Systems analysis of the Glycoside Hydrolase family 18 enzymes from *Cellvibrio japonicus* characterizes essential chitin degradation functions

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Running Title
Chitin degradation in *C. japonicus*

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Cellvibrio japonicus, chitin, chitinase, enzyme, gene knockout, glycosyl hydrolase, polysaccharide

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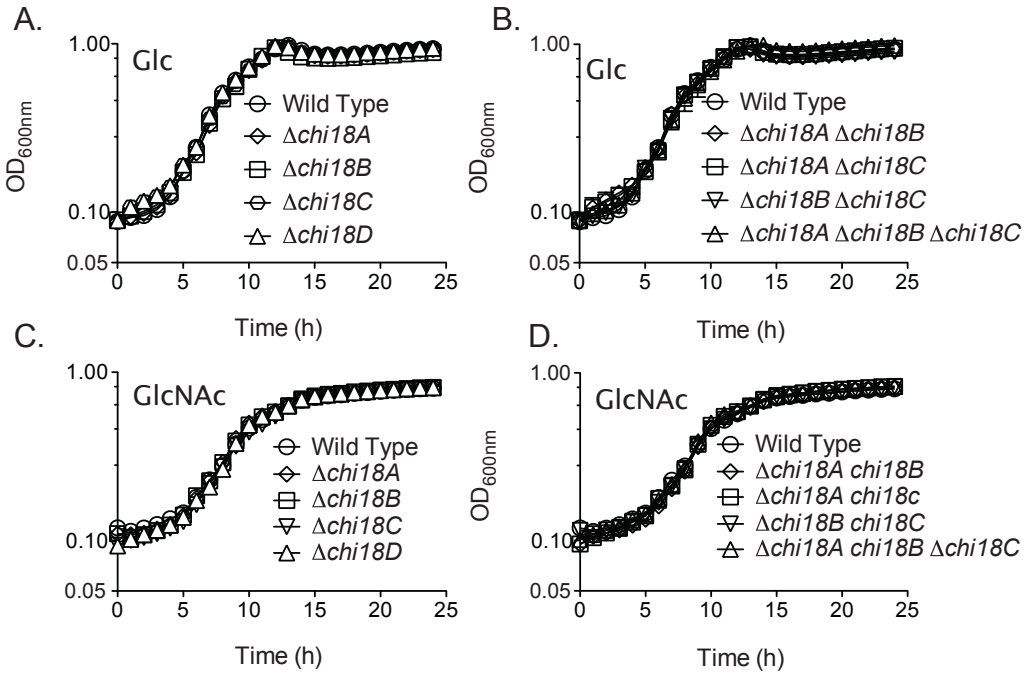

Figure S1. Growth of *C. japonicus* mutants on Glc and GlcNAc. Growth analysis was conducted on MOPS minimal medium supplemented with glucose (Glc; 0.20%) **(A&B)** or *N*-acetylglucosamine (GlcNAc; 0.50%) **(C&D)** as the sole source of carbon. While all belonging to the same experiment, the glucose experimental data is shown as two panels: single **(A)** and multiple **(B)** deletion mutants. The same applies to the GlcNAc experiments (panels **C** and **D**, respectively). All experiments were performed in biological triplicates; error bars represent standard deviations, but are not visible in many cases due to errors being <10%. These growth experiments were performed simultaneously, but are separated into multiple panels for clarity. As a consequence, the same control strain (wild type) is repeated in each set of panels (**A**&**B** and **C**&**D**).

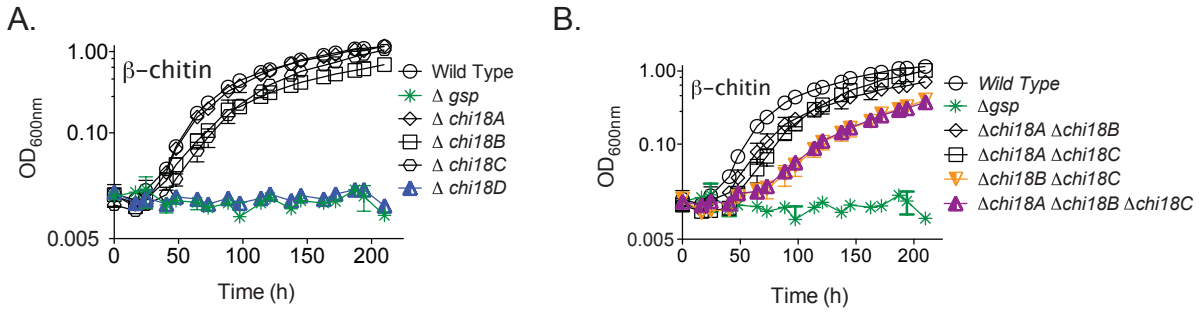

Figure S2. Growth of *C. japonicus* mutants on β-chitin. Growth analysis of family GH18 deletion mutants of *C. japonicus* was performed in MOPS minimal medium supplemented with 0.2% β-chitin as the sole source of carbon. All experiments were performed in biological triplicates; error bars represent standard deviations. The experimental data is shown as two panels: **(A)** single and **(B)** double deletion mutants, although they all belong to the same experiment. As a consequence, the same control strains (wild type and Δ*gsp*) are repeated in each panel.

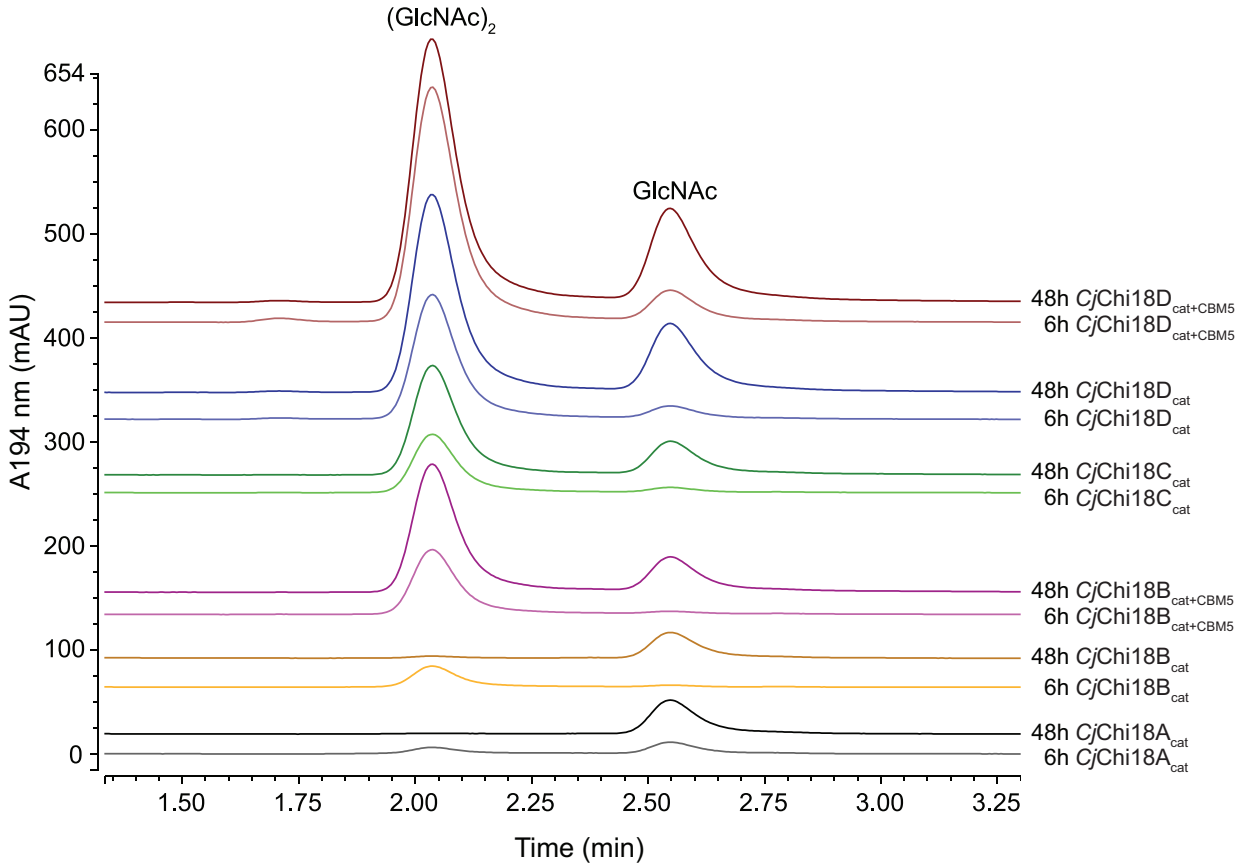

Figure S3. Product profile after degradation of α-chitin. Chromatograms of samples taken after 6h and 48h reaction time are shown, representing the product profile early and late in the degradation process. GlcNAc and/or $(GlcNAc)_2$ are the main products for all enzymes. Reactions were done at 30 °C in 20 mM BisTris pH 6.5, 0.1 mg/mL BSA at 30 °C. The enzyme concentration was 0.5 μM.

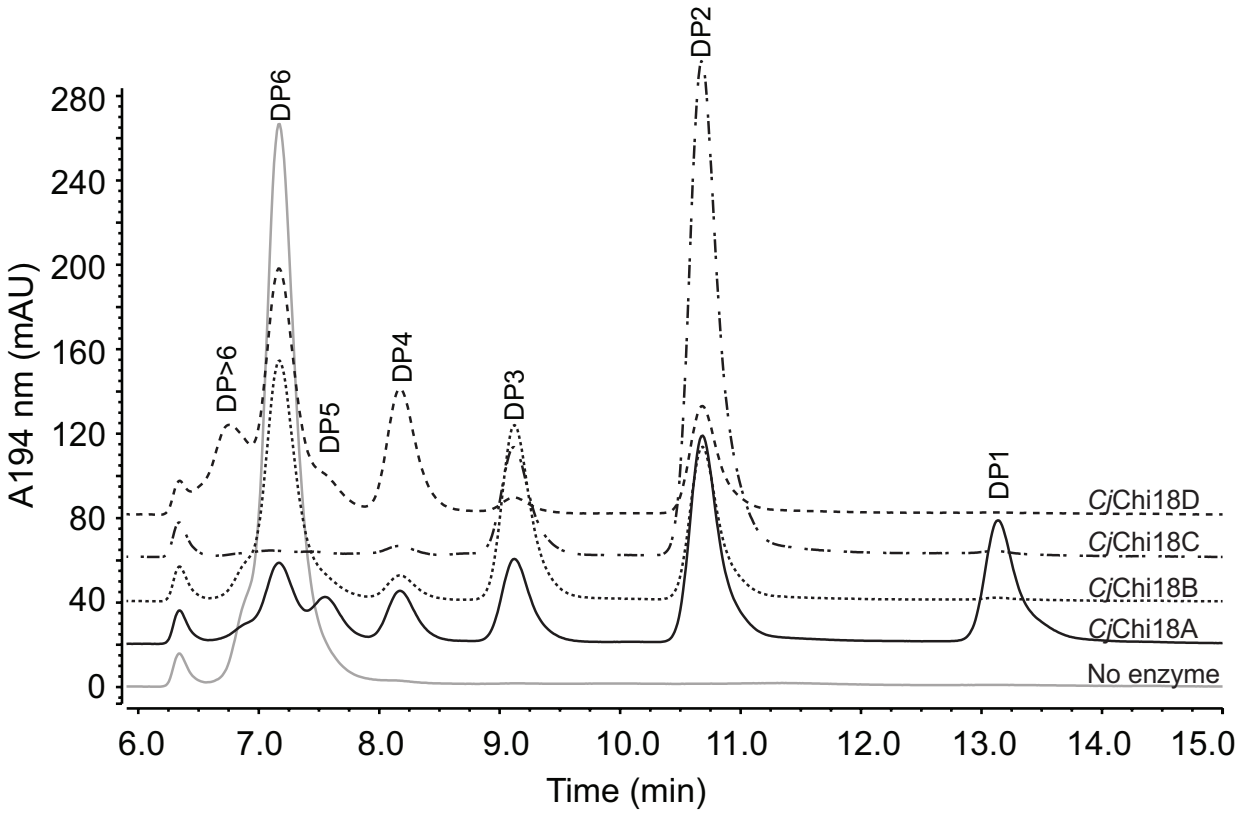

Figure S4. Product profile after degradation of $(GlcNAc)_6$. Chromatograms showing the product profile obtained 60 minutes after mixing chitinases with substrate. Degree of polymerization (DP1-6) represent $(GlcNAc)_{1-6}$. A chromatogram for a reaction without enzyme is also shown (grey line). These experiments were done with the catalytic domains of the chitinases. The reactions contained 2 mM $(GlcNAc)_6$, 10 mM BisTris pH 6.5, 0.1 mg/mL BSA and 50 nM enzyme, and were done in triplicates. Data for samples taken after 2 min of reaction appear in **Fig. 7** of the main manuscript.

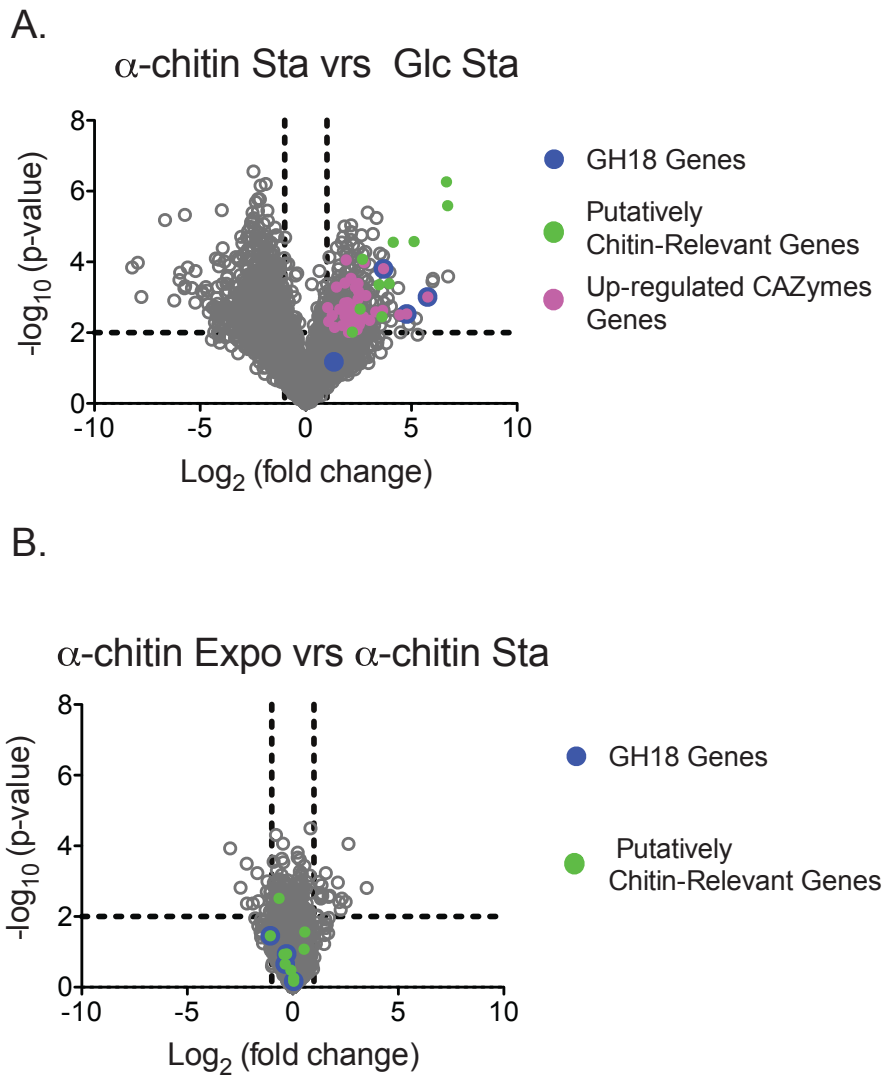

Figure S5. Differential gene expression in *C. japonicus*. **(A)** Comparison of early stationary phase (Sta) transcriptomes during growth on glucose (Glc) or α-chitin **(B)** Comparison of Sta and exponential phase (Exp) transcriptomes during growth on α-chitin. These volcano plots show the $\log_2$(fold change) plotted against the $-\log_{10}$(p-value) of all expressed genes in *C. japonicus* and each gray circle represents the expression of a gene. The dashed lines indicate significance cut-off values: $-\log_{10}$(p-value)> 2 and $\log_2$(fold change)> 1.

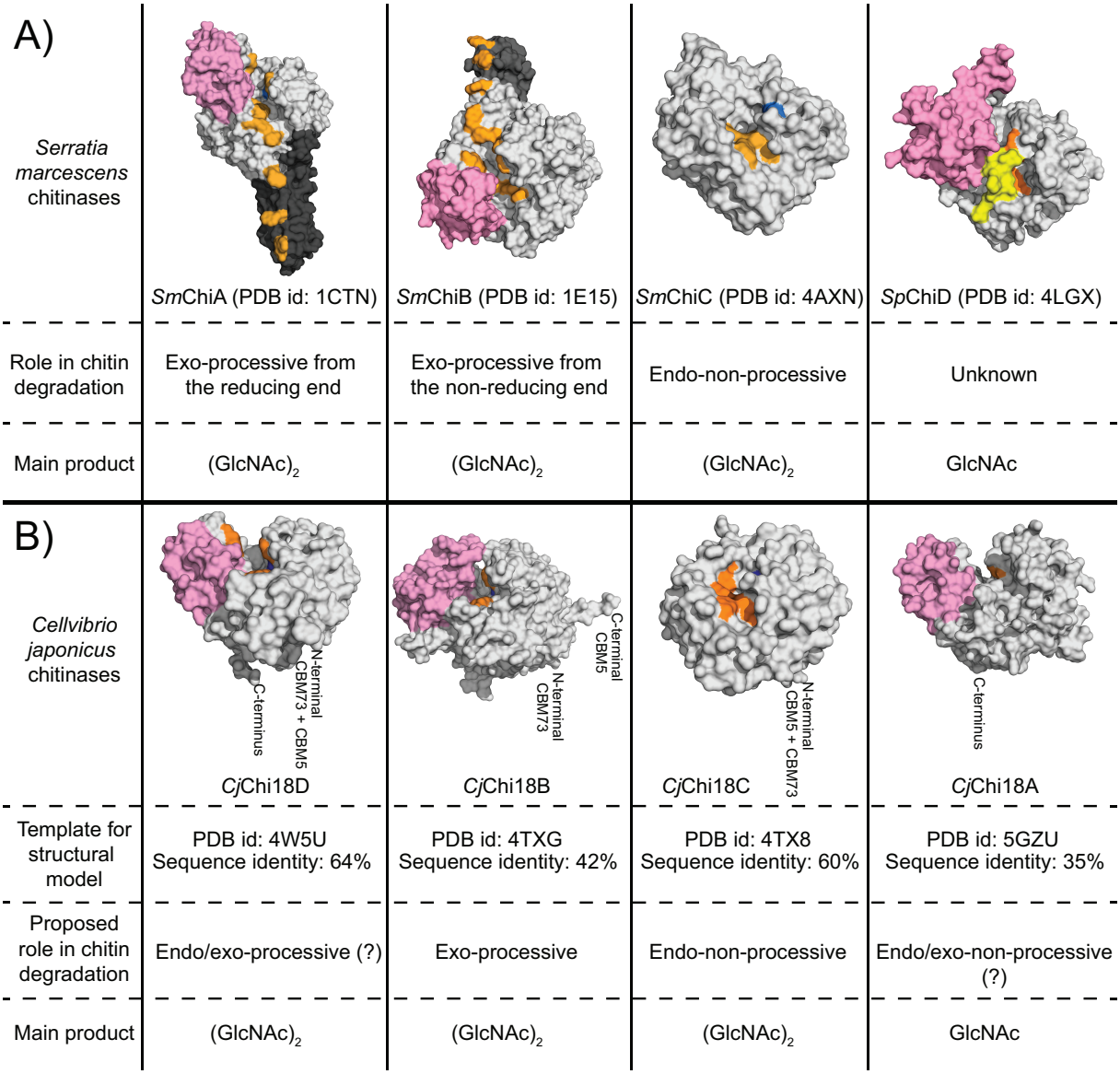

Figure S6. Structural comparison of models of the *C. japonicus* chitinases with crystal structures of *S. marcescens* chitinases. Aromatic residues in the substrate-binding cleft and on the surface of CBMs are shown in orange. The catalytic Glu, which sometimes is almost hidden, is shown in blue, while the α+β domain discussed in the text, and lacking in some of the enzymes, is colored pink. **(A)** Structures of the *S. marcescens* chitinases and key enzyme features. The structures *Sm*ChiA of *Sm*ChiB show complete two-domain proteins containing an FnIII and a CBM5 domain, respectively, which is shown in dark grey. *Sm*ChiC has an FnIII and a CBM5/12 domain located at the C-terminal end of the catalytic domain that is lacking from the structure. The structure of *Sm*ChiD has not been determined, however, the structure of a homologue from *S. proteamaculans* with 86% sequence identity (1,2) is available and is used here for illustration purposes. An extra loop occluding the active site in *Sp*ChiD is shown in yellow. **(B)** Structural models of the catalytic domains of the four *C. japonicus* GH18 chitinases. The models of the catalytic domains of the *C. japonicus*

114 chitinases were built using PyMod 2.0 (1). The positions of lacking domains and, when
115 visible, the N- and/or C-terminus of the protein are indicated.
116

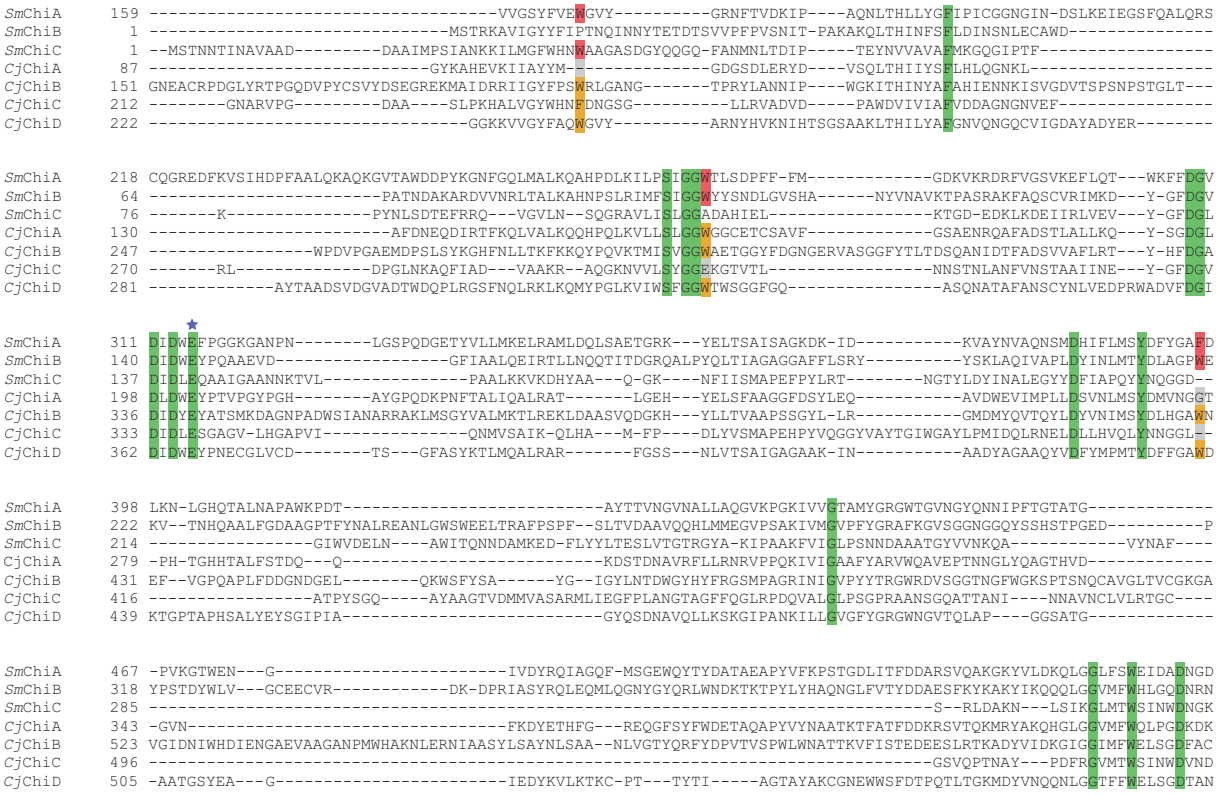

Figure S7. Sequence alignment. The catalytic domains of four *C. japonicus* chitinases are aligned with the catalytic domains of three well-characterized chitinases from *S. marcescens*. Fully conserved residues are shown with green background, and a blue star indicates the catalytic Glu acting as the catalytic acid/base. Trp residues in *Sm*ChiA and *Sm*ChiB known to be important for processivity (3-5) are shown with a red background, and aligned Trp residues in *C. japonicus* chitinases are shown on orange background.

Table S1A. Growth of *C. japonicus* strains grown in MOPS defined media supplemented with α-chitin[a]

Strain	Growth Rate (gen h^{-1})	Lag time (h)	Max OD_{600}
Wild Type[b]	0.051 ± 0.009	17.5	1.14 ± 0.14
Δ*gsp*	Not determined	Not determined	0.02 ± 0.001
Δ*chi18A*[c]	0.059 ± 0.016	17.5	1.20 ± 0.21
Δ*chi18B*[d]	0.050 ± 0.012	41	0.84 ± 0.09
Δ*chi18C*[d]	0.058 ± 0.009	49	1.08 ± 0.16
Δ*chi18D*	Not determined	Not determined	0.002 ± 0.001
Δ*chi18A* Δ*chi18B*[c]	0.046 ± 0.008	17.5	0.88 ± 0.05
Δ*chi18A* Δ*chi18C*[e]	0.047 ± 0.002	41	1.41 ±0 .43
Δ*chi18B* Δ*chi18C*[f]	0.032 ± 0.007	41	0.50 ± 0.06
Δ*chi18A* Δ*chi18B* Δ*chi18C*[g]	0.022 ± 0.004	41	0.51 ± 0.001

[a] Experiments were performed in biological triplicates; the Table shows average values and standard deviations
[b] Time points used to calculate growth rate were T_i=24 and T_f=66
[c] Time points used to calculate growth rate were T_i=41 and T_f=66
[d] Time points used to calculate growth rate were T_i=41 and T_f=72
[e] Time points used to calculate growth rate were T_i=41 and T_f=96
[f] Time points used to calculate growth rate were T_i=41 and T_f=137
[g] Time points used to calculate growth rate were T_i=96 and T_f=120

Table S1B. Growth of *C. japonicus* strains grown in MOPS defined media supplemented with crab shell[a]

Strain	Growth Rate (gen h^{-1})	Lag time (h)	Max OD_{600}
Wild Type[b]	0.011 ± 0.001	25	0.707 ± 0.011
Δ*gsp*	Not determined	Not determined	0.011 ± 0.004
Δ*chi18A*[c]	0.009 ± 0.0009	25	0.480 ± 0.104
Δ*chi18B*[d]	0.0134 ± 0.004	25	0.514 ± 0.051
Δ*chi18C*[d]	0.011 ± 0.002	25	0.612 ± 0.072
Δ*chi18D*	Not determined	Not determined	0.014 ± 0.008
Δ*chi18A* Δ*chi18B*[b]	0.012 ± 0.002	25	0.539 ± 0.153
Δ*chi18A* Δ*chi18C*[b]	0.023 ± 0.018	25	0.732 ± 0.182
Δ*chi18B* Δ*chi18C*[b]	0.013 ± 0.011	25	0.342 ± 0.101
Δ*chi18A* Δ*chi18B* Δ*chi18C*[d]	0.025 ± 0.008	25	0.485 ± 0.236

[a] Experiments were performed in biological triplicates; the Table shows average values and standard deviations
[b] Time points used to calculate growth rate were T_i=53 and T_f=125
[c] Time points used to calculate growth rate were T_i=53 and T_f=148
[d] Time points used to calculate growth rate were T_i=53 and T_f=103

Table S2. Quantification of the zone of clearance generated by various *C. japonicus* strains grown on colloidal chitin plates[a]

Strain	Zone of Clearing (cm^2)
Wild Type	1.77 ± 0.08
Δ*gsp*	None detectable
Δ*chi18A*	1.93 ± 0.14
Δ*chi18B*	1.61 ± 0.13
Δ*chi18C*	1.54 ± 0.22
Δ*chi18D*	None detectable
Δ*chi18A* Δ*chi18B*	1.54 ± 0.01
Δ*chi18A* Δ*chi18C*	1.47 ± 0.12
Δ*chi18B* Δ*chi18C*	0.89 ± 0.10
Δ*chi18A* Δ*chi18B* Δ*chi18C*	0.92 ± 0.10

[a] Experiments were performed in biological triplicates, and the table shows average values and standard deviations

138 **Table S3A. Up-regulated putative CAZyme-encoding genes during exponential**
139 **growth on α-chitin compared to glucose**[a]
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Substrate	CAZy name[b]	Fold change[c]	p-value[d]	Putative activity	Locus ID[e]
Chitin	*lpmo10A*	6.7	5.6	Lytic polyssaccharide mono-oxygenase	CJA_2191
Chitin	*chi18D*	6.7	6.3	Chitinase	CJA_2611
Chitin	*chi18C*	5.1	4.6	Chitinase	CJA_2993
Chitin	*hex20B*	4.1	4.6	Hexosaminidase	CJA_0287
Chitin	*chi18A*	4.0	3.4	Chitinase	CJA_1182
Chitin	*chi18B*	3.6	2.5	Chitinase	CJA_0988
Chitin	*nag9A*	3.5	3.4	Deacetylase	CJA_1163
Arabinan	*abf43L*	3.4	6.0	α-Arabinofuranidase	CJA_0806
Starch	*amy13A*	3.3	2.5	α-Amylase	CJA_2618
Arabinan	*abf43M*	3.0	3.2	α-Arabinofuranidase	CJA_0819
Transglycosylase	*lmt23D*	2.9	5.2	Transglycosylase	CJA_2884
Pectin	*bgl2C*	2.9	4.5	β-Galactosidase	CJA_2610
Arabinan	*gly43D*	2.8	2.1	α-Arabinofuranidase	CJA_0818
Arabinan	*gly43C*	2.7	3.6	α-Arabinofuranidase	CJA_0816
Cellulose	*cbp2E*	2.7	2.8	Predicted redox	CJA_2615
Chitin	*hex20A*	2.7	4.1	Hexosaminidase	CJA_0350
Carbohydrate biding protein	*cbp6B*	2.7	2.8	Carbohydrate biding protein	CJA_0276
Xylan	*abf62A*	2.6	3.2	α-Arabinofuranidase	CJA_3281
Chitin	*csn46F*	2.6	2.	Chitosanase	CJA_2611
Pectin	*pga28A*	2.6	3.7	Polygalacturonase	CJA_0172
Cellulose	*cbp2D*	2.6	2.2	Predicted redox	CJA_2616
β-Glucan	*ebg98*	2.6	3.5	Endogalactosidase	CJA_3286
Arabinan	*arb43A*	2.5	2.5	α-Arabinofuranidase	CJA_0805
Glycosyl transferase	*gt5B*	2.3	2.1	Glycosyl transferase	CJA_3255
Starch	*pul13B*	2.3	3.2	Pullanase	CJA_3161
Xylan	*xyn11B*	2.3	2.6	Endoxylanase	CJA_3762
Chitin	*chi19A*	2.2	2.0	Chitinase	CJA_0996
Starch	*agd31A*	2.2	3.1	α-Glucosidase	CJA_3248
Xyloglucan	*gly74A*	2.1	4.1	Endoxyloglucanase	CJA_2477
Carbohydrate biding protein	*cbp6A*	2.0	2.3	Carbohydrate biding protein	CJA_1191
Starch	*gla15*	2.0	4.2	α-Glucosidase	CJA_0731
Starch	*cbp26A*	2.0	4.3	Carbohydrate biding	CJA_2869

[a] RNAseq sampling experiments were performed in biological triplicates
[b] Names as described by Deboy *et al.* and Henrissat (6,7)
[c] $\log_2$ of the fold change of the gene expression when grown in α- chitin versus glucose
[d] The adjusted $-\log_{10}$(p-value) was calculated using ArrayStar software. An adjusted p-value < 0.01 was selected as the significance cut-off value
[e] Locus IDs from Deboy *et al.* (7)

				protein	
Xylan	*abf51A*	1.9	4.7	α-Arabinofuranidase	CJA_2769
Xylan	*xyn10C*	1.9	2.0	Endoxylanase	CJA_3066
Xylan	*gla67A*	1.9	3.9	α-Glucuronidase	CJA_2887
Arabinan	*gly43E*	1.8	2.0	α-Arabinofuranidase	CJA_0799
Xylan	*xyn10A*	1.8	2.0	Endoxylanase	CJA_2471
Mannan	*man26A*	1.7	3.8	Endomannanase	CJA_2770
Xylan	*xyn5A*	1.7	2.5	Endoxylanase	CJA_3279
Starch	*glu13A*	1.7	2.2	α-Glucosidase	CJA_0732
Starch	*mal77Q*	1.7	6.0	Amylomaltase	CJA_1882
Mannan	*aga27A*	1.6	2.0	α-Galactosidase	CJA_0246
Starch	*amy13J*	1.6	3.0	α-Amylase	CJA_0398
Cellulose	*LPMO10B*	1.6	2.5	Lytic polyssaccharide mono-oxygenase	CJA_3139
Xylan	*xyn11A*	1.6	2.7	Endoxylanase	CJA_3763
Miscellaneous	*gly57A*	1.6	3.2	Glycoside hydrolase	CJA_1883
Mannan	*man5B*	1.6	2.1	Endomannanase	CJA_2480
Polysaccharide deacetylase	*pda4C*	1.6	2.7	Deacetylase	CJA_3428
Cellulose	*cel3B*	1.6	2.1	β-Glucosidase	CJA_1497
Xylan	*xyn10B*	1.6	3.3	Endoxylanase	CJA_3280
Pectin	*bgl2A*	1.6	3.1	β-Galactosidase	CJA_0496
Glycosyl transferases	*gt5A*	1.6	2.4	Glycosyl transferase	CJA_1886
Pectin	*gal53A-2*	1.6	3.2	Endogalactosidase	CJA_0491
Starch	*pul13A*	1.5	2.0	Pullanase	CJA_2160
Glycosyl transferases	*gt1B*	1.5	2.1	Rhamnosyltransferase	CJA_0772
Glycosyl transferases	*gt4A*	1.5	2.5	Glycosyl transferase	CJA_3411
Pectin	*pel1G*	1.5	2.3	Pectate lyase	CJA_3120
Starch	*amy13B*	1.5	2.7	α-Amylase	CJA_1522
Pectin	*pel10B*	1.5	3.8	Pectate lyase	CJA_2040
Pectin	*pel3B*	1.5	2.7	Pectate lyase	CJA_2413
Glycosyl transferases	*gt4B*	1.5	2.1	Glycosyl transferase	CJA_3410
Polysaccharide deacetylase	*pda4E*	1.4	2.9	Deacetylase	CJA_3408
Xylan	*cbp35A*	1.4	3.0	Carbohydrate biding protein	CJA_0020
Starch	*glc13A*	1.3	3.1	α-glucosidase	CJA_0257
Pectin	*bgl35A*	1.3	2.0	β-Galactosidase	CJA_2707
Cellulose	*cbp2A*	1.3	2.7	Carbohydrate biding protein	CJA_0007
Starch	*gbe13A*	1.3	2.3	Transglycosylase	CJA_1885
Cellulose	*cel45A*	1.2	2.2	Cellulase	CJA_0374
Cellulose	*cel6A*	1.2	2.8	Cellobiohydrolase	CJA_2473
Starch	*amy13F*	1.2	3.0	α-Amylase	CJA_0398
Transglycosylase	*lmt23B*	1.1	2.3	Transglycosylase	CJA_2053
Cellulose	*cel5D*	1.0	3.1	Cellulase	CJA_3010
Starch	*amy13D*	1.0	3.3	α-Amylase	CJA_0737

Table S3B. Up-regulated putative CAZyme-encoding genes during early stationary growth on α-chitin compared to glucose[a]

Substrate	CAZy name[b]	Fold change[c]	p-value[d]	Putative activity	Locus ID[e]
Chitin	*lpmo10A*	10.6	4.3	Lytic polyssaccharide mono-oxygenase	CJA_2191
Chitin	*chi18D*	5.8	3.0	Chitinase	CJA_2611
Chitin	*chi18C*	4.8	2.5	Chitinase	CJA_2993
Arabinan	*abf43L*	4.5	2.5	α-Arabinofuranidase	CJA_0806
Chitin	*chi18B*	3.7	3.8	Chitinase	CJA_0988
Pectin	*pel3B*	3.6	2.6	Pectate lyase	CJA_2413
Starch	*amy13A*	3.3	2.6	α-Amylase	CJA_2618
Xylan	*axe2C*	3.0	2.4	Acetylxylan esterase	CJA_0450
Arabinan	*gly43C*	2.8	3.1	α-Arabinofuranidase	CJA_0816
β-Glucans	*ebg98*	2.8	4.0	Endogalactosidase	CJA_3286
Arabinan	*gly43D*	2.7	2.5	α-Arabinofuranidase	CJA_0818
Glycosyl Transferase	*gt9B*	2.7	2.4	Glycosyl Transferase	CJA_1369
Chitin	*nag9A*	2.6	3.1	Deacetylase	CJA_1163
Pectin	*bgl2C*	2.6	2.3	β-Galactosidase	CJA_2610
Glycosyl Transferase	*gt5B*	2.5	2.2	Glycosyl Transferase	CJA_3255
Starch	*amy13H*	2.5	3.2	α-Amylase	CJA_3247
Starch	*cgt13B*	2.5	3.4	Glucanotransferase	CJA_3263
Transglycosylase	*lmt23D*	2.5	3.0	Transglycosylase	CJA_2884
Pectin	*pme8C*	2.4	2.1	Pectinesterase	CJA_0181
Starch	*agd31A*	2.4	3.3	α-Glucosidase	CJA_3248
Xylan	*cpb35A*	2.3	2.2	Carbohydrate biding protein	CJA_0020
β-Glucan	*cgs94A*	2.3	2.4	Glucan synthetase	CJA_0849
Pectin	*pme8A*	2.3	2.6	Pectin methylesterase	CJA_0041
β-Glucan	glu16A	2.3	2.2	β-Glucanase	CJA_0225
Arabinan	*gly43G*	2.2	2.2	α-Arabinofuranidase	CJA_3070
Arabinan	*abf43M*	2.2	2.4	α-Arabinofuranidase	CJA_0819
Arabinan	*gly43J*	2.2	2.0	α-Arabinofuranidase	CJA_3067
Mannan	*man5C*	2.1	3.5	Endomannanase	CJA_3470
Chitin	*hex20A*	2.1	2.8	Hexosaminidase	CJA_0350
Chitin	*chi19A*	2.0	2.7	Chitinase	CJA_0996
Arabinan	*afc95A*	2.0	2.0	α-fucosidase	CJA_2710
Arabinan	*abf51A*	2.0	2.9	α-fucosidase	CJA_2769

[a] RNAseq sampling experiments were performed in biological triplicate
[b] Names as described by Deboy *et al.* and Henrissat (6,7)
[c] $\log_2$ of the fold change of the gene expression when grown in α- chitin versus glucose
[d] The adjusted $-\log_{10}$(p-value) was calculated using ArrayStar software. An adjusted p-value < 0.001 was selected as the significance cut-off value
[e] Locus IDs from Deboy *et al.* (7)

Pectin	*pel10B*	2.0	2.2	Pectate lyase	CJA_2040
Pectin	*pel1D*	2.0	2.3	Pectate lyase	CJA_2040
Polysaccharide deacetylase	*pda4C*	1.9	4.1	Deacetylase	CJA_3428
Cellulose	*cel5D*	1.9	2.7	Cellulase	CJA_3010
Xylan	*abf62A*	1.9	2.5	α-Arabinofuranidase	CJA_3281
Cellulose	*cbp2D*	1.9	3.4	Predicted redox	CJA_2616
Xylan	*gla67A*	1.9	2.8	α-Glucuronidase	CJA_2887
Xylan	*cbp35B*	1.8	2.7	Carbohydrate biding protein	CJA_0559
Pectin	*bgl35A*	1.7	2.2	β-Galactosidase	CJA_2707
Cellulose	*cel6A*	1.6	2.7	Cellulase	CJA_2473
Starch	*amy13B*	1.5	3.3	α-Amylase	CJA_1522
Mannan	*man5D*	1.4	2.2	Endomannanase	CJA_0244
Mannan	*man5B*	1.3	2.5	Endomannanase	CJA_2475
Carbohydrate Biding Protein	*cbp35C*	1.1	2.3	Carbohydrate binding protein	CJA_0494
Cellulose	*cel3B*	1.0	2.7	β-Glucosidase	CJA_1497

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Table S4. Product ratios after degradation of α-chitin and $(GlcNAc)_6$

Chitinase	Chitin degradation	$(GlcNAc)_6$ degradation
	$(GlcNAc)_2$/GlcNAc ratio, 12 h	$(GlcNAc)_2$/$(GlcNAc)_4$ ratio, 2 min
*Cj*Chi18A$_{cat}$	0.2 ± 0.002	1.8 ± 0.048
*Cj*Chi18B$_{cat}$	7.0 ± 0.409	13.4 ± 0.692
*Cj*Chi18C$_{cat}$	7.3 ± 0.148	1.5 ± 0.067
*Cj*Chi18D$_{cat}$	2.8 ± 0.031	1.1 ± 0.048
*Cj*Chi18B$_{cat+CBM5}$	19.1 ± 0.034	Not determined
*Cj*Chi18D$_{cat+CBM5}$	3.4 ± 0.109	Not determined

148 **Table S5. Strains, plasmids and primers used in this study**
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Strains, plasmid or primer	Genotype	Source or Reference
Strains		
E. coli DH5α	λ-Φ80dlacZΔM15 Δ(lacZYA-argF)U169 recA1 endA1 hsdR17(rk-mk) supE44 thi-1gyrA relA1	Laboratory collection
E. coli S17 *λpir*	Tpr Smr recA thi pro hsdR hsdM+ RP4-2-TC::Mu::Km Tn7 λpri	Laboratory collection
C. japonicus Ueda 107	Wild Type	Laboratory collection
C. japonicus Δ*gsp*	Ueda 107 Δ*gsp*	(8)
C. japonicus Δ*chi18A*	Ueda 107 Δ*chi18A*[a]	This study
C. japonicus Δ*chi18B*	Ueda 107 Δ*chi18B*[b]	This study
C. japonicus Δ*chi18C*	Ueda 107 Δ*chi18C*[c]	This study
C. japonicus Δ*chi18D*	Ueda 107 Δ*chi18D*[d]	This study
C. japonicus Δ*chi18A*Δ*chi18B*	Ueda 107 Δ*chi18A*Δ*chi18B*	This study
C. japonicus Δ*chi18A*Δ*chi18C*	Ueda 107 Δ*chi18A*Δ*chi18C*	This study
C. japonicus Δ*chi18B*Δ*chi18C*	Ueda 107 Δ*chi18B*Δ*chi18C*	This study
C. japonicus Δ*chi18A*Δ*chi18B*Δ*chi18C*	Ueda 107 Δ*chi18A*Δ*chi18B*Δ*chi18C*	This study
Plasmids		
pK2013	ColE1 RK2-Mob$^+$ RK2-Tra$^+$; Km^r	(9)
pK18mobsacB	pMB1 ori mob$^+$ sacB$^+$; Km^r	(10)
pK18/Δ*chi18A*	Contains 500bp upstream and downstream of *chi18A* cloned into pK18mobsacB; Km^r	This study
pK18/Δ*chi18B*	Contains 500bp upstream and downstream of *chi18B* cloned into pK18mobsacB; Km^r	This study
pK18/Δ*chi18C*	Contains 500bp upstream and downstream of *chi18C* cloned into pK18mobsacB; Km^r	This study
pK18/Δ*chi18D*	Contains 1000bp upstream and downstream of *chi18D* cloned into pK18mobsacB; Km^r	This study
Primers		
(5') to amplify 750 bp upstream of *chi18D*	GCTATGACATGATTACGGGTGGTTATACGCGTAATAACCTTC	This study
(3') to amplify 750 bp upstream of *chi18D*	GAATTAGCGTTTCATAGTGTTTTCCTCAACGTTTTTATATAAATACG	This study

[a] BioCyc accession number CJA_1182
[b] BioCyc accession number CJA_0988
[c] BioCyc accession number CJA_2993
[d] BioCyc accession number CJA_2611

(5') to amplify 750 bp downstream of *chi18D*	CACTATGAAACGCTAATTCATGATTACCGGAAGC	This study
(3') to amplify 750 bp downstream of *chi18D*	GCCTGCAGGTCGACTGGTGATATCGATATAGCTGGCGTTG	This study
Δ*chi18A*_CONF_ (5')	ATCATGGGCAGCTTTC	This study
Δ*chi18A*_CONF_ (3')	AGCAGGAGCCTGGTA	This study
Δ*chi18B*_CONF_ (5')	CAATTGGAAATTGGTAATC	This study
Δ*chi18B*_CONF_ (3')	ATATAGTCACGCCCTATTTTG	This study
Δ*chi18C*_CONF_ (5')	AAGGGCATCTGGTTATT	This study
Δ*chi18C*_CONF_ (3')	GTATTTCTATCTGCGTTCAC	This study
Δ*chi18D*_CONF_ (5')	CTGATTGTCCCCTATCTGC	This study
Δ*chi18D*_CONF_ (3')	ATTTCCCAGCGATTGTTAC	This study
chi18A INT_(5')	GGTGGTTCTAGAGCTTGTATCAGTGCG	This study
chi18A INT_(3')	GGTGGTGAATTCCAAGCATCCTTCACATC	This study
chi18B INT_(5')	GGTGGTGAATTCGCTATGTGGCGTTGA	This study
chi18B INT_(3')	GGTGGTTCTAGACTATGTCGTGCCAAATA	This study
chi18C INT_(5')	GGTGGTAAGCTTAGTTTGGGACAACTG	This study
chi18C INT_(3')	GGTGGTTCTAGATGGAGTTATTCAGCG	This study
chi18D INT_(5')	GGTGGTAAGCTTCGACATCCTCTGTTG	This study
chi18D INT_(3')	GGTGGTTCTAGAATAGGCATCACCAATA	This study

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Table S6. Name and description of expressed and characterized versions of the *C. japonicus* GH18 chitinases

Name	Description
*Cj*Chi18A$_{cat}$	Chi18A catalytic domain (residues 87-432 of totally 432)
*Cj*Chi18B$_{cat+CBM5}$	Chi18B catalytic domain + CBM5 domain (residues 151-890 of totally 890)
*Cj*Chi18B$_{cat}$	Chi18B catalytic domain (residues 151-808 of totally 890)
*Cj*Chi18C$_{cat}$	Chi18C catalytic domain (residues 218-537 of totally 537)
*Cj*Chi18D$_{cat+CBM5}$	Chi18D CBM5 + catalytic domain (residues 119-588 of totally 588)
*Cj*Chi18D$_{cat}$	Chi18D catalytic domain (residues 222-588 of totally 588)

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