The Discovery, Enzymatic Characterization and Functional Analysis of a Newly Isolated Chitinase from Marine-Derived Fungus *Aspergillus fumigatus* df347

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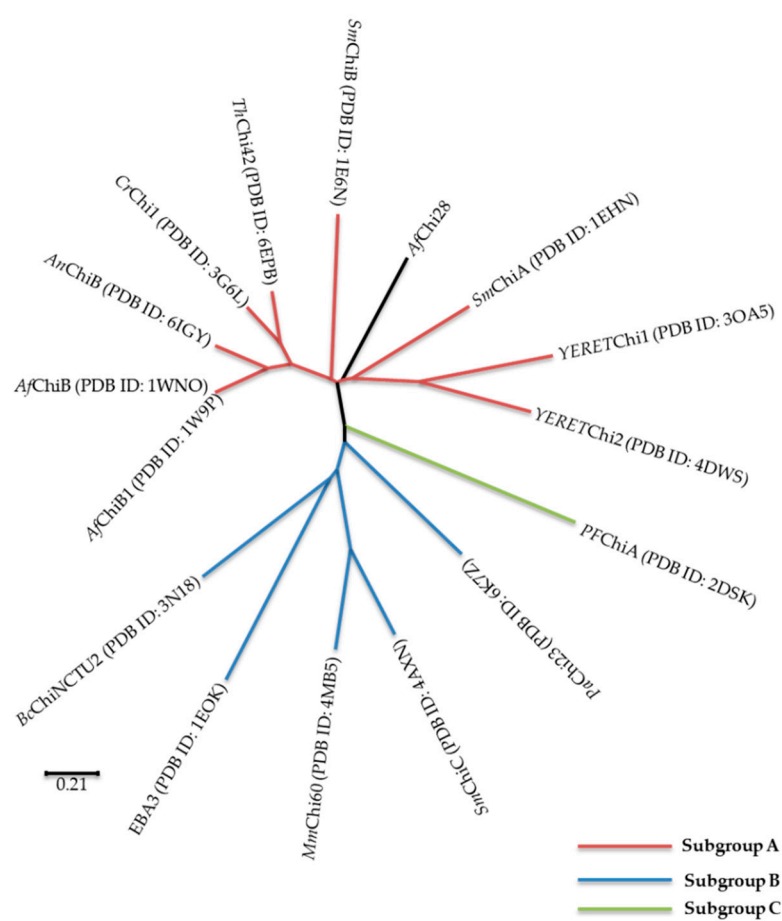† These authors contributed equally to this work.

Contents

Figure S1. Phylogenetic tree analysis of *Af*Chi28 and 15 different protein sequences with high identities in Protein Data Bank. The PDB ID also show in the figure. The Gene Bank number of *Af*chi28 from NCBI database is OK 302920.

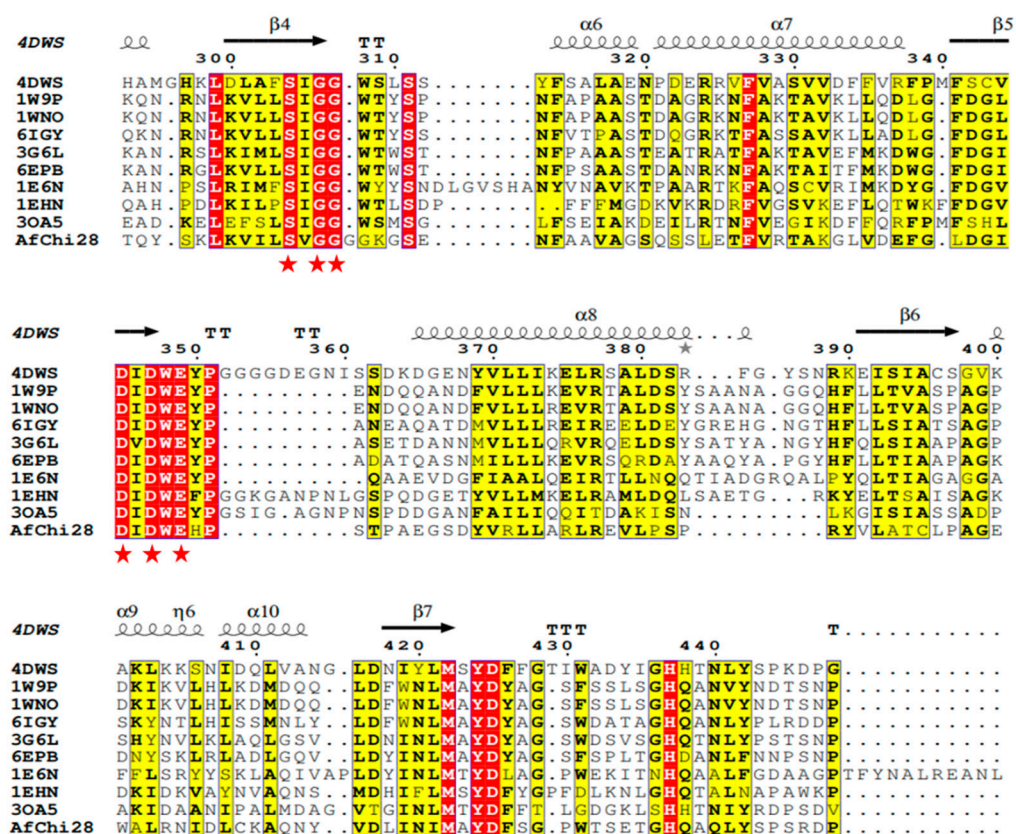Tree scale: 0.21 substitutions per nucleotide position.

Figure S2. Sequence and structural analysis of domains of *Af*Chi28. Sequence alignment analysis of *Af*Chi28 with other subgroup A chitinases, respectively. The different sources of chitinase are indicated by their corresponding Protein Data Bank ID. The motif that was predicted to be associated with substrate specificity is indicated by red star (★).
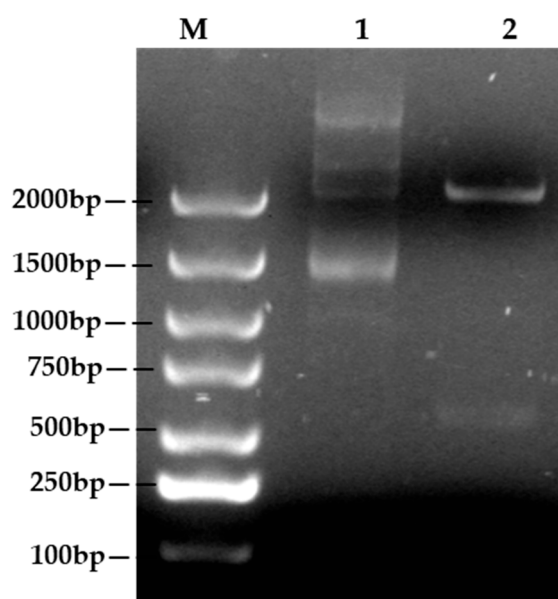

Figure S3. Agarose gel verification diagram after restriction digestion; Lane M: DNA Marker; Lane 1: pET28a (+)- *Af*chi28 (1G2800); Lane 2: Target gene.
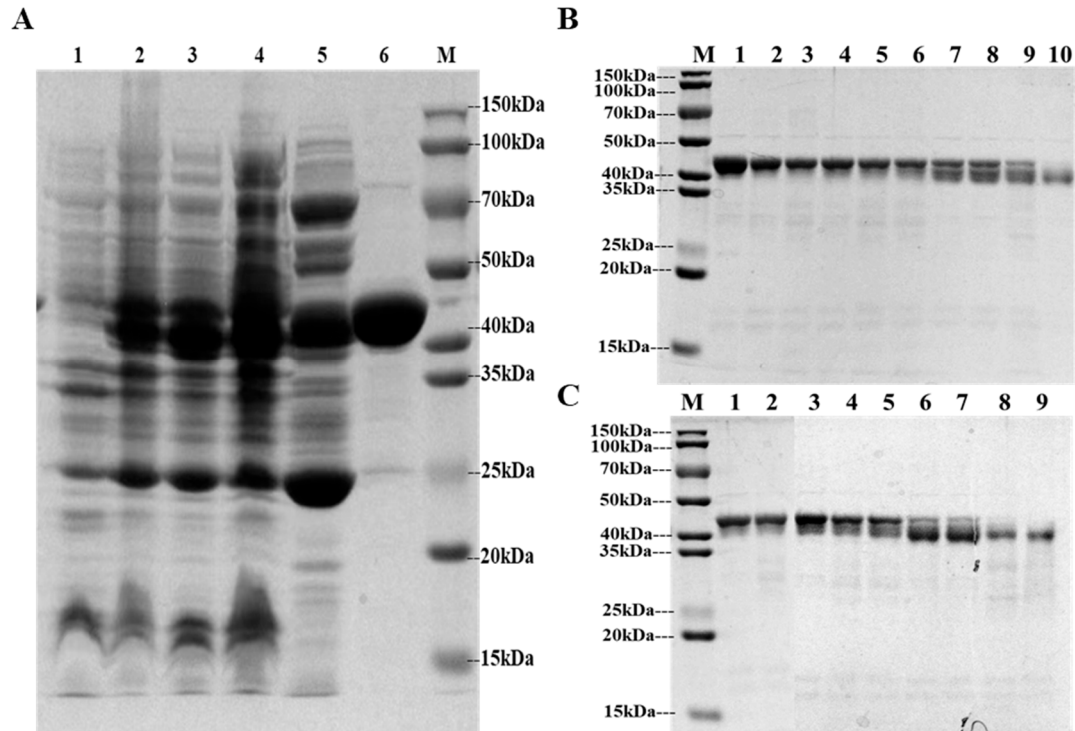
Figure S4. SDS-PAGE analysis of *Af*Chi28. Lane M: low molecular weight protein standards (150, 100, 70, 50, 40,35, 25 and 15 kDa). (A)SDS-PAGE analysis of *AfChi28* protein expression and purification. Lane 1, Cell lysates; Lane 2, The crude enzyme in the supernatant of lysates; Lane 3, the flow through; Lane 4 and 5, the flow through of impurity protein eluted by 50 mM phosphate buffer; Lane 6, purified protein eluate. (B) SDS-PAGE analysis of the stability of purified *AfChi28* protein at 30 °C incubation. Lane 1 is for the purified *AfChi28* with the concentration was 0.242 mg/ml. Lane 2 to lane 10 was supernatants collected after different incubation time (from left to right：2 h, 4 h, 6 h, 8 h, 12 h, 16 h, 24 h, 36 h and 48 h). (C) SDS-PAGE analysis of the stability of purified *AfChi28* protein at 45 °C incubation. Lane 1 to lane 9 was supernatants collected after different incubation times (from left to right：2 h, 4 h, 6 h, 8 h, 12 h, 16 h, 24 h, 36 h and 48 h).
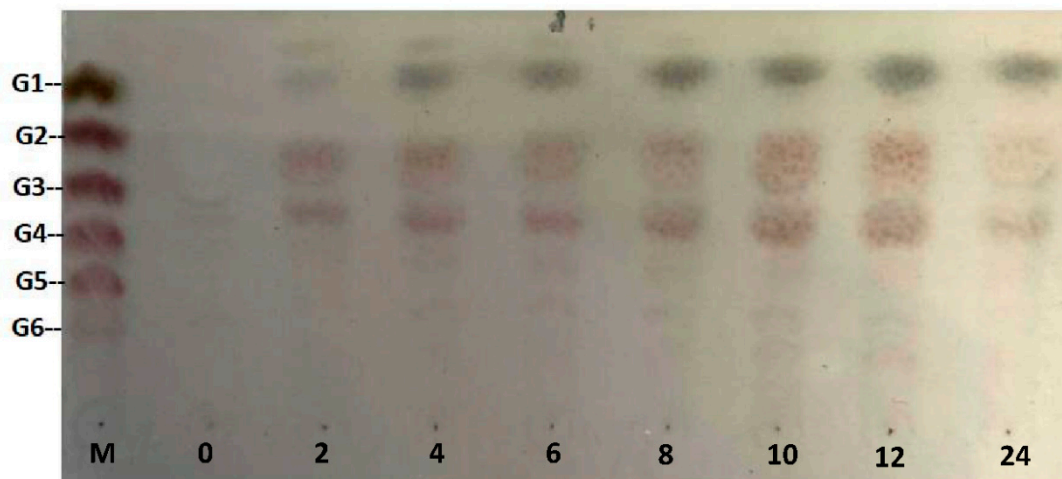

Figure S5. TLC of colloidal chitin hydrolysates produced by *Af*Chi28. Lane M, the standard of (GlcNAc)n

(n = 1–6); Lane 0, control of chitin; Lane 2 to lane 24 represent the products collected at different degradation times respectively (different degradation times from left to right:2h, 4h, 6h, 8h, 10h, 12h and 24h).
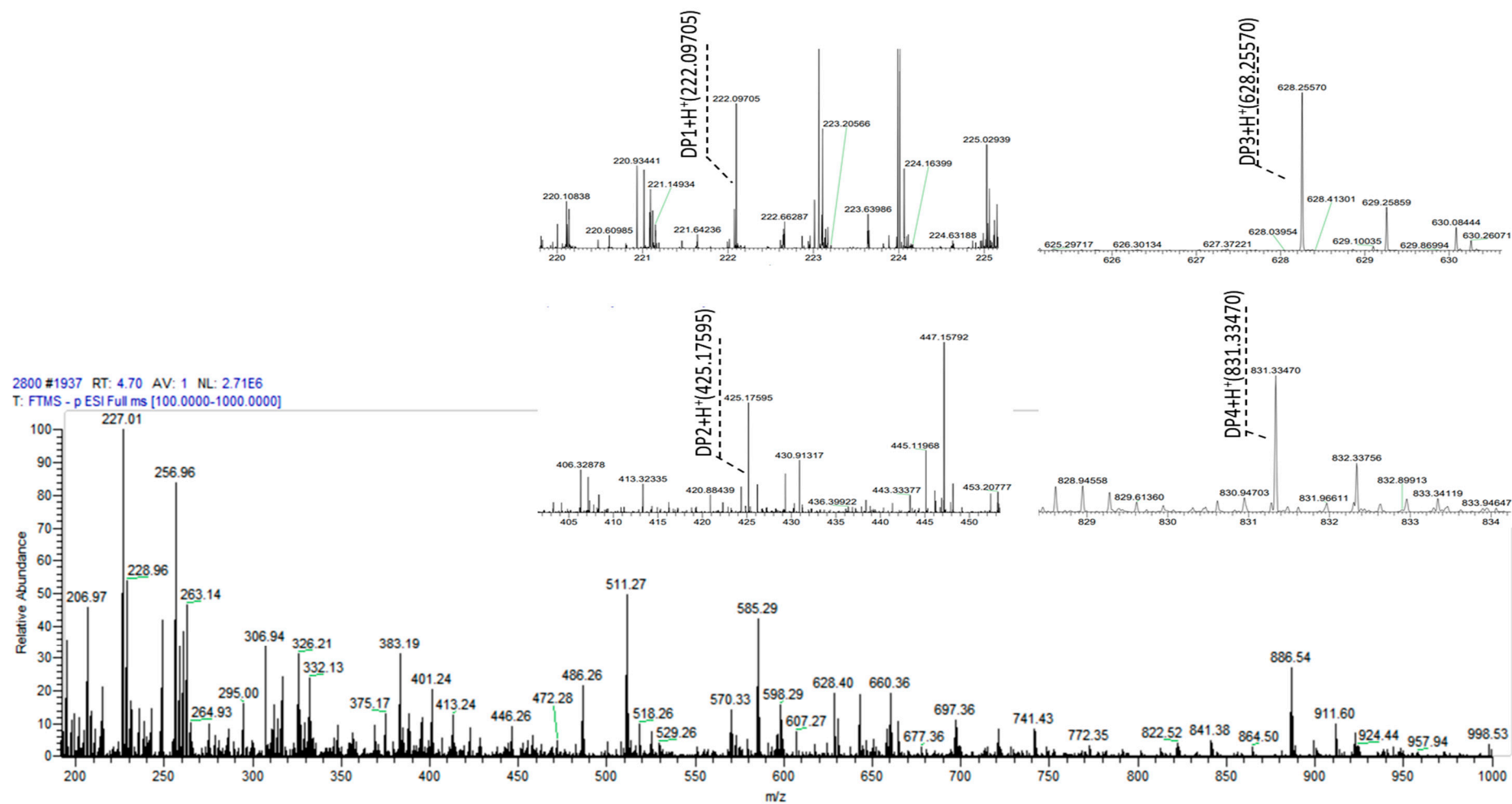
Figure S6. The degree of polymerization of the hydrolysis products after 6 h of reaction of *Af*Chi28 with colloidal chitin were analyzed by Q Exactive LC-MS.

Table S1. Kinetic parameters of *Af*Chi28 towards colloidal chitin.

Parameters	AfChi28
K_m(mg/mL)	2.003±0.01
K_{cat}(s^{-1})	0.0093
K_{cat}/K_m(mL/s·mg)	0.0349