

SUPPLEMENTARY INFORMATION

Transglycosylation by a chitinase from *Enterobacter cloacae* subsp. *cloacae* generates longer chitin oligosaccharides

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Keywords:

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Supplementary figure 1:

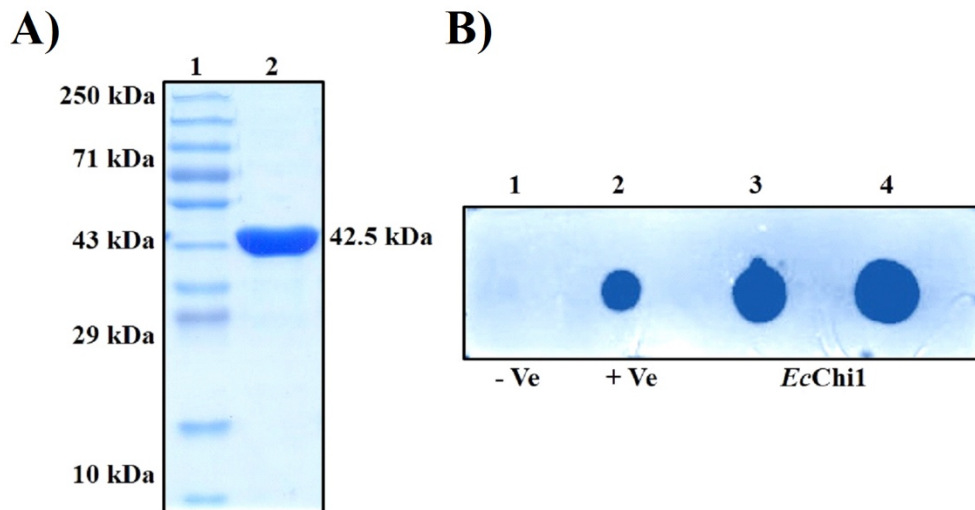


Fig. S1: Purification and activity analysis of *EcChi1*. (A) Recombinant *EcChi1* (MW 42.5 kDa) was purified by Ni-NTA agarose affinity chromatography. Lane 1. Prestained protein marker, lane 2. Purified *EcChi1*. (B) Purified *EcChi1* was spotted on glycol chitin substrate containing polyacrylamide gel and incubated overnight at 37°C in a humid chamber, and stained with Calcofluor white. The gel was placed on UV transilluminator to visualize lysis zone. Lane 1. Negative control (50 mM sodium citrate buffer pH 5.0), lane 2. Positive control (ChiA from *Enterobacter cloacae*), lane 3. 5 µg *EcChi1*, lane 4. 10 µg *EcChi1*.

Supplementary figure 2:

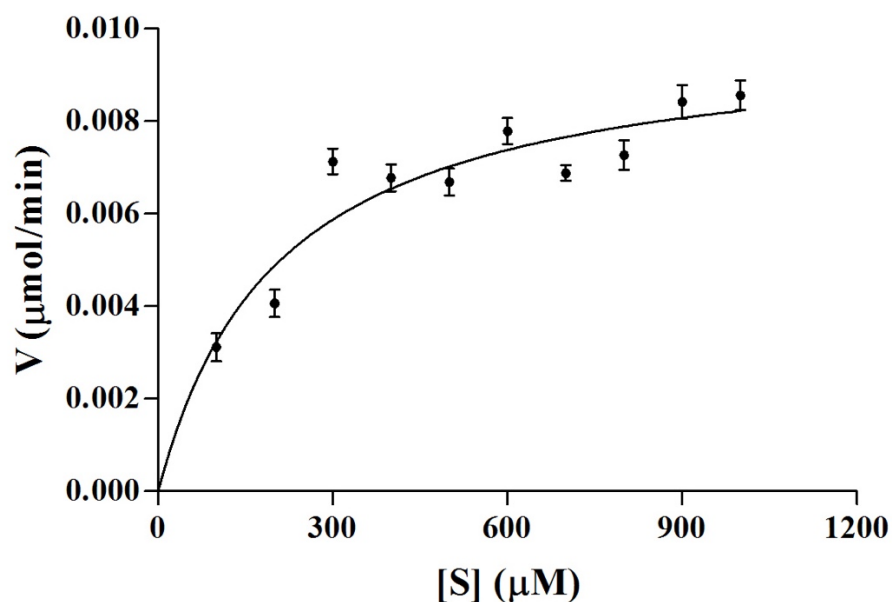


Fig. S2: Michaelis-Menten plot for chitobiose hydrolysis with *EcChi1*. Solid symbols are experimentally determined data for the rate of chitobiose hydrolysis versus substrate concentration. The solid line is the best nonlinear fit using the Michaelis-Menten equation. The error bars represent the standard deviations from three individual experiments.

Supplementary figure 3:

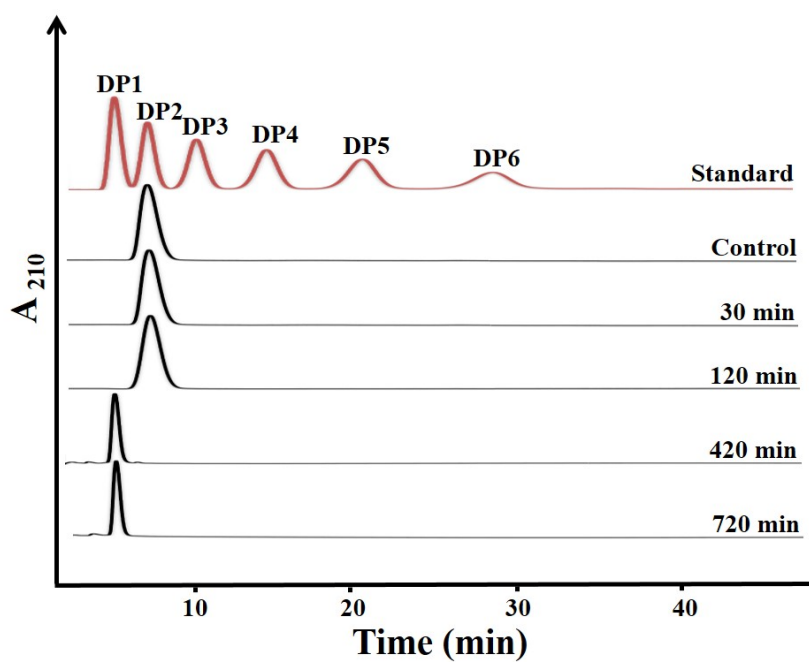


Fig. S3: Time course hydrolysis of *EcChi1* with DP2 substrate. The reaction mixture products were analyzed by binary gradient HPLC. The topmost profile shows a standard mixture of CHOS ranging from DP1 to DP6. The other profiles show the reaction products at the indicated incubation times.

Supplementary figure 4:

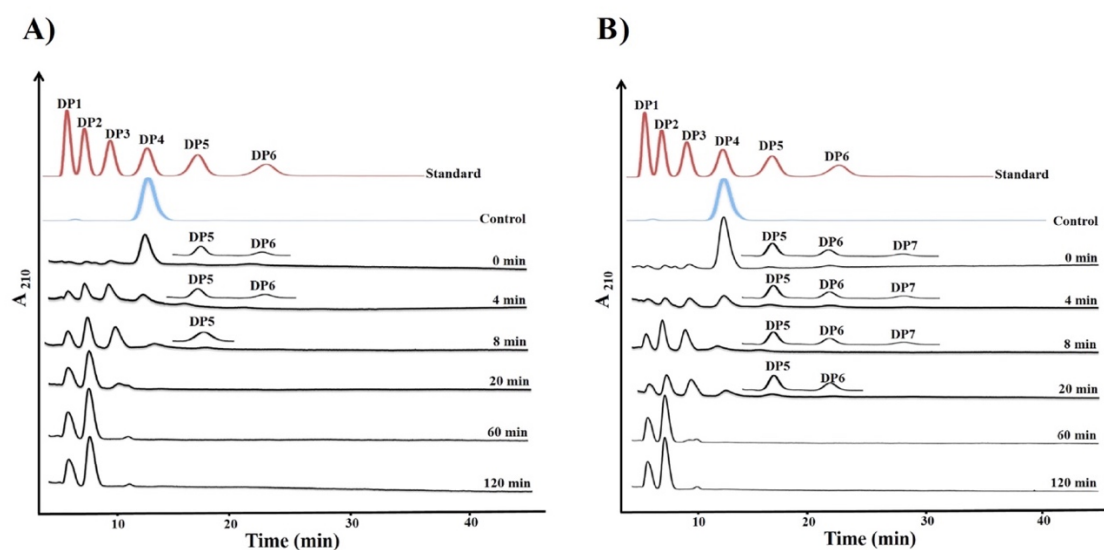


Fig. S4: Dose-responsive effects of *EcChi1* with the low substrate (DP4) and enzyme concentrations on hydrolytic and TG activity. Fractions collected from reaction mixture were analyzed by binary gradient HPLC. (A) *EcChi1* (20 nM) and DP4 substrate (100 μ M); (B) *EcChi1* (20 nM) and DP4 substrate (50 μ M). The top most profile in each chromatogram shows a standard mixture of CHOS ranging from DP1-DP6. Insets show the magnified view of the low peak area products.