

AA16, a new Lytic Polysaccharide Monooxygenase family identified in fungal secretomes

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Additional data

Table S1: Protein concentration (determined by BCA assay) of the secretomes of five *Aspergillus* spp. strains grown on three inducers, after seven days of culture.

Code	Species	Strain number	Inducer	Concentration (mg/mL)
405_Avi	<i>Aspergillus japonicus</i>	CIRM-BRFM 405	Avicel	0.55
405_MB	<i>Aspergillus japonicus</i>	CIRM-BRFM 405	Maize bran	12.56
405_SBP	<i>Aspergillus japonicus</i>	CIRM-BRFM 405	Sugar beet pulp	4.96
430_Avi	<i>Aspergillus niger</i>	CIRM-BRFM 430	Avicel	0.27
430_MB	<i>Aspergillus niger</i>	CIRM-BRFM 430	Maize bran	17.63
430_SBP	<i>Aspergillus niger</i>	CIRM-BRFM 430	Sugar beet pulp	7.98
1487_Avi	<i>Aspergillus japonicus</i>	CIRM-BRFM 1487	Avicel	0.25
1487_MB	<i>Aspergillus japonicus</i>	CIRM-BRFM 1487	Maize bran	17.24
1487_SBP	<i>Aspergillus japonicus</i>	CIRM-BRFM 1487	Sugar beet pulp	8.43
1489_Avi	<i>Aspergillus japonicus</i>	CIRM-BRFM 1489	Avicel	0.29
1489_MB	<i>Aspergillus japonicus</i>	CIRM-BRFM 1489	Maize bran	13.47
1489_SBP	<i>Aspergillus japonicus</i>	CIRM-BRFM 1489	Sugar beet pulp	12.37
1490_Avi	<i>Aspergillus japonicus</i>	CIRM-BRFM 1490	Avicel	0.34
1490_MB	<i>Aspergillus japonicus</i>	CIRM-BRFM 1490	Maize bran	12.31
1490_SBP	<i>Aspergillus japonicus</i>	CIRM-BRFM 1490	Sugar beet pulp	8.50

Figure S1: Hydrolysis of three pretreated lignocellulosic substrates by a cellulolytic cocktail of *Trichoderma reesei* strain CL847 produced on lactose. Error bars represent standard deviations calculated on 3 replicates.

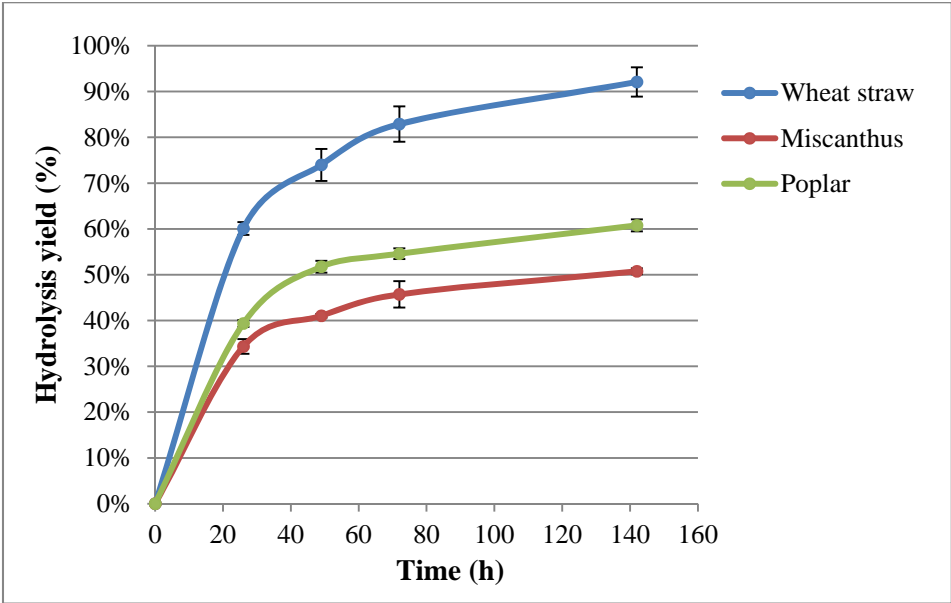


Figure S2: Graphical representation of AA16 module consensus amino acids, based on the alignment of 213 sequences, generated using the WebLogo application [1]. The strictly conserved histidine residues are shown in positions 1 and 107.

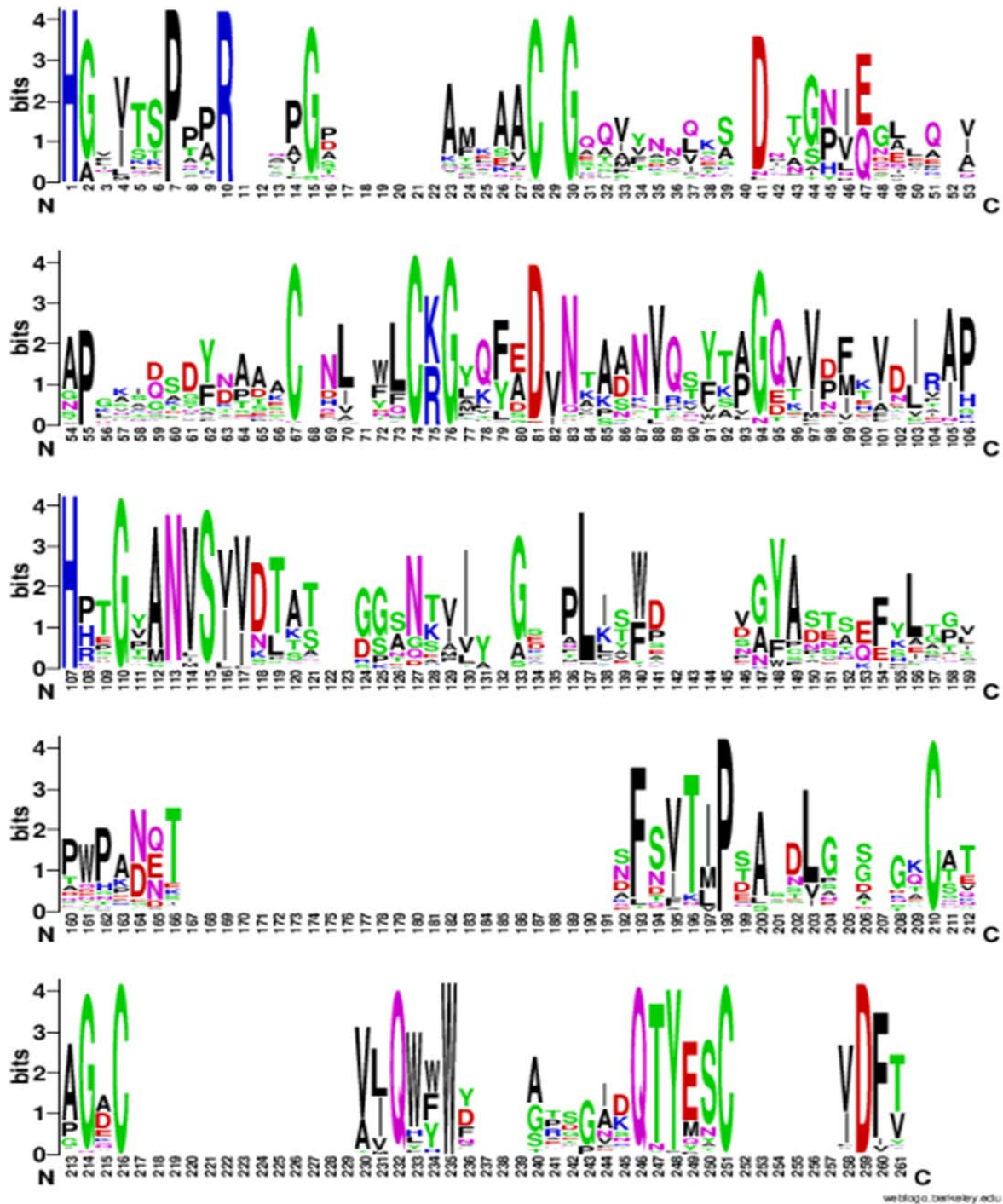


Figure S3: Time profiles of dissolved oxygen (DO), temperature and optical density (OD) at 600 nm during the production of AaAA16 by *Pichia pastoris* in a 1.3-L bioreactor. 1: Glycerol batch phase; 2 : Sorbitol and methanol transition phase; 3 : Methanol fed-batch phase.

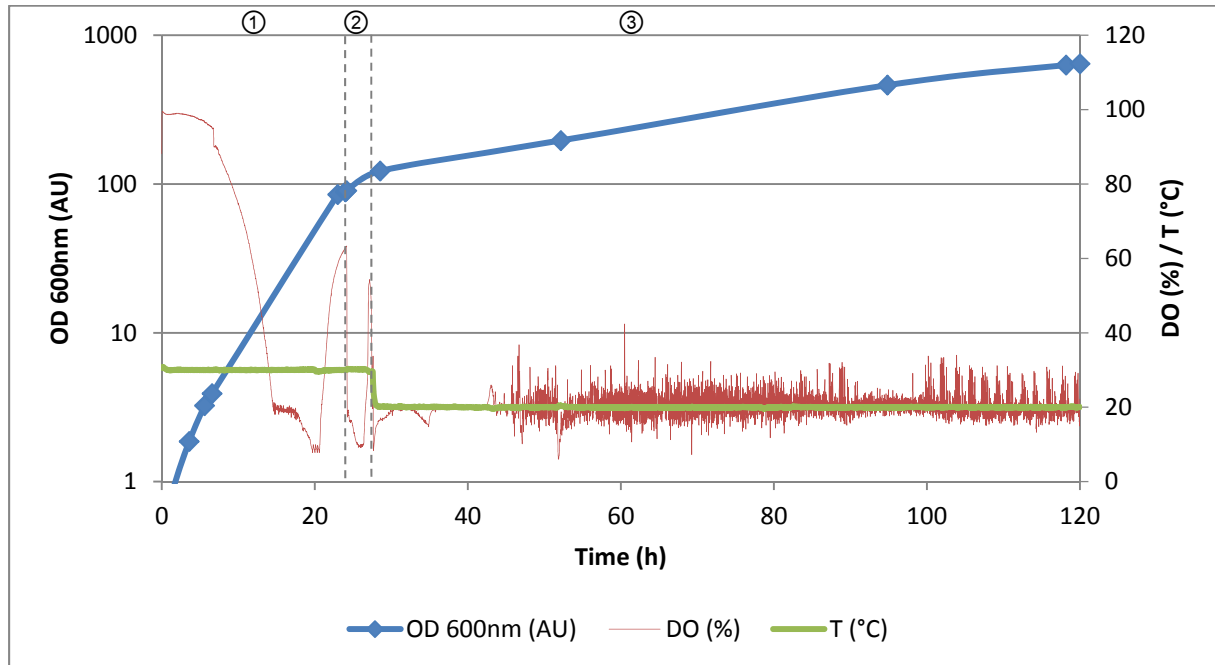


Figure S4: HPAEC-PAD chromatograms of 0.1% PASC soluble degradation products, after 70h incubation with *P. pastoris* bioreactor supernatant (SN, blue chromatogram) or with AaAA16 protein purified by affinity chromatography using a nickel HisTrap column (black chromatogram).

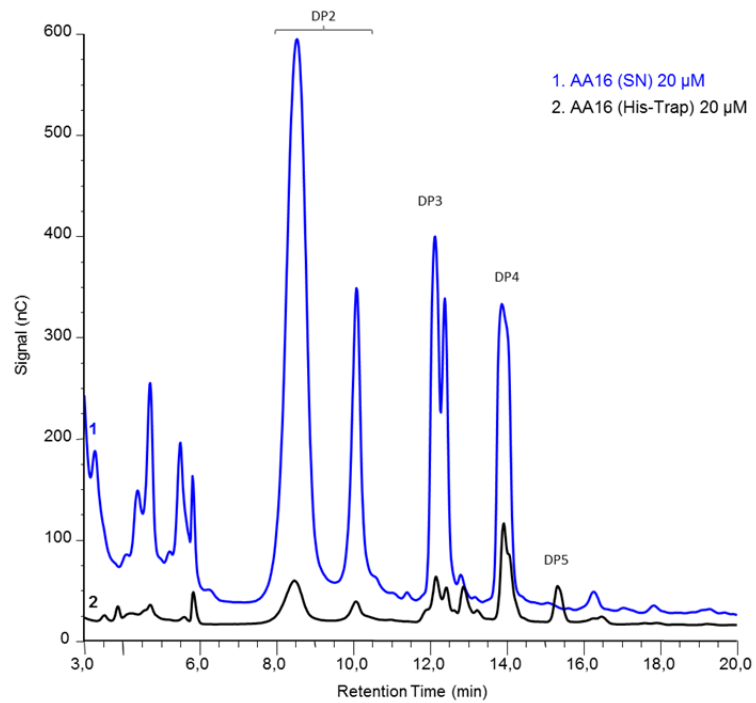


Figure S5: SDS-PAGE analysis of AaAA16 before and after purification. Lane A, *Pichia pastoris* bioreactor supernatant; lane B, AaAA16 purified by ion exchange chromatography; lane M, Molecular weight protein ladder.

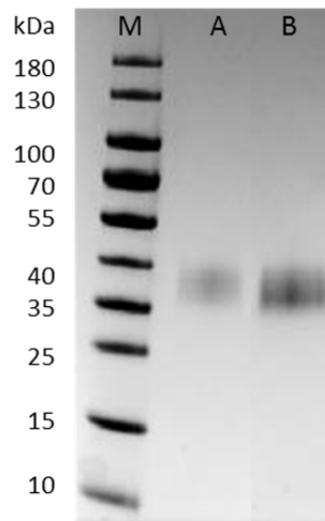


Figure S6: Production of H₂O₂ by AaAA16 in the presence of ascorbate or L-cysteine, visualized using the Amplex red coupled assay. The produced H₂O₂ is used by horse radish peroxidase to transform Amplex Red into fluorescent resorufin, which is measured by fluorimetric counts at 560 nm.

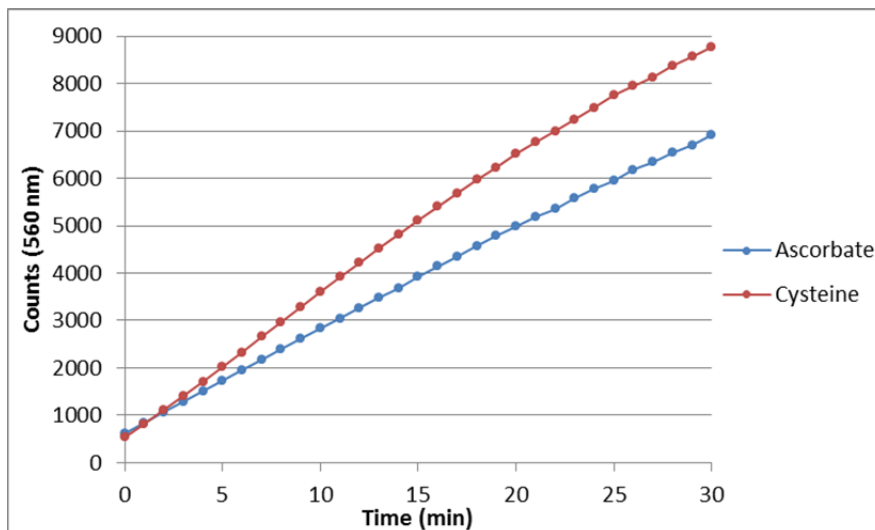


Figure S7: HPAEC-PAD chromatograms showing soluble products generated from 0.5 mM cellohexaose (G6) using 4.4 μ M AaAA16 and 1 mM L-cysteine. The LPMO9H from *Podospora anserina* (PaAA9H) [2] was used as a reference of LPMO acting on cello-oligosaccharides (producing native sugars and C4-oxidized products, visible at 26 and 40 min in the box at the top right).

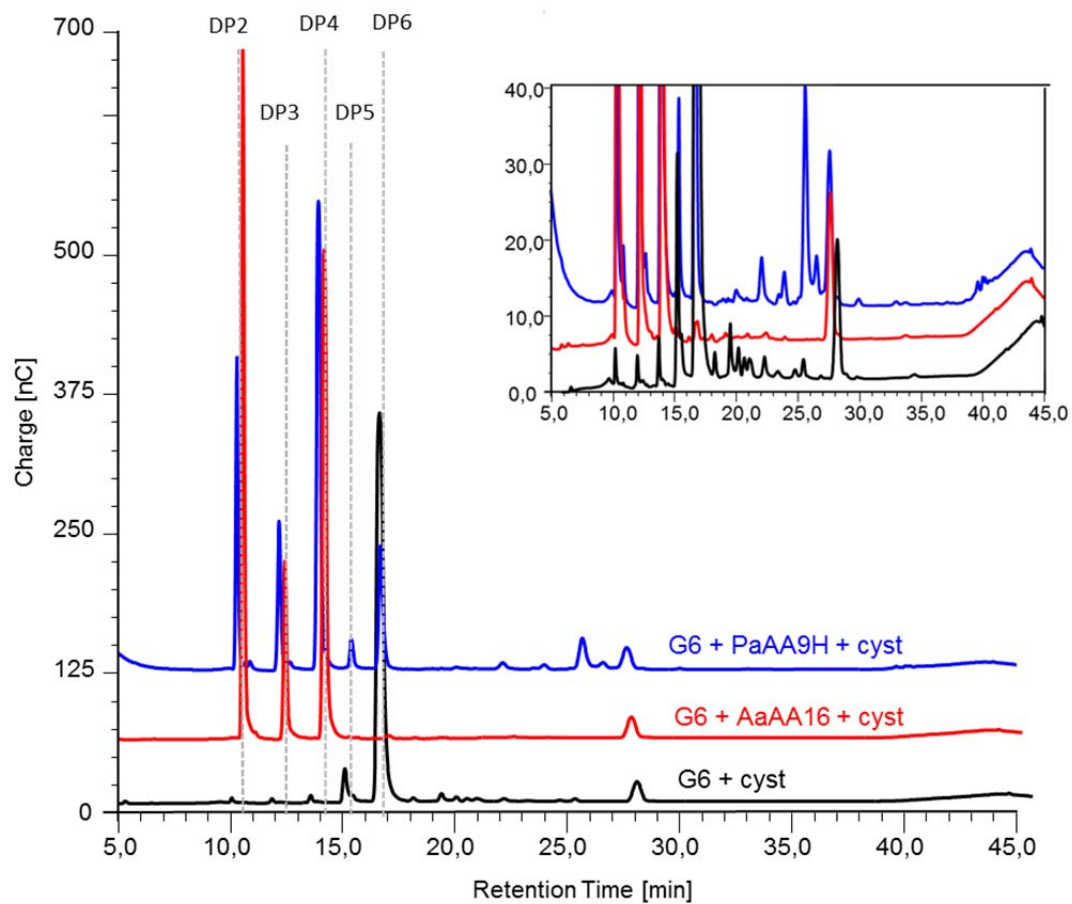
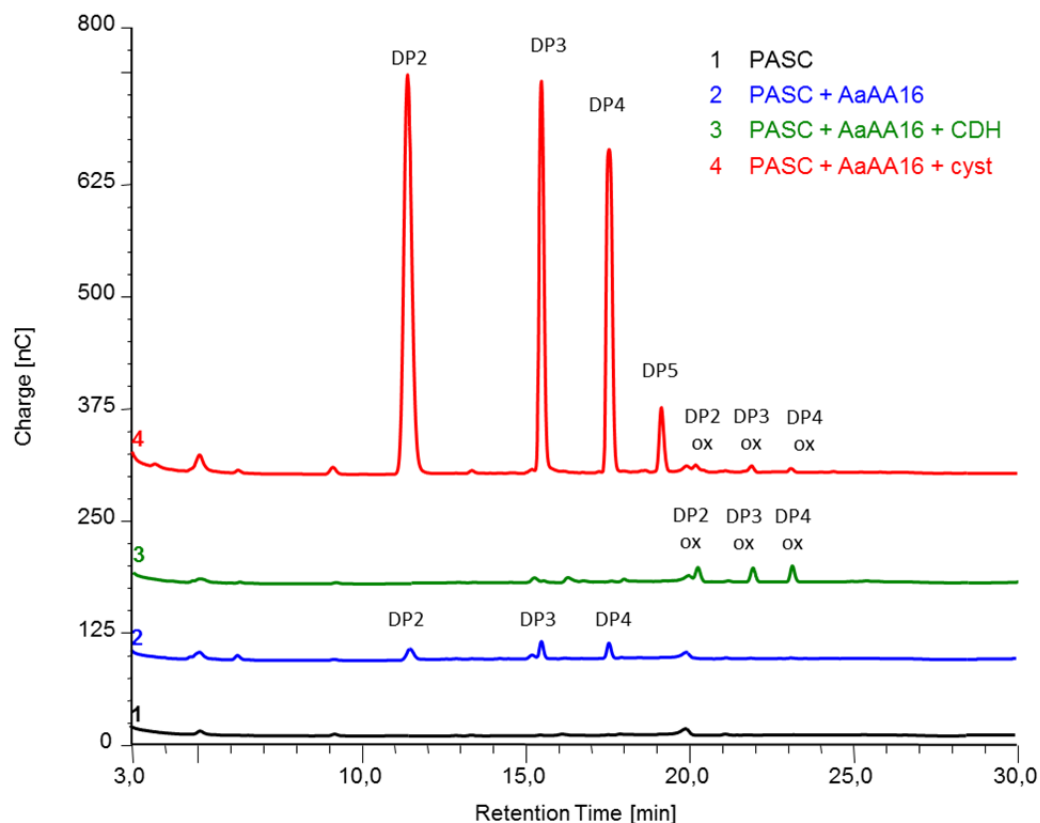


Figure S8: HPAEC-PAD chromatograms of PASC soluble degradation products, after incubation with AaAA16 alone or using *Podospira anserina* cellobiodehydrogenase (PaCDHB) [2] or L-cysteine as electron donors. The oxidized products visible in the CDH condition (green chromatogram) are the result of the action of CDH on cellodextrins released from PASC by AaAA16 (blue chromatogram). The proper LPMO activity is only visible after activation by L-cysteine (red chromatogram).



References

1. Crooks GE, Hon G, Chandonia J-M, Brenner SE. WebLogo: A Sequence Logo Generator. *Genome Res.* 2004;14:1188–90.
2. Bennati-Granier C, Garajova S, Champion C, Grisel S, Haon M, Zhou S, et al. Substrate specificity and regioselectivity of fungal AA9 lytic polysaccharide monoxygenases secreted by *Podospira anserina*. *Biotechnol Biofuels.* 2015;8:90.