Supporting information for:

Chitinase Chi1 from *Myceliophthora thermophila* C1, a thermostable enzyme for chitin and chitosan depolymerization

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Figure S1. Protein oligomers detected after proteolytic digestion of chitinase Chi1 with HPLC-ESI-MS/MS analysis. Peptides (indicated in yellow) were only found matching the C-terminus of the enzyme.

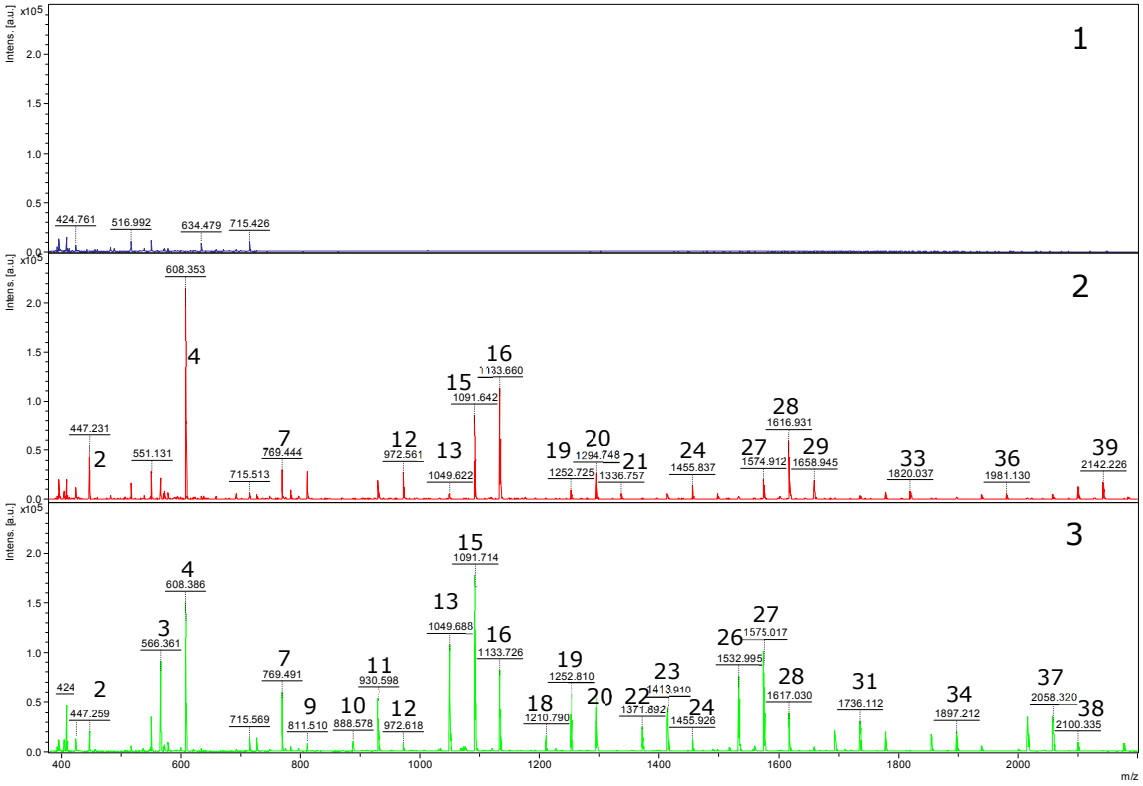

Figure S2. Spectra from matrix assisted laser-desorption time-of-flight mass spectrometry (MALDI-TOF-MS) obtained for chito-oligosaccharides released by chitinase Chi1 from chitosan 90 DDA/100. Chitosan (0.45% (w/v)) in 1 mL 0.05 M sodium phosphate buffer pH 6.0 was incubated with purified chitinase Chi1 (100 nM) at 50 °C. Presented spectra are obtained for reaction at time 0 (1), 240 min incubation (2) and 1,440 min incubation (3). The type of chito-oligosaccharides and their corresponding masses are shown in Table 1S. The most predominant peaks were assigned.

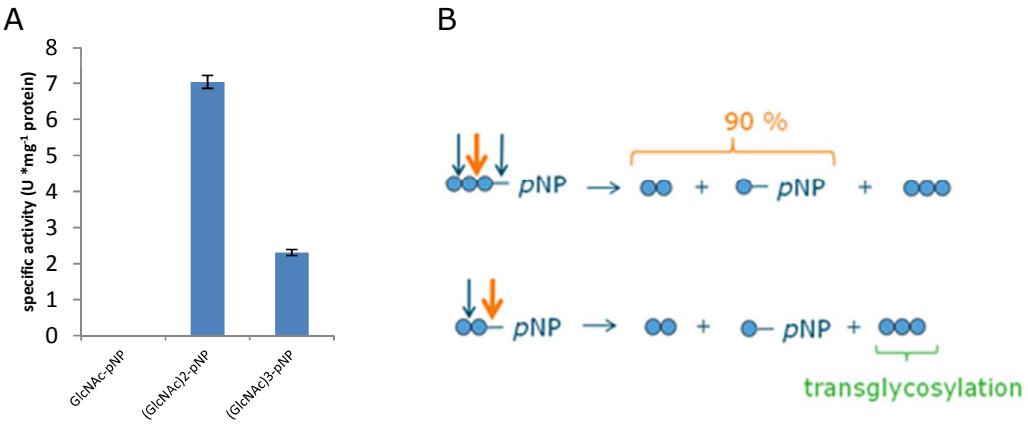

Figure S3. Specific activity of chitinase Chi1 for GlcNAc-*p*NP, $(GlcNAc)_2$-*p*NP and $(GlcNAc)_3$-*p*NP (A) and mode of action of chitinase Chi1 on $(GlcNAc)_3$-*p*NP and $(GlcNAc)_2$-*p*NP (B). Reactions with GlcNAc-*p*NP, $(GlcNAc)_2$-*p*NP, and $(GlcNAc)_3$-*p*NP (2 mM) in 0.5 mL 50 mM sodium phosphate buffer pH 6.0 were performed with purified chitinase Chi1 (17 nM) at 50 °C for 15 min. Arrows indicate place of cleavage.

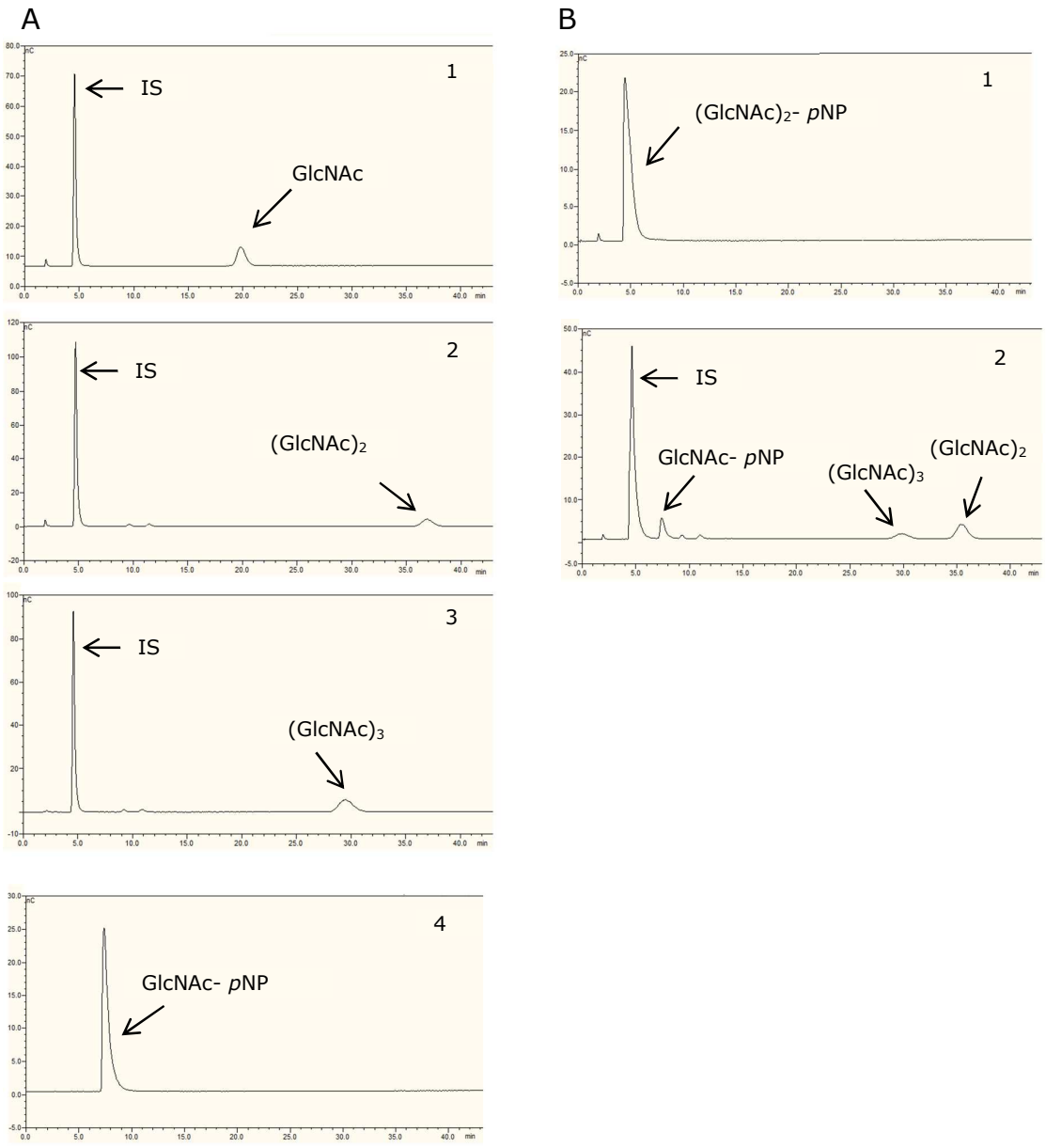

Figure S4. High Performance Anion Exchange (HPAEC) elution profiles of standards of chito-oligosaccharides $(GlcNAc)_{1-3}$ and GlcNAc-*p*NP (A) and reaction products released after 60 min incubation of $(GlcNAc)_2$-*p*NP with purifed chitinase Chi1 (B). A. Elution time recorded for:1, GlcNAc; 2, $(GlcNAc)_2$; 3, $(GlcNAc)_3$; 4, GlcNAc-*p*NP. B. Elution time recorded for: 1, substrate $(GlcNAc)_2$-*p*NP; 2, products released from $(GlcNAc)_2$-*p*NP by chitinase Chi1. Reaction with $(GlcNAc)_2$-*p*NP (1 mM) in 0.5 mL 50 mM sodium phosphate buffer pH 6.0 was performed with purified chitinase Chi1 (25 nM) at 50 °C for 60 min. For Figure A1, A2, A3 and B2, fucose was used as the internal standard (IS). For Figure A4 and B1 the IS was not used.

Table S1. Chito-oligosaccharides released by chitinase Chi1 from chitosan 90 DDA/100. Chitosan (0.45% (w/v)) in 1 mL 0.05 M sodium phosphate buffer pH 6.0 was incubated with purified chitinase Chi1 (100 nM) at 50 °C. Aliquots were taken at different time intervals and the hydrolysis products were analyzed by matrix assisted laser-desorption time-of-flight mass spectrometry (MALDI-TOF-MS). Sign (+) indicates the presence of chito-oligosaccharide in sample.

m/*z*	Composition	Peak Nr.	Type of adduct	Hydrolysis time (min)								
				0	15	30	60	90	120	240	360	1440
405.2	GlcNAc,GlcN	1	$[M+Na]^+$						+	+	+	+
447.2	$(GlcNAc)_2$	2	$[M+Na]^+$		+	+	+	+	+	+	+	+
566.3	GlcNAc,$(GlcN)_2$	3	$[M+Na]^+$					+	+	+	+	+
608.3	$(GlcNAc)_2$,GlcN	4	$[M+Na]^+$		+	+	+	+	+	+	+	+
650.3	$(GlcNAc)_3$	5	$[M+Na]^+$		+							
727.1	GlcNAc,$(GlcN)_3$	6	$[M+Na]^+$		+		+	+	+		+	+
769.3	$(GlcNAc)_2$,$(GlcN)_2$	7	$[M+Na]^+$		+		+	+		+	+	+
784.0	$(GlcNAc)_2$,$(GlcN)_2$	8	$[M+K]^+$					+	+	+	+	+
811.4	$(GlcNAc)_3$,GlcN	9	$[M+Na]^+$		+	+	+	+	+	+	+	
888.6	GlcNAc,$(GlcN)_4$	10	$[M+Na]^+$									+
930.0	$(GlcNAc)_2$,$(GlcN)_3$	11	$[M+Na]^+$						+	+	+	
972.4	$(GlcNAc)_3$,$(GlcN)_2$	12	$[M+Na]^+$		+	+	+	+	+	+	+	
1014.4	$(GlcNAc)_4$,GlcN	13	$[M+Na]^+$		+							
1049.7	GlcNAc,$(GlcN)_5$	14	$[M+Na]^+$									+
1091.5	$(GlcNAc)_2$,$(GlcN)_4$	15	$[M+Na]^+$		+	+	+	+	+	+	+	+
1133.5	$(GlcNAc)_3$,$(GlcN)_3$	16	$[M+Na]^+$		+	+	+	+	+	+	+	+
1175.5	$(GlcNAc)_4$,$(GlcN)_2$	17	$[M+Na]^+$		+	+	+		+			
1210.7	GlcNAc,$(GlcN)_6$	18	$[M+Na]^+$									+
1252.0	$(GlcNAc)_2$,$(GlcN)_5$	19	$[M+Na]^+$							+	+	+
1294.6	$(GlcNAc)_3$,$(GlcN)_4$	20	$[M+Na]^+$		+	+	+	+	+	+	+	+
1336.6	$(GlcNAc)_4$,$(GlcN)_3$	21	$[M+Na]^+$		+	+	+	+	+	+		
1371.9	GlcNAc,$(GlcN)_7$	22	$[M+Na]^+$									+
1413.0	$(GlcNAc)_2$,$(GlcN)_6$	23	$[M+Na]^+$						+	+	+	+
1455.7	$(GlcNAc)_3$,$(GlcN)_5$	24	$[M+Na]^+$		+		+	+	+	+	+	+
1497.7	$(GlcNAc)_4$,$(GlcN)_4$	25	$[M+Na]^+$		+	+	+	+	+	+		
1532.9	GlcNAc,$(GlcN)_8$	26	$[M+Na]^+$									+
1575.0	$(GlcNAc)_2$,$(GlcN)_7$	27	$[M+Na]^+$					+	+	+	+	+
1616.7	$(GlcNAc)_3$,$(GlcN)_6$	28	$[M+Na]^+$		+	+	+	+	+	+	+	+
1658.8	$(GlcNAc)_4$,$(GlcN)_5$	29	$[M+Na]^+$		+	+	+	+	+	+	+	
1700.8	$(GlcNAc)_5$,$(GlcN)_4$	30	$[M+Na]^+$		+							
1736.1	$(GlcNAc)_2$,$(GlcN)_8$	31	$[M+Na]^+$									+
1777.9	$(GlcNAc)_3$,$(GlcN)_7$	32	$[M+Na]^+$						+	+	+	+
1820.0	$(GlcNAc)_4$,$(GlcN)_6$	33	$[M+Na]^+$		+			+	+	+	+	
1897.2	$(GlcNAc)_2$,$(GlcN)_9$	34	$[M+Na]^+$									+
1939.0	$(GlcNAc)_3$,$(GlcN)_8$	35	$[M+K]^+$						+	+	+	+
1981.1	$(GlcNAc)_2$,$(GlcN)_9$	36	$[M+Na]^+$		+				+	+	+	

2058.3	$(GlcNAc)_2,(GlcN)_{10}$	37	$[M+Na]^+$										+
2100.0	$(GlcNAc)_3,(GlcN)_9$	38	$[M+Na]^+$		+			+	+	+	+	+	
2142.2	$(GlcNAc)_4,(GlcN)_8$	39	$[M+Na]^+$		+		+	+	+	+	+		
2184.0	$(GlcNAc)_5,(GlcN)_7$	40	$[M+Na]^+$		+	+	+	+	+				