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Online Resources

Enzyme Kinetics of Fungal Glucuronoyl Esterases on Natural Lignin-Carbohydrate Complexes

Applied Microbiology and Biotechnology

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Contents

Online Resource 1: Codon optimized DNA sequences

*Armillaria fuscipes, Af*GE

>GAATTCGGTTGTTCTGATTTGCCATCTAACATTCCATTGACTCCTAATTCTATGTTGCCAGATCCTTTTACTTTCTTGAAT
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*Thielavia terrestris, Tt*GE

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*Punctularia strigosonata, Ps*GE

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*Cerrena unicolor, Cu*GE

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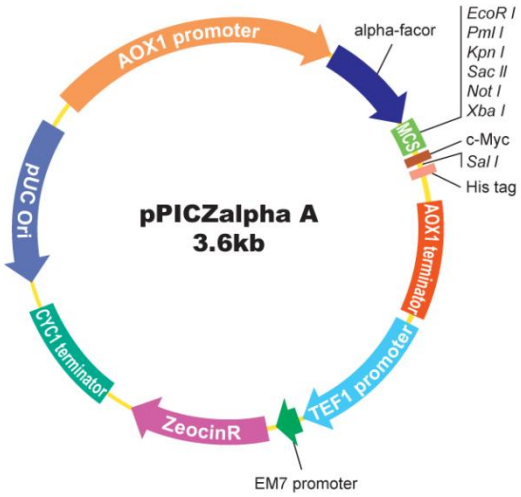
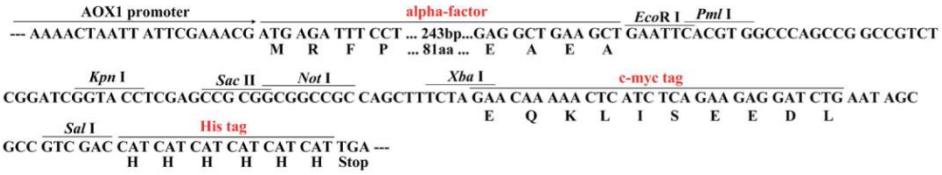
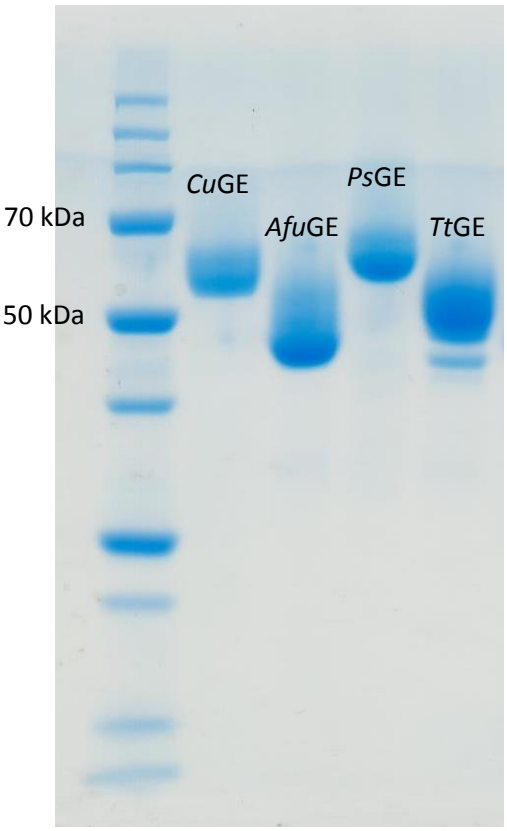
Online Resource 2: Cloning vector and SDS gel



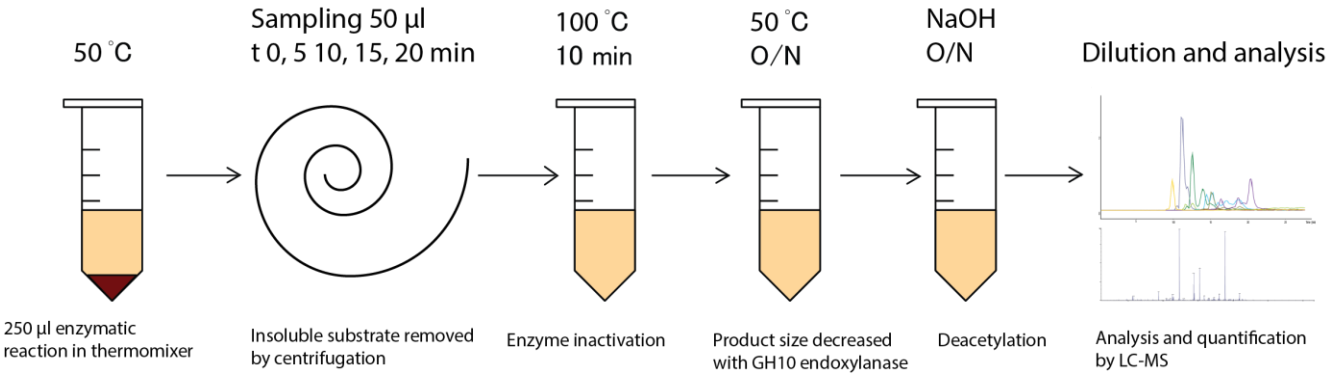


Graphic representation of the *p*PICZalphaA vector delivered by Genscript holding the CE15 genes between the *Eco*RI and *Sal*I restriction sites
(pPICZalpha-A vector (2019) Genscript https://www.genscript.com/gsfiles/vector-map/yeast/pPICZalpha-A.pdf?1897614803 Accessed March 12th 2019)



SDS gel of the expressed enzymes after purification. Loaded in the following order with theoretical molecular weight given in bracket; *Cu*GE (48 kDa), *Afu*GE (40 kDa), *Ps*GE (47 kDa), *Tt*GE (42 kDa). The four enzymes are all glycosylated resulting in the smear and the increase in size compared to the theoretical weight.

Online Resource 3: Assay flow diagram



Schematic overview of assay procedure for the kinetic study. Varying substrate concentrations of LRP with a fixed enzyme concentration. Assay conducted in eppendorph tubes in thermomixer. 50 µL samples taken every 5 minutes. Reaction stopped after initial removal of substrate by centrifugation and heat inactivation at 100 °C for 10 min. GH10 endo-xylanase added to the reaction supernatant to decrease the size of the products released by CE15 enzymes. Acetylation removed from the products by alkaline treatment to simplify the products profile. Characterization and quantification of products done by LC-MS.

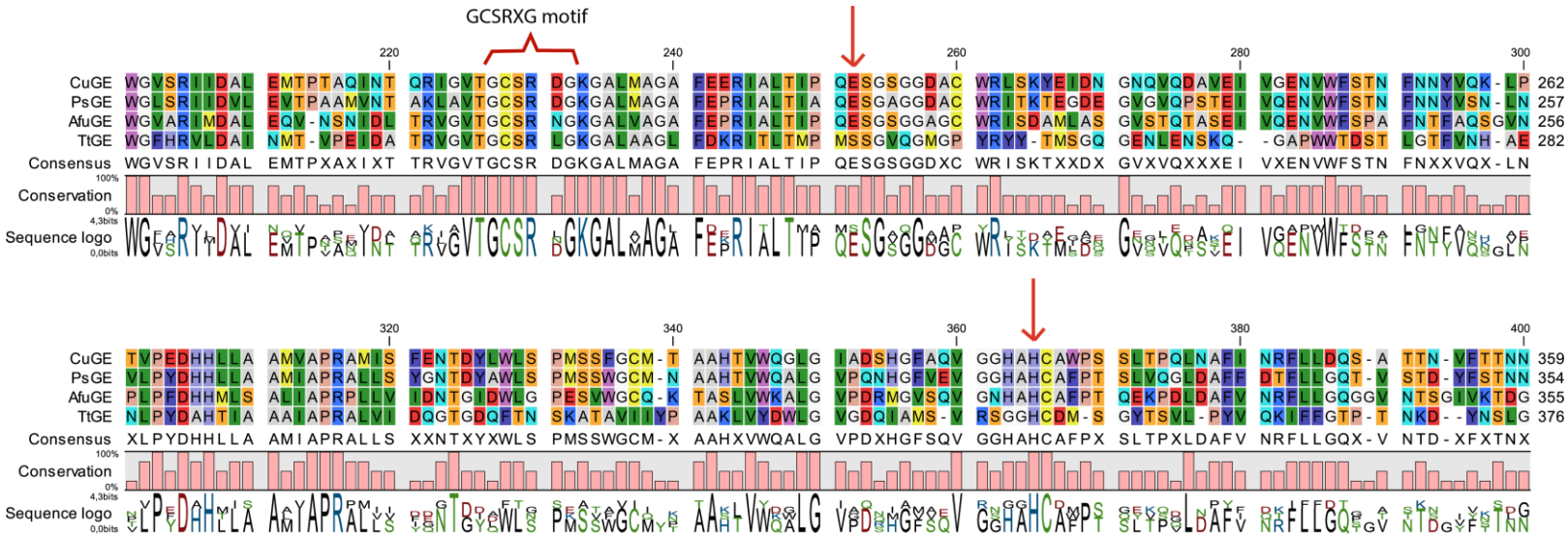
Online Resource 4: Ions for quantification.

Complete list of adducts used for quantification of enzyme reaction products and their corresponding retention times.

Analyte	*m/z* $[M+Na]^+$	*m/z* $[M+NH_4]^+$	Retention time (min), peak max
MeGlcAXyl	363	358	7.6
$MeGlcAXyl_2$	495	490	9.8
$MeGlcAXyl_3$	627	622	12.6
Reduced $MeGlcAXyl_3$	629	624	11.4

Online Resource 5: Sequence alignment

Alignment of segments of the amino acid sequences from CE15 characterized in this study. Conserved GCSRXG motif with a catalytic nucleophile serine marked with red curly bracket. Catalytic glutamic acid and histidine marked with red arrow. *Tt*GE belonging to PPR group 8 holds a serine at the position of the glutamic acid. Aligment and sequence logo made with CLC Workbench 7.5.

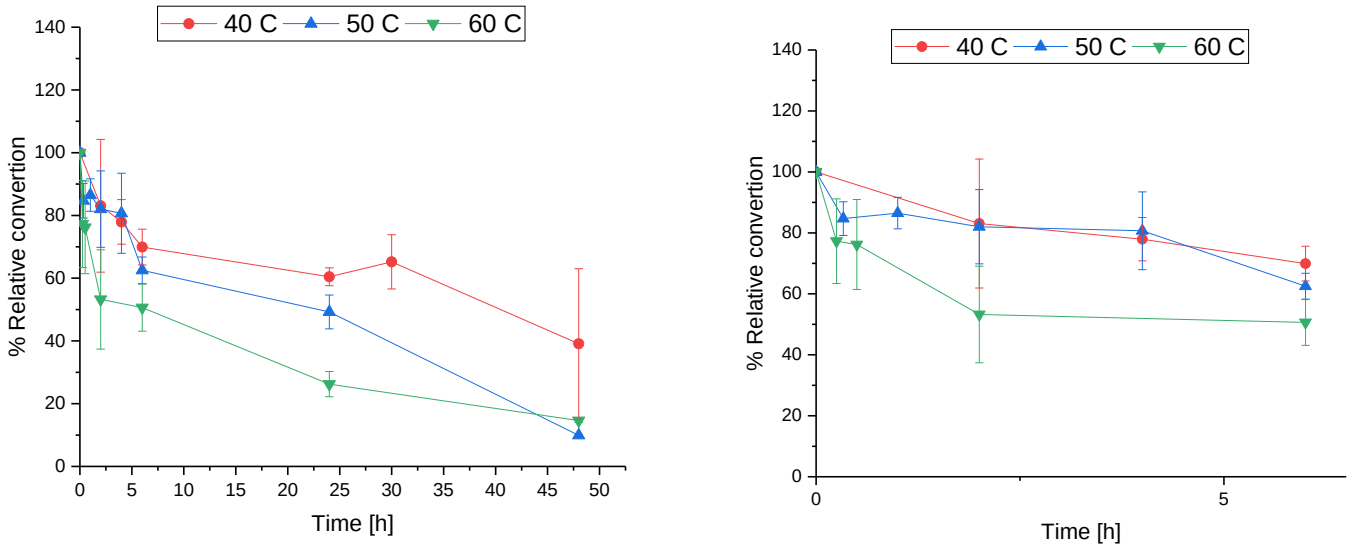


Online Resource 6: Percent Identity Matrix (PIM).

Percent query coverage given in brackets. Color scale from light to dark orange indicate percent sequence identity from the lowest value 28% in light orange to the highest value 100% in dark orange. Values obtained using BLAST (https://blast.ncbi.nlm.nih.gov).

	*Tt*GE	*Afu*GE	*Ps*GE	*Cu*GE
*Tt*GE	100 (100)	35 (84)	31(79)	28 (96)
*Afu*GE	35 (84)	100 (100)	51 (99)	49 (99)
*Ps*GE	31 (77)	51 (89)	100 (100)	65 (88)
*Cu*GE	28 (98)	49 (99)	65 (97)	100 (100)

Online Resource 7: *Cu*GE Temperature Stability.



Thermostability of *Cu*GE obtained by incubation at specific temperatures for 48 hours and followed by activity measurement on Me-MeGlcA (left graph). % conversion calculated relative to activity with *Cu*GE not pre-exposed to incubation. Rigth graph: A zoom of the first 6 hours of incubation. The study was conducted in triplicates. The kinetic assays (Fig 2) were conducted for 20 min at 50 ˚C. Under these conditions *Cu*GE still retained around 85 % of its relative activity. After 25 hours at 50 ˚C approx. 50% relative activity remained.