



## Characterization of the Specific Mode of Action of a Chitin Deacetylase and Separation of the Partially Acetylated Chitosan Oligosaccharides

Xian-Yu Zhu <sup>1,2</sup>, Yong Zhao <sup>1</sup>, Huai-Dong Zhang <sup>1,3</sup>, Wen-Xia Wang <sup>1</sup>, Hai-Hua Cong <sup>2</sup> and Heng Yin <sup>1,\*</sup>

- <sup>1</sup> Liaoning Provincial Key Laboratory of Carbohydrates, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian 116023, China; zhuxy0721@126.com (X.-Y.Z.); zhaoyong\_2019@163.com (Y.Z.); huaidongzhang@yahoo.com (H.-D.Z.); wangwx@dicp.ac.cn (W.-X.W.)
- <sup>2</sup> College of Food Science and Engineering, Dalian Ocean University, Dalian 116023, China; haihuacong780@gmail.com
- <sup>3</sup> Engineering Research Center of Industrial Microbiology, Ministry of Education; College of Life Sciences, Fujian Normal University, Fujian 350117, China
- \* Correspondence: yinheng@dicp.ac.cn; Tel./Fax: +86-0411-84379061











**Figure S1.** The model was further evaluated for protein geometry by SAVES (A comprehensive measurement website for the quality of a protein structure). 97.3% Residues in additional allowed regions and 85.57% of the residues have averaged 3D-1D score  $\geq$  0.2, and the quality factor is 91.2214.







**Figure S2.** Compare deacetylase charge distribution. These pictures show the surface charge distributions of chitin deacetylase from *Saccharomyces cerevisiae* (*Sc*CDA<sub>2</sub>) and chitin deacetylase from *Aspergillus Nidulans* (*An*CDA, PDB ID: 2Y8U) calculated using ABPS (The Adaptive Poisson-Boltzmann Solver to generate electrostatic surface displayed) in VMD. Red represents a negative charge and blue represents a positive charge.



**Figure S3.** *Sc*CDA<sub>2</sub> substrate specificity on chitin oligomers. 0.5mg/ml chitin oligomers as substrates were incubated with 0. 75  $\mu$ M *Sc*CDA<sub>2</sub> at 37 °C for 30 min. The data represents the mean SD values of the results from three independent experiments.

Relative enzyme activity (%)

Α

100-

90-

80-

70-

60-50-

40-

30-20-

10-0+ 0

2

**90** 

80

**70** 

**60** 

Na⁺

 $NH_4^+$ 

Ba<sup>2+</sup>



Figure S4. Effects of pH, temperature and metal ion on enzyme activity. (A) The optimum pH was determined by incubating the 0.75µM ScCDA2 with A4 chitin oligomer (0.5 mg/mL) for 60 min at pH 3-11 in universal buffer. (B) To obtain the optimal temperature, the enzyme (075 µmol) was incubated for 60 min at different temperatures in 50mM Tris-HCl buffer (pH 8.0) containing chitin oligomer mixture (0.5 mg/mL) as a substrate. (C) Relative activity with different metal cations. Proteins were incubated with 1 mm metallized cations, and activity was determined in 50 mM Tris-HCl buffer (pH 8.0) using 0.5 mg/mL A4 as a substrate.

Co<sup>2+</sup>

Mn<sup>2+</sup>

Zn<sup>2+</sup>

Cu<sup>2+</sup>

Mg<sup>2+</sup>

Fe<sup>3+</sup>



**Figure S5.** Spectra of N-glycosylation of *Sc*CDA<sub>2</sub>. Mass spectrometry showed that *Sc*CDA<sub>2</sub> have N-glycosylation post-translational modification at Asn 181, Asn 199 and Asn 203.