

## **Supplementary Data (Electronic Annex)**

### **A recombinant fungal chitin deacetylase produces fully defined chitosan oligomers with novel patterns of acetylation**

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#### **Keywords**

Fungal chitin deacetylase; mode of action; chitin oligomers; pattern of acetylation; UHPLC-ELSD-ESI-MS<sup>n</sup>

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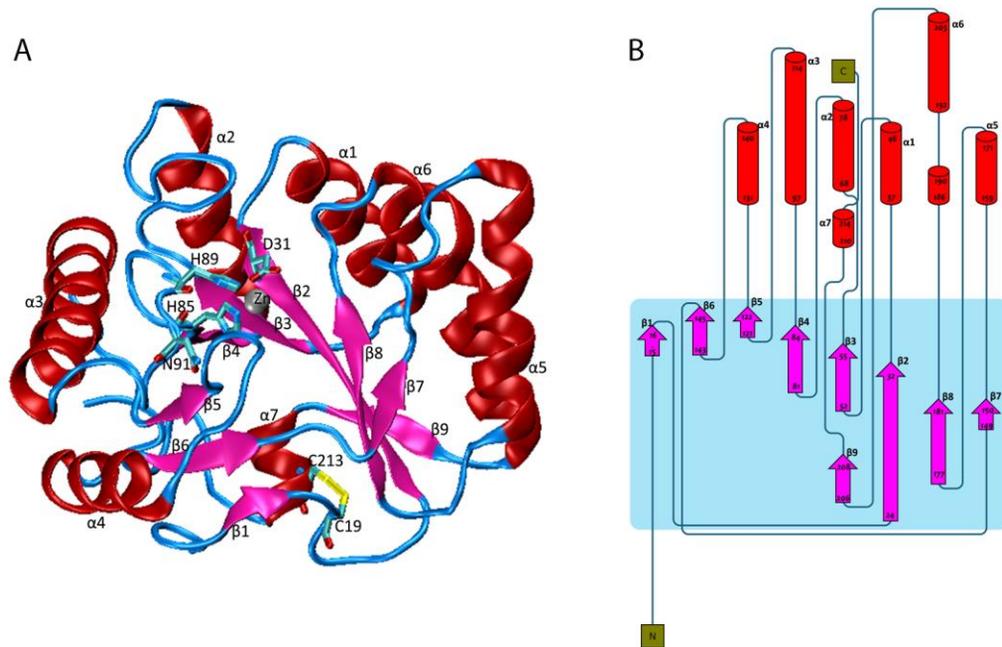
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**Fig. S1. Codon optimized nucleotide sequence of MBP-*Pgt*-StrepII**

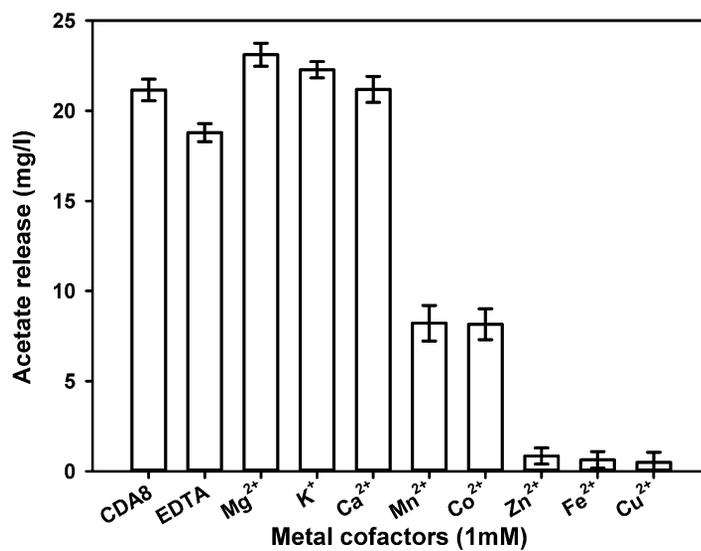
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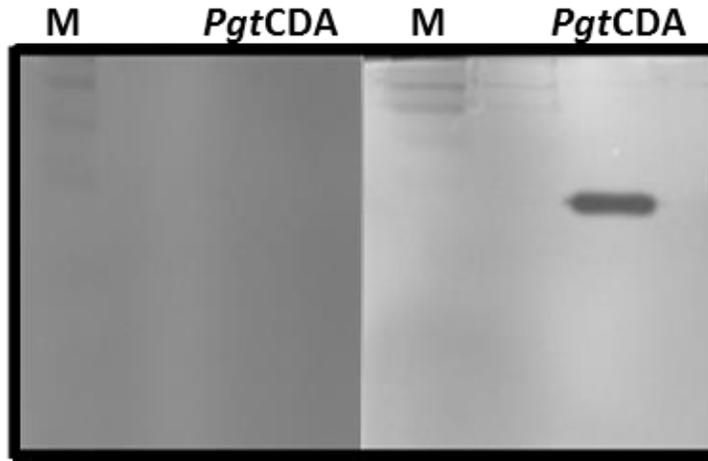
**Fig. S2.** (A) Homology model of *PgtCDA* 3D structure displaying the metal binding site (D31, H85, H89), putative *N*-glycosylation site (N91) and disulfide bond (C19-C213) (B) *PgtCDA* topological diagram generated by Pro-Origami where the alpha helices are in red and beta sheets are in pink, elucidating the relative positions and the connections between the helices and sheets in the enzyme.

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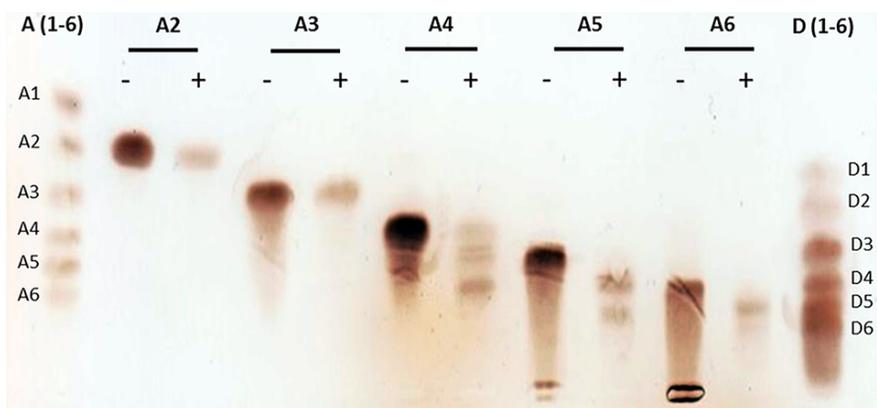
**Fig. S3. Effect of EDTA and metal ions on enzyme activity.** The enzyme (0.207 nmol) was pre-incubated with 1 mM metal co-factors or EDTA before A<sub>5</sub>/A<sub>4</sub> chitin oligomer mix was added as a substrate and incubation for 1 h at 37 °C.

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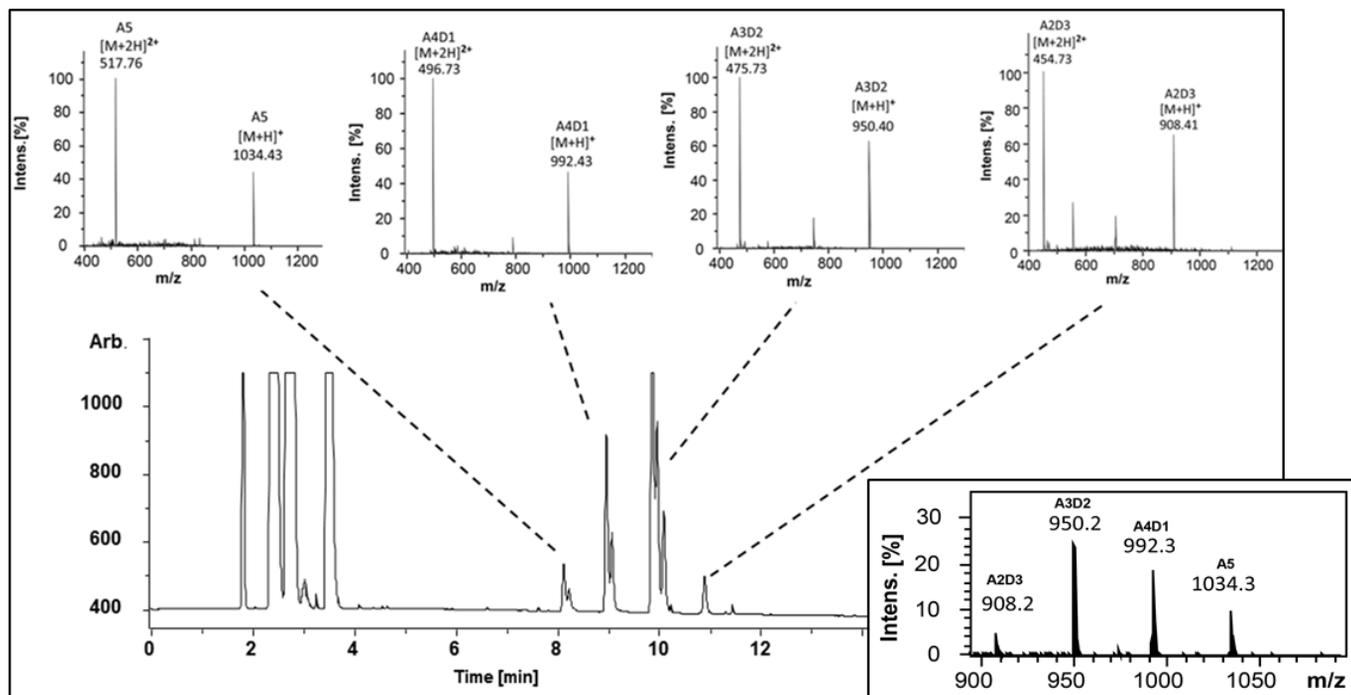
**Fig. S4. Zymography on semi native PAGE containing 1% glycol chitin.** A semi-native polyacrylamide gel containing 1% glycol chitin as substrate was run at 4 °C at 20 mA after loading 2 µg of each recombinant enzyme per lane. The gel was washed with TEA buffer (20 mM, pH 8.0) containing 10% Triton X-100 and incubated overnight in TEA buffer (20 mM, pH 8.0). The protein activity was observed under UV light after staining the gel with Calcofluor white M2R (Sigma) before (left) and after (right) treatment with sodium nitrite and sulfuric acid that depolymerized glycol chitosan produced by the action of CDA. M is the BIO-RAD Precision Plus Protein™ All Blue standard marker and *PgtCDA* is the purified recombinant enzyme.

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**Fig. S5. HP-TLC showing activity of recombinant *PgtCDA* on chitin oligomers (A<sub>2</sub>-A<sub>6</sub>).** The chitin oligomers (1 mg ml<sup>-1</sup>) were incubated at 37 °C with 0.34 nmol recombinant enzyme overnight in a 20 µl reaction mixture in TEA buffer (20 mM, pH 8.0). For HP-TLC, 10 µl sample was used after removing enzyme using Modified PES 3K VWR filter. The enzyme treated samples are marked with plus sign (+) while untreated controls with minus sign (-). A distinct shift and formation of multiple new bands is observed for the treated samples for A<sub>4</sub>-A<sub>6</sub>.

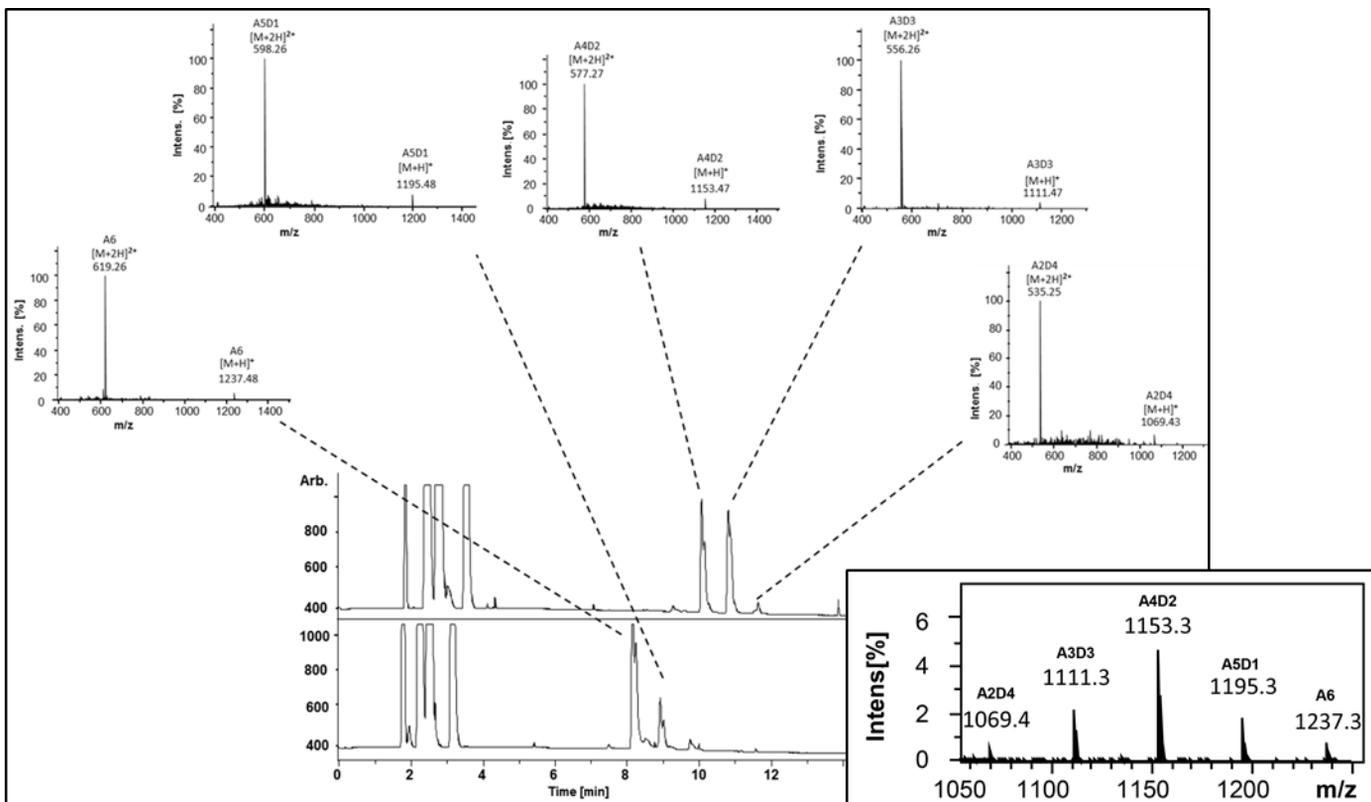
## Supplementary Data (Electronic Annex)



**Fig. S6. UHPLC-ELSD-ESI-MS analysis of chitin pentamer ( $A_5$ ) treated with *PgtCDA*.**

The ELSD chromatogram depicts time point of elution with initial buffer peaks (2-6 min) followed by target peaks. The  $m/z$  ratios in the MS spectra correspond to the mass of the substrate ( $A_5$ ) which after incubation with enzyme gives rise to differentially deacetylated products. A combined spectrum is also attached for an overview.

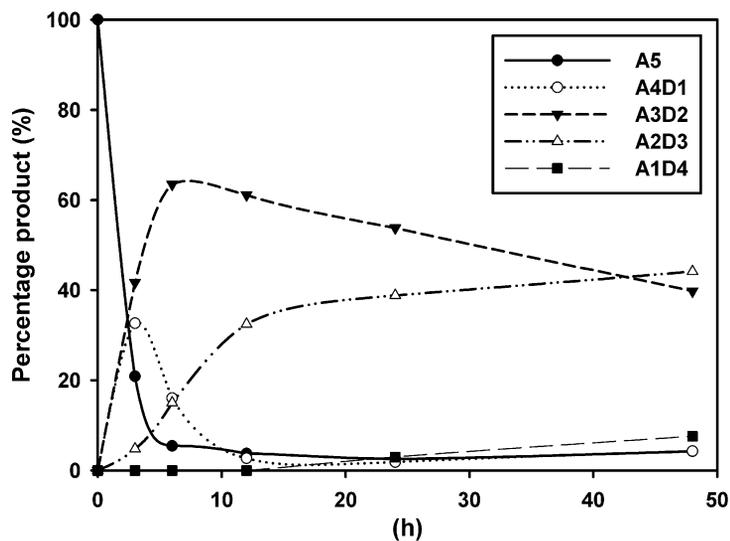
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**Fig. S7.** UHPLC-ELSD-ESI-MS analysis of chitin hexamer ( $A_6$ ) treated with *PgtCDA*.

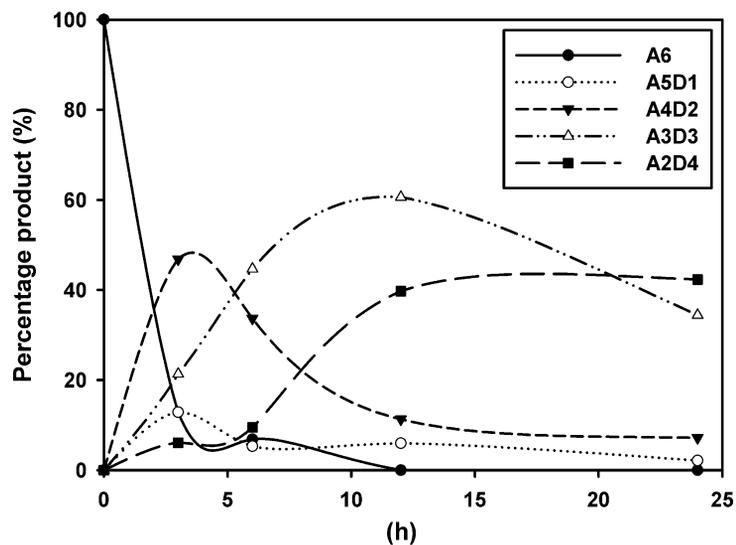
The ELSD chromatogram depicts time point of elution with initial buffer peaks (2-6 min) followed by target peaks. The  $m/z$  ratios in the MS spectra correspond to the mass of the substrate ( $A_6$ ) which after incubation with enzyme gives rise to differentially deacetylated products. A combined spectrum is also attached for an overview.

## Supplementary Data (Electronic Annex)



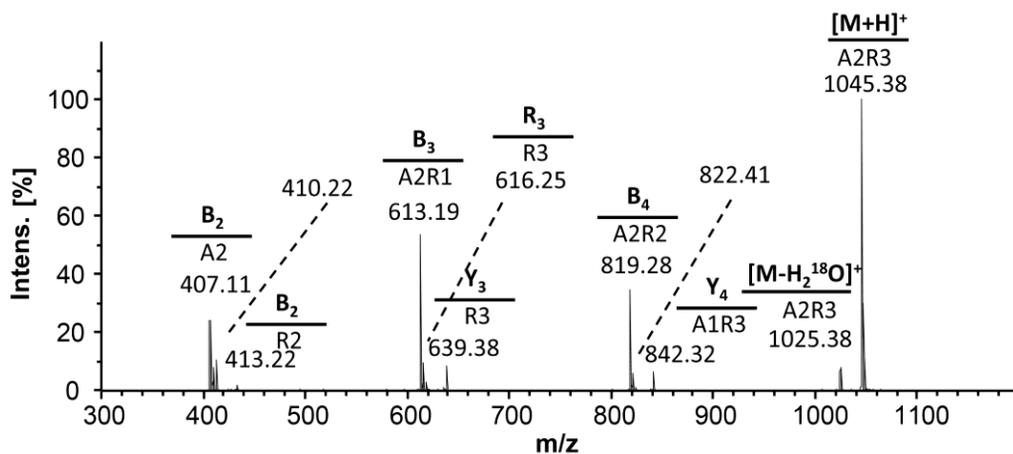
**Fig. S8. Time curve of deacetylation of chitin pentamer.** Chitin pentamer ( $1 \text{ mg ml}^{-1}$ ) was incubated with *PgtCDA* ( $0.6 \text{ nmol}$ ) in  $200 \text{ }\mu\text{l}$  TEA buffer ( $50 \text{ mM}$ ,  $\text{pH } 8.0$ ) for different lengths of time. The samples were reacylated,  $^{18}\text{O}$ -labelled followed by UHPLC-ELSD-ESI-MS<sup>n</sup>. The generation of differentially deacetylated products was studied with time.

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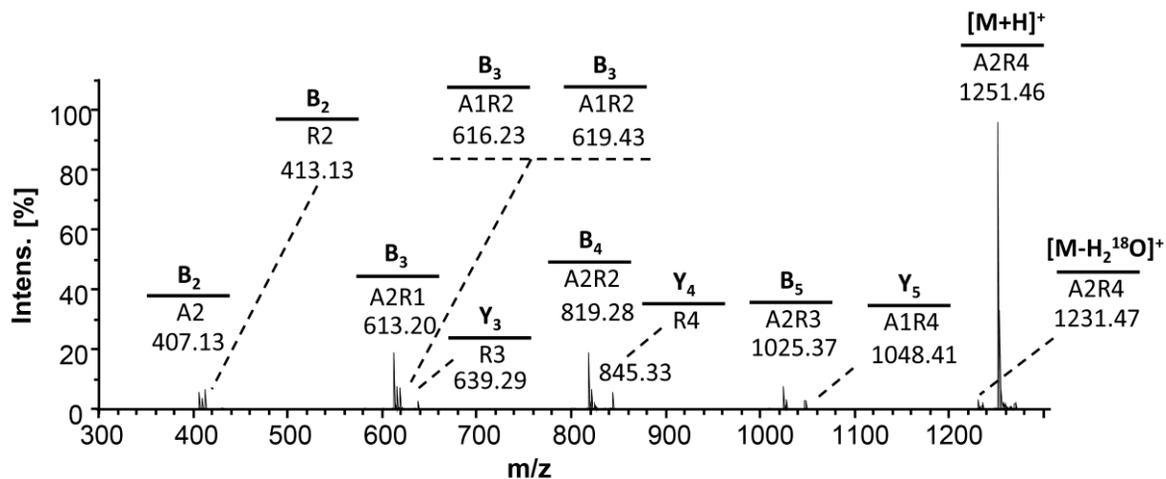
**Fig. S9. Time curve of deacetylation of chitin hexamer.** Chitin hexamer ( $1 \text{ mg ml}^{-1}$ ) was incubated with *PgtCDA* ( $0.6 \text{ nmol}$ ) in  $200 \text{ }\mu\text{l}$  TEA buffer ( $50 \text{ mM}$ ,  $\text{pH } 8.0$ ) for different lengths of time. The samples were reacylated,  $^{18}\text{O}$ -labelled followed by UHPLC-ELSD-ESI-MS<sup>n</sup>. The generation of differentially deacetylated products was studied with time.

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**Fig. S10. HILIC-MS-MS chromatogram of the deacetylation products from *Pgt*CDA with chitin pentamer ( $A_5$ ) as substrate. The precursor ion m/z values and the MS<sup>2</sup> spectra for sequencing the precursor ion are shown. The chromatograms is only for the end product, i.e. A-A-D-D-D for  $A_5$  ( $A_2R_3$ ).**

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**Fig. S11.** HILIC-MS-MS chromatogram of the deacetylation products from *PgtCDA* with chitin hexamer ( $A_6$ ) as substrate. The precursor ion  $m/z$  values and the  $MS^2$  spectra for sequencing the precursor ion are shown. The chromatogram is only for the end product, i.e. A-A-D-D-D-D for  $A_6$  ( $A_2R_4$ ).