1, *Coniochaeta pulveracea* (decaying acacia); PKS12973.1, *Lomentospora prolificans* (opportunistic human pathogen); EMR68601.1, *Eutypa lata* UCREL1 (eutypa dieback of grapevines); RYP85000.1, *Monosporascus* sp. CRB-8-3 (arid plant roots); KAF2664086.1, *Microthyrium microscopicum* (tree leaves/stems).