

1 **Systems analysis of the Glycoside Hydrolase family 18 enzymes from**
2 ***Cellvibrio japonicus* characterizes essential chitin degradation functions**
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17 **Running Title**

18 Chitin degradation in *C. japonicus*
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23 **Keywords:**

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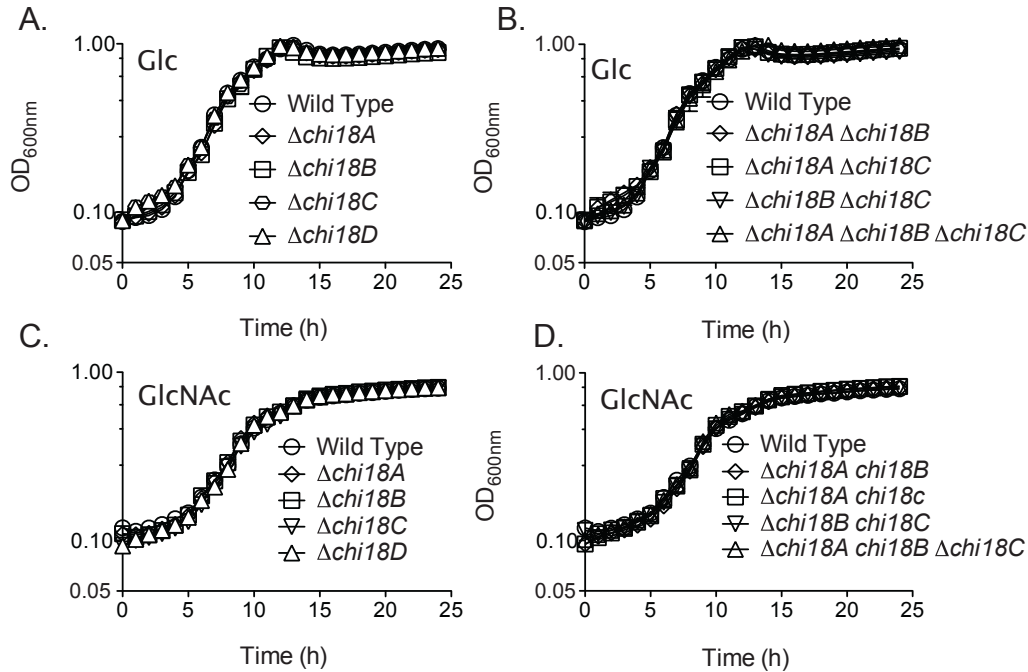
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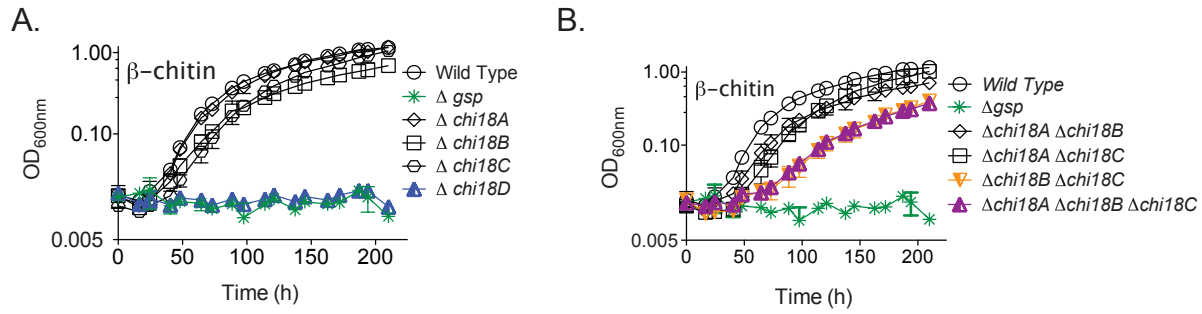
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49 **Figure S1. Growth of *C. japonicus* mutants on Glc and GlcNAc.** Growth analysis was
 50 conducted on MOPS minimal medium supplemented with glucose (Glc; 0.20%) (A&B) or N-
 51 acetylglucosamine (GlcNAc; 0.50%) (C&D) as the sole source of carbon.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

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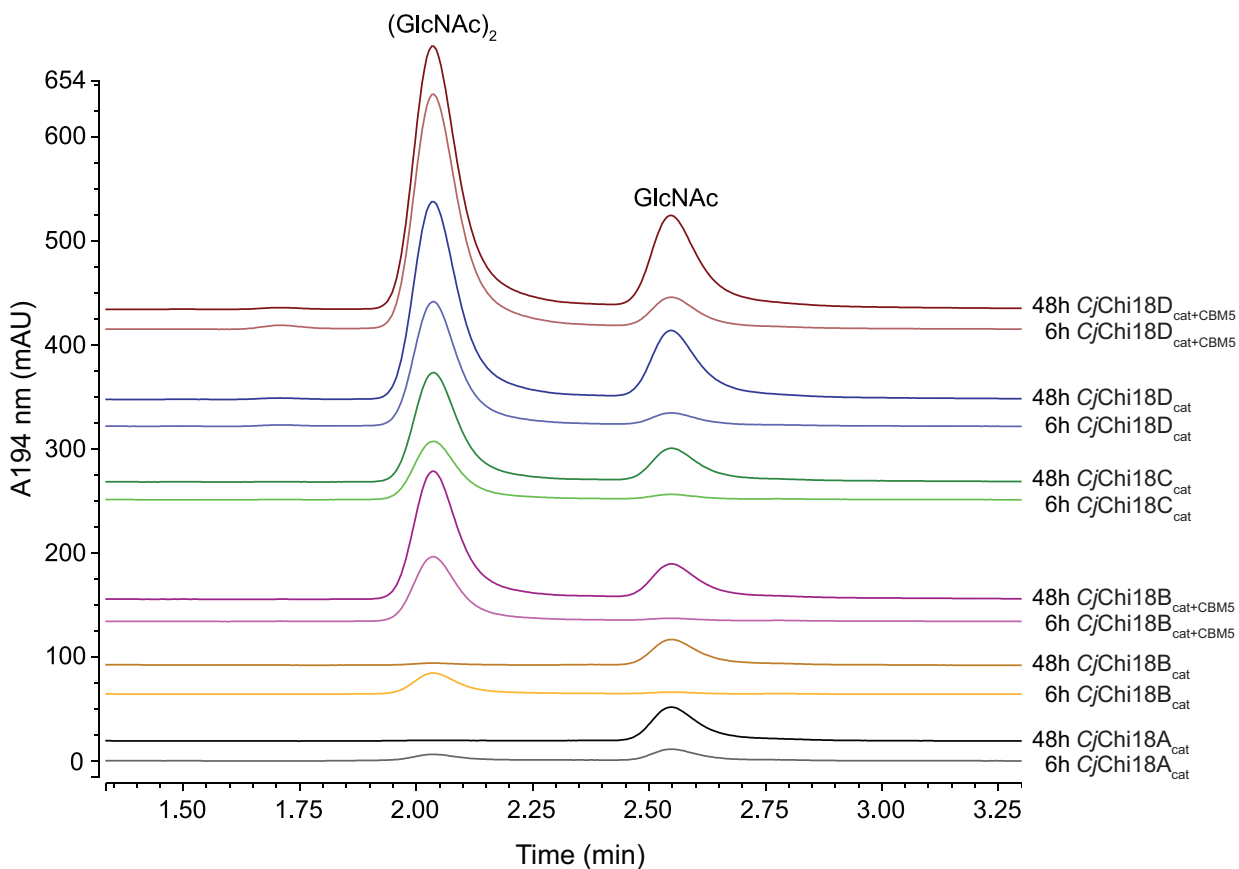


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62 **Figure S2. Growth of *C. japonicus* mutants on β -chitin.** Growth analysis of family GH18
 63 deletion mutants of *C. japonicus* was performed in MOPS minimal medium supplemented
 64 with 0.2% β -chitin as the sole source of carbon. All experiments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

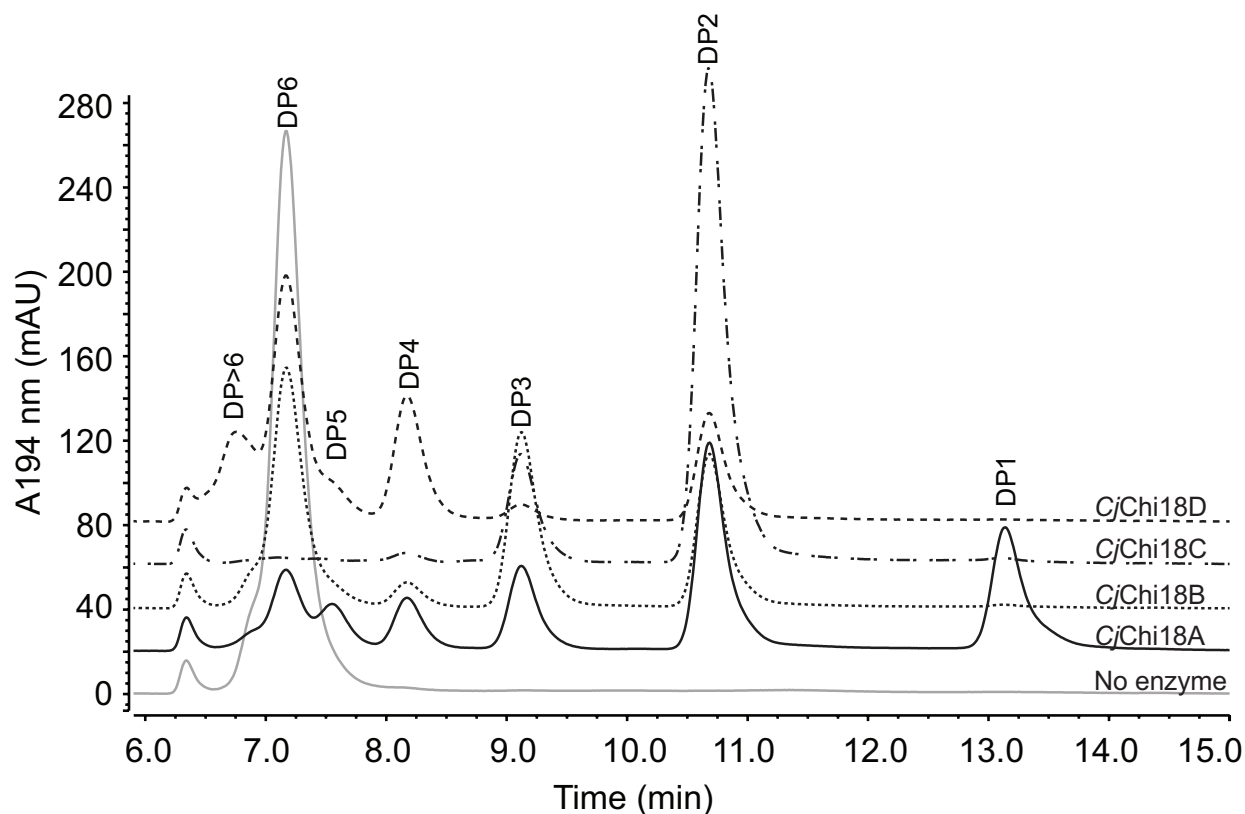
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72 **Figure S3. Product profile after degradation of α -chitin.** Chromatograms of samples
73 taken after 6h and 48h reaction time are shown, representing the product profile early and
74 late in the degradation process. GlcNAc and/or $(\text{GlcNAc})_2$ are the main products for all
75 enzymes. Reactions were done at 30 °C in 20 mM BisTris pH 6.5, 0.1 mg/mL BSA at 30 °C.
76 The enzyme concentration was 0.5 μM .
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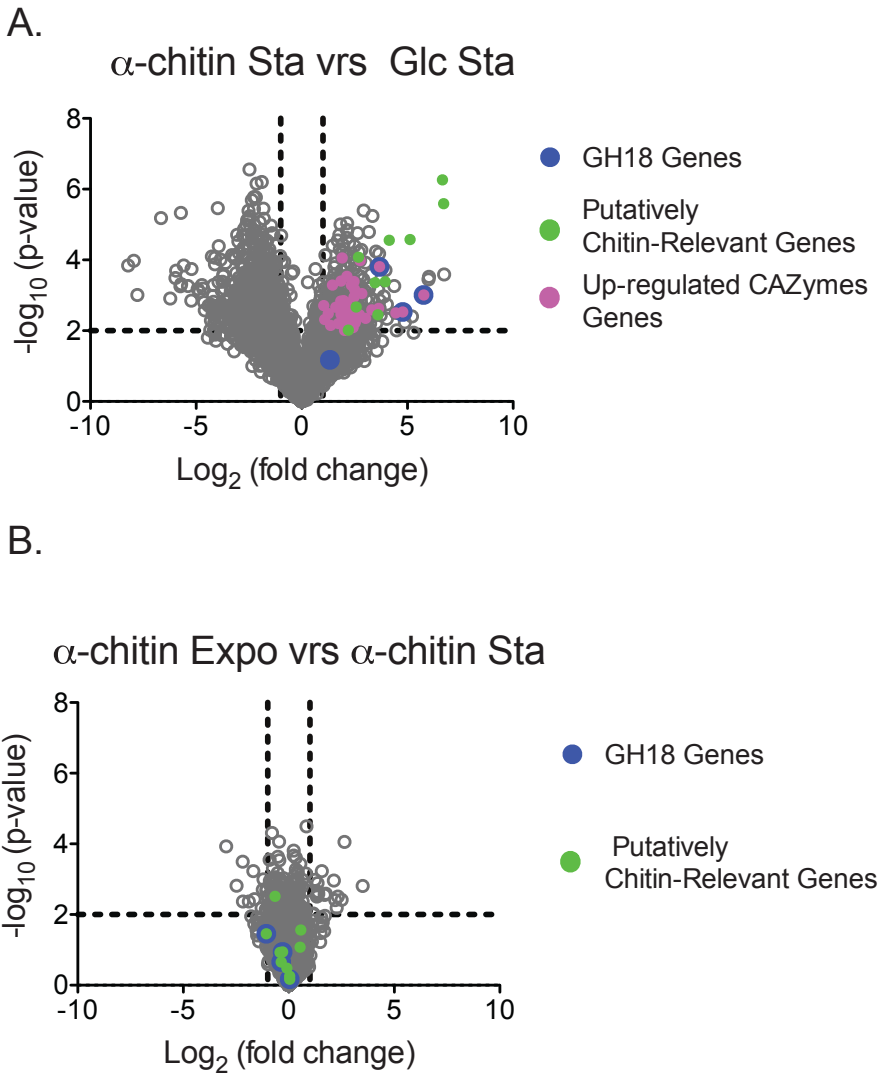
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Figure S4. Product profile after degradation of (GlcNAc)₆. Chromatograms showing the product profile obtained 60 minutes after mixing chitinases with substrate. Degree of polymerization (DP1-6) represent (GlcNAc)₁₋₆. A chromatogram for a reaction without 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.

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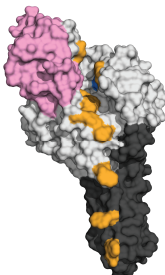
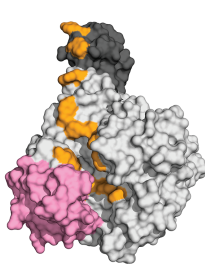
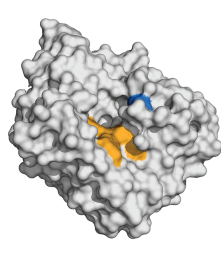
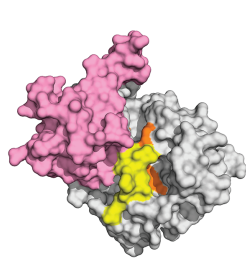
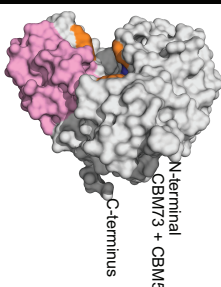
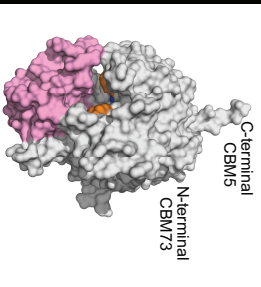
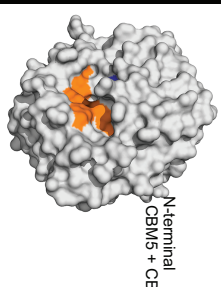
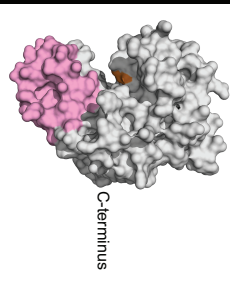


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90 **Figure S5. Differential gene expression in *C. japonicus*.** (A) Comparison of early91 stationary phase (Sta) transcriptomes during growth on glucose (Glc) or α -chitin (B)92 Comparison of Sta and exponential phase (Exp) transcriptomes during growth on α -chitin.93 These volcano plots show the $\log_2(\text{fold change})$ plotted against the $-\log_{10}(\text{p-value})$ of all94 expressed genes in *C. japonicus* and each gray circle represents the expression of a gene.95 The dashed lines indicate significance cut-off values: $-\log_{10}(\text{p-value}) > 2$ and $\log_2(\text{fold}$ 96 $\text{change}) > 1$.

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A)					
	<i>SmChiA</i> (PDB id: 1CTN)	<i>SmChiB</i> (PDB id: 1E15)	<i>SmChiC</i> (PDB id: 4AXN)	<i>SpChiD</i> (PDB id: 4LGX)	
	Role in chitin degradation	Exo-processive from the reducing end	Exo-processive from the non-reducing end	Endo-non-processive	Unknown
	Main product	(GlcNAc) ₂	(GlcNAc) ₂	(GlcNAc) ₂	GlcNAc
B)					
	<i>CjChi18D</i>	<i>CjChi18B</i>	<i>CjChi18C</i>	<i>CjChi18A</i>	
	Template for structural model	PDB id: 4W5U Sequence identity: 64%	PDB id: 4TXG Sequence identity: 42%	PDB id: 4TX8 Sequence identity: 60%	PDB id: 5GZU Sequence identity: 35%
	Proposed role in chitin degradation	Endo/exo-processive (?)	Exo-processive	Endo-non-processive	Endo/exo-non-processive (?)
Main product	(GlcNAc) ₂	(GlcNAc) ₂	(GlcNAc) ₂	GlcNAc	

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101 **Figure S6. Structural comparison of models of the *C. japonicus* chitinases with crystal**
 102 **structures of *S. marcescens* chitinases.** Aromatic residues in the substrate-binding cleft
 103 and on the surface of CBMs are shown in orange. The catalytic Glu, which sometimes is
 104 almost hidden, is shown in blue, while the $\alpha+\beta$ domain discussed in the text, and lacking in
 105 some of the enzymes, is colored pink. (A) Structures of the *S. marcescens* chitinases and
 106 key enzyme features. The structures *SmChiA* of *SmChiB* show complete two-domain
 107 proteins containing an FnIII and a CBM5 domain, respectively, which is shown in dark grey.
 108 *SmChiC* has an FnIII and a CBM5/12 domain located at the C-terminal end of the catalytic
 109 domain that is lacking from the structure. The structure of *SmChiD* has not been determined,
 110 however, the structure of a homologue from *S. proteamaculans* with 86% sequence identity
 111 (1,2) is available and is used here for illustration purposes. An extra loop occluding the active
 112 site in *SpChiD* is shown in yellow. (B) Structural models of the catalytic domains of the four
 113 *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.
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SmChiA 159 -----VVGYSYFVEIGVY-----GRNFTVDKIP---AQNLTHTLLYGLIPICGGNGIN-DLSKEIEGSFQALQRS
SmChiB 1 -----MSTRKAVIGYFIPETNQINNYTEIDTSVVPFPVSNIT-PAKAKQLTHINFSLDINSNLECAWD-----
SmChiC 1 --MSTNNTINAVAAD-----DAAIMPSTANKKLLMGFWHNAAGASDGYQQGQ-FANMMLTDIP-----TEYNVVAVAKMGQGIPTF-----
CjChiA 87 -----GYKAHEVKKIIAYYM-----GDGSDLERYD---VSQTLTHIYSLHLQGNKL-----
CjChiB 151 GNEACRPDGLYRTPGQDVPYCSVYDSEGREKMAIDRRRIIGYFPSRLGANG-----TPRYLANNIP---WGKITHINYAFAHIENKISVGDVTSPSNPSTGLT---
CjChiC 212 -----GNARVPG-----DAA---SLPKHALVGYWHNFDNGSG-----LLRVADVD---PAWDVIVIAVDDAGNGNVEF-----
CjChiD 222 -----GGKKVVGYFAQIGVY-----ARNYHVKNIHSTSGSAAKLTHILYAGNVQNGQCVIGDAYADYER-----

SmChiA 218 CQGREDFKVISIHDPFAALQKAQKGVTAWDDPYKGNFQGLMALKQAHPLDKILFSLGGWTLSDPFF-FM-----GDKVKRDRFVGSVKEFLQT---WKFFDGV
SmChiB 64 -----PATNDAKARDVNRRLTALKAHNPSLRIMFSIIGWYYINDLVGSHA-----NYVNAVKT PASRAKFAQSCVRIMKD---Y-GFDGV
SmChiC 76 -----K-----PYNLSDTEFRRQ---VGVLN---SQGRAVLISLGGADAHIEL-----KTGD-EDKLDKEIIRLVEV---Y-GFDGL
CjChiA 130 -----AFDNEQDIRTFKQLVALKQQHPQLKVLVLSLGGWGGCETCSAVF-----GSAENRQAFADSTLALLKQ---Y-SGDGL
CjChiB 247 -----WPDVPGAEMDPSLSYKGFHLLTKFKQYPQVKTMISVGGWAEETGGYFDGNGERVASGGFYTLTDSQANIDTFADSVVAFRLT---Y-HFDGA
CjChiC 270 -----RL-----DPGLNKAQFIAD---VAAKR---AQQKNVVL SYGGEKGTVTL-----NNSTNLANFVNSTAAIINE---Y-GFDGV
CjChiD 281 -----AYTAADSVDGVDATWQPLRGSFNQLRKLKQMYPLGLKVIWVSGGWTWSGGFGQ-----ASQNATAFANSCYNLVEDPRWADVFDGI

SmChiA 311 DIDWEFPGGKGANPN-----LGSPQDGETYVLLMKELRAMLDQLSAETGRK---YELTSAISAGKDK-ID-----KVAYNVAQNSMHIFLMSDFYGAFD
SmChiB 140 DIDWEYPQAAEVD-----GFIAALQEIIRLLNQQTITDGRQALPYQLTIAGAGGAFPLSRY-----YSKLAQIVAPLBYINLMTDLAGPME
SmChiC 137 DIDLEQAAGI GAANNKTVL-----PAALKVKVDHYAA---Q-GK---NFIISMAPEFPYLRT-----NGTYLDYINALEGYYDFIAPQYVNGGDD--
CjChiA 198 DIDWEYPTVPGYPGH-----AYGPQDKPNFTALIQALRAT---LGEH---YELSFAGGFDSYLEQ-----AVDWEVIMPLLSVSNLMSYDMVNGGT
CjChiB 336 DIDYRYATSMKDGANPADWSIANARRAKLMSGYVALMKTLEKLDAAASVDQDKH---YLLTVAAPSSGYL-LR-----GMDYQVQTYLRYVNIIMSVDLHGANN
CjChiC 333 DIDLESGAGV-LHGAPVI-----QNMVSAIK-QLHA---M-FP---DLVYSMAPEHPYVQGGYVAYTGIWGAYLEPMDQLRNELELLHVQLNNGGL--
CjChiD 362 DIDWEYVNECGLVCD-----TS---GFASYKTLMQALRAR-----FGSS---NLV TSAIGAGAAK-IN-----AADYAGAAQYVDFYMPMTDFFGAVD

SmChiA 398 LKN-LGHQTALNAPAWKPD-----AYTTVNGVNALLAQGVKPKKIVVGTAMYGRGWTGVNGYQNNIPFTGTATG-----
SmChiB 222 KV--TNHQALFGDAAGPTFYNALREANLGSWEELTRAFSPFF--SLTVDAVQOHLMMEGVPSAKIVMGVPPYGRAFKVSGGNGGOYSSHSHTPGED-----P
SmChiC 214 -----GIWVDELN---AWITQNN DAMKED-FLYLTESLVTGTRGYA-KIPAAKFVIGLPSNNDAAATGYVVKQA-----VYNAF-----
CjChiA 279 -PH-TGHH TALFSTDQ---Q-----KDS TDNAVRFLLRNRVFPQKIVIGAAFYARVWQAVEPTNNGLYQAGTHVD-----
CjChiB 431 EF--VGPQAPLFDGDNDGDEL-----QKWSFYSA-----YG---IGYLN TDWGYHYFRGSM PAGRINIGVPPYTRGWRDVS GGTNGFWGKSPTSNOCAVGLTVCGKGA
CjChiC 416 -----ATPYSGQ-----AYAAGTVDMVMVASARMLIEGFPLANGTAGFFQGLRPDQVALGLPSGPRAANSQGATTANI-----NNAVNCLVLRGTG---
CjChiD 439 KTGPTAPH S ALYEYSGIPIA-----GYQSDNAVQLLKS KGI PANKILLGVGYGRGWNGVTQLAP-----GGSATG-----

SmChiA 467 -PVKGTWEN---G-----IVDYRQIAGQF-MSGEWQTYTDATAEAPYVFKPSTGDLITFDDARSVQAKGKYVLDKQLGLLFSWEIDAINGD
SmChiB 318 YPSTDYWL V---GCEECV R-----DK-DPRIASRYQL EQLMQNGYQRLWNDKTKTPYLYHAQNGLFVTYDDAESFKYKAKYIKQQQLGQVVMFHLGQDNRN
SmChiC 285 -----S---RLDAKN---LSIKELMTWSINWNGK
CjChiA 343 -GVN-----FKDYETHFG---REQGFSYFWDETAQAPYVYNAATKTFATFDDKRSVTQMRMYAKQHGLGQVVMFQWLPGRDKD
CjChiB 523 VGI DNIWHD IENGA EVAAGANPMHAKNLERINIAASYLSAYNLSAA---NLVGTYQRFPYDPTVSPWLWNATTKVPISTEDEESLRTKADYVIDKIGIGIMFWELSGDFAC
CjChiC 496 -----GVSQPTNAY---PDRFRVMTWSINWVND
CjChiD 505 -AATGSYEA---G-----IEDYKVLKTKC-PT---TYTI-----AGTAYAKCGNEWWSFDTPTQLTGM D YV NQNLGQTFEWFELSGDTAN

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117
118 **Figure S7. Sequence alignment.** The catalytic domains of four *C. japonicus* chitinases are aligned
119 with the catalytic domains of three well-characterized chitinases from *S. marcescens*. Fully conserved
120 residues are shown with green background, and a blue star indicates the catalytic Glu acting as the
121 catalytic acid/base. Trp residues in *SmChiA* and *SmChiB* known to be important for processivity (3-5)
122 are shown with a red background, and aligned Trp residues in *C. japonicus* chitinases are shown on
123 orange background.
124

125 **Table S1A. Growth of *C. japonicus* strains grown in MOPS defined media**
 126 **supplemented with α -chitin^a**
 127

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
Δgsp	Not determined	Not determined	0.02 ± 0.001
$\Delta chi18A^c$	0.059 ± 0.016	17.5	1.20 ± 0.21
$\Delta chi18B^d$	0.050 ± 0.012	41	0.84 ± 0.09
$\Delta chi18C^d$	0.058 ± 0.009	49	1.08 ± 0.16
$\Delta chi18D$	Not determined	Not determined	0.002 ± 0.001
$\Delta chi18A \Delta chi18B^c$	0.046 ± 0.008	17.5	0.88 ± 0.05
$\Delta chi18A \Delta chi18C^e$	0.047 ± 0.002	41	1.41 ± 0.43
$\Delta chi18B \Delta chi18C^f$	0.032 ± 0.007	41	0.50 ± 0.06
$\Delta chi18A \Delta chi18B$ $\Delta chi18C^g$	0.022 ± 0.004	41	0.51 ± 0.001

128
 129

^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

130 **Table S1B. Growth of *C. japonicus* strains grown in MOPS defined media**
 131 **supplemented with crab shell^a**
 132

Strain	Growth Rate (gen h ⁻¹)	Lag time (h)	Max OD ₆₀₀
Wild Type ^b	0.011 ± 0.001	25	0.707 ± 0.011
<i>Δgsp</i>	Not determined	Not determined	0.011 ± 0.004
<i>Δchi18A</i> ^c	0.009 ± 0.0009	25	0.480 ± 0.104
<i>Δchi18B</i> ^d	0.0134 ± 0.004	25	0.514 ± 0.051
<i>Δchi18C</i> ^d	0.011 ± 0.002	25	0.612 ± 0.072
<i>Δchi18D</i>	Not determined	Not determined	0.014 ± 0.008
<i>Δchi18A Δchi18B</i> ^b	0.012 ± 0.002	25	0.539 ± 0.153
<i>Δchi18A Δchi18C</i> ^b	0.023 ± 0.018	25	0.732 ± 0.182
<i>Δchi18B Δchi18C</i> ^b	0.013 ± 0.011	25	0.342 ± 0.101
<i>Δchi18A Δchi18B</i> <i>Δchi18C</i> ^d	0.025 ± 0.008	25	0.485 ± 0.236

133

^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

134 **Table S2. Quantification of the zone of clearance generated by various *C.***
 135 ***japonicus* strains grown on colloidal chitin plates^a**
 136

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

137

^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	<i>lpmo10A</i>	6.7	5.6	Lytic polyssaccharide mono-oxygenase	CJA_2191
Chitin	<i>chi18D</i>	6.7	6.3	Chitinase	CJA_2611
Chitin	<i>chi18C</i>	5.1	4.6	Chitinase	CJA_2993
Chitin	<i>hex20B</i>	4.1	4.6	Hexosaminidase	CJA_0287
Chitin	<i>chi18A</i>	4.0	3.4	Chitinase	CJA_1182
Chitin	<i>chi18B</i>	3.6	2.5	Chitinase	CJA_0988
Chitin	<i>nag9A</i>	3.5	3.4	Deacetylase	CJA_1163
Arabinan	<i>abf43L</i>	3.4	6.0	α -Arabinofuranidase	CJA_0806
Starch	<i>amy13A</i>	3.3	2.5	α -Amylase	CJA_2618
Arabinan	<i>abf43M</i>	3.0	3.2	α -Arabinofuranidase	CJA_0819
Transglycosylase	<i>lmt23D</i>	2.9	5.2	Transglycosylase	CJA_2884
Pectin	<i>bg12C</i>	2.9	4.5	β -Galactosidase	CJA_2610
Arabinan	<i>gly43D</i>	2.8	2.1	α -Arabinofuranidase	CJA_0818
Arabinan	<i>gly43C</i>	2.7	3.6	α -Arabinofuranidase	CJA_0816
Cellulose	<i>cbp2E</i>	2.7	2.8	Predicted redox	CJA_2615
Chitin	<i>hex20A</i>	2.7	4.1	Hexosaminidase	CJA_0350
Carbohydrate binding protein	<i>cbp6B</i>	2.7	2.8	Carbohydrate binding protein	CJA_0276
Xylan	<i>abf62A</i>	2.6	3.2	α -Arabinofuranidase	CJA_3281
Chitin	<i>csn46F</i>	2.6	2.	Chitosanase	CJA_2611
Pectin	<i>pga28A</i>	2.6	3.7	Polygalacturonase	CJA_0172
Cellulose	<i>cbp2D</i>	2.6	2.2	Predicted redox	CJA_2616
β -Glucan	<i>ebg98</i>	2.6	3.5	Endogalactosidase	CJA_3286
Arabinan	<i>arb43A</i>	2.5	2.5	α -Arabinofuranidase	CJA_0805
Glycosyl transferase	<i>gt5B</i>	2.3	2.1	Glycosyl transferase	CJA_3255
Starch	<i>pul13B</i>	2.3	3.2	Pullanase	CJA_3161
Xylan	<i>xyn11B</i>	2.3	2.6	Endoxylanase	CJA_3762
Chitin	<i>chi19A</i>	2.2	2.0	Chitinase	CJA_0996
Starch	<i>agd31A</i>	2.2	3.1	α -Glucosidase	CJA_3248
Xyloglucan	<i>gly74A</i>	2.1	4.1	Endoxyloglucanase	CJA_2477
Carbohydrate binding protein	<i>cbp6A</i>	2.0	2.3	Carbohydrate binding protein	CJA_1191
Starch	<i>gla15</i>	2.0	4.2	α -Glucosidase	CJA_0731
Starch	<i>cbp26A</i>	2.0	4.3	Carbohydrate binding protein	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	<i>abf51A</i>	1.9	4.7	α -Arabinofuranidase	CJA_2769
Xylan	<i>xyn10C</i>	1.9	2.0	Endoxylanase	CJA_3066
Xylan	<i>gla67A</i>	1.9	3.9	α -Glucuronidase	CJA_2887
Arabinan	<i>gly43E</i>	1.8	2.0	α -Arabinofuranidase	CJA_0799
Xylan	<i>xyn10A</i>	1.8	2.0	Endoxylanase	CJA_2471
Mannan	<i>man26A</i>	1.7	3.8	Endomannanase	CJA_2770
Xylan	<i>xyn5A</i>	1.7	2.5	Endoxylanase	CJA_3279
Starch	<i>glu13A</i>	1.7	2.2	α -Glucosidase	CJA_0732
Starch	<i>mal77Q</i>	1.7	6.0	Amylomaltase	CJA_1882
Mannan	<i>aga27A</i>	1.6	2.0	α -Galactosidase	CJA_0246
Starch	<i>amy13J</i>	1.6	3.0	α -Amylase	CJA_0398
Cellulose	<i>LPMO10B</i>	1.6	2.5	Lytic polyssaccharide mono-oxygenase	CJA_3139
Xylan	<i>xyn11A</i>	1.6	2.7	Endoxylanase	CJA_3763
Miscellaneous	<i>gly57A</i>	1.6	3.2	Glycoside hydrolase	CJA_1883
Mannan	<i>man5B</i>	1.6	2.1	Endomannanase	CJA_2480
Polysaccharide deacetylase	<i>pda4C</i>	1.6	2.7	Deacetylase	CJA_3428
Cellulose	<i>cel3B</i>	1.6	2.1	β -Glucosidase	CJA_1497
Xylan	<i>xyn10B</i>	1.6	3.3	Endoxylanase	CJA_3280
Pectin	<i>bgl2A</i>	1.6	3.1	β -Galactosidase	CJA_0496
Glycosyl transferases	<i>gt5A</i>	1.6	2.4	Glycosyl transferase	CJA_1886
Pectin	<i>gal53A-2</i>	1.6	3.2	Endogalactosidase	CJA_0491
Starch	<i>pul13A</i>	1.5	2.0	Pullanase	CJA_2160
Glycosyl transferases	<i>gt1B</i>	1.5	2.1	Rhamnosyltransferase	CJA_0772
Glycosyl transferases	<i>gt4A</i>	1.5	2.5	Glycosyl transferase	CJA_3411
Pectin	<i>pel1G</i>	1.5	2.3	Pectate lyase	CJA_3120
Starch	<i>amy13B</i>	1.5	2.7	α -Amylase	CJA_1522
Pectin	<i>pel10B</i>	1.5	3.8	Pectate lyase	CJA_2040
Pectin	<i>pel3B</i>	1.5	2.7	Pectate lyase	CJA_2413
Glycosyl transferases	<i>gt4B</i>	1.5	2.1	Glycosyl transferase	CJA_3410
Polysaccharide deacetylase	<i>pda4E</i>	1.4	2.9	Deacetylase	CJA_3408
Xylan	<i>cbp35A</i>	1.4	3.0	Carbohydrate binding protein	CJA_0020
Starch	<i>glc13A</i>	1.3	3.1	α -glucosidase	CJA_0257
Pectin	<i>bgl35A</i>	1.3	2.0	β -Galactosidase	CJA_2707
Cellulose	<i>cbp2A</i>	1.3	2.7	Carbohydrate binding protein	CJA_0007
Starch	<i>gbe13A</i>	1.3	2.3	Transglycosylase	CJA_1885
Cellulose	<i>cel45A</i>	1.2	2.2	Cellulase	CJA_0374
Cellulose	<i>cel6A</i>	1.2	2.8	Cellobiohydrolase	CJA_2473
Starch	<i>amy13F</i>	1.2	3.0	α -Amylase	CJA_0398
Transglycosylase	<i>lmt23B</i>	1.1	2.3	Transglycosylase	CJA_2053
Cellulose	<i>cel5D</i>	1.0	3.1	Cellulase	CJA_3010
Starch	<i>amy13D</i>	1.0	3.3	α -Amylase	CJA_0737

141 **Table S3B. Up-regulated putative CAZyme-encoding genes during early**
 142 **stationary growth on α -chitin compared to glucose^a.**
 143

Substrate	CAZy name ^b	Fold change ^c	p-value ^d	Putative activity	Locus ID ^e
Chitin	<i>lpmo10A</i>	10.6	4.3	Lytic polysaccharide mono-oxygenase	CJA_2191
Chitin	<i>chi18D</i>	5.8	3.0	Chitinase	CJA_2611
Chitin	<i>chi18C</i>	4.8	2.5	Chitinase	CJA_2993
Arabinan	<i>abf43L</i>	4.5	2.5	α -Arabinofuranidase	CJA_0806
Chitin	<i>chi18B</i>	3.7	3.8	Chitinase	CJA_0988
Pectin	<i>pel3B</i>	3.6	2.6	Pectate lyase	CJA_2413
Starch	<i>amy13A</i>	3.3	2.6	α -Amylase	CJA_2618
Xylan	<i>axe2C</i>	3.0	2.4	Acetylxytan esterase	CJA_0450
Arabinan	<i>gly43C</i>	2.8	3.1	α -Arabinofuranidase	CJA_0816
β -Glucans	<i>ebg98</i>	2.8	4.0	Endogalactosidase	CJA_3286
Arabinan	<i>gly43D</i>	2.7	2.5	α -Arabinofuranidase	CJA_0818
Glycosyl Transferase	<i>gt9B</i>	2.7	2.4	Glycosyl Transferase	CJA_1369
Chitin	<i>nag9A</i>	2.6	3.1	Deacetylase	CJA_1163
Pectin	<i>bgl2C</i>	2.6	2.3	β -Galactosidase	CJA_2610
Glycosyl Transferase	<i>gt5B</i>	2.5	2.2	Glycosyl Transferase	CJA_3255
Starch	<i>amy13H</i>	2.5	3.2	α -Amylase	CJA_3247
Starch	<i>cgt13B</i>	2.5	3.4	Glucanotransferase	CJA_3263
Transglycosylase	<i>lmt23D</i>	2.5	3.0	Transglycosylase	CJA_2884
Pectin	<i>pme8C</i>	2.4	2.1	Pectinesterase	CJA_0181
Starch	<i>agd31A</i>	2.4	3.3	α -Glucosidase	CJA_3248
Xylan	<i>cpb35A</i>	2.3	2.2	Carbohydrate binding protein	CJA_0020
β -Glucan	<i>cgs94A</i>	2.3	2.4	Glucan synthetase	CJA_0849
Pectin	<i>pme8A</i>	2.3	2.6	Pectin methylesterase	CJA_0041
β -Glucan	<i>glu16A</i>	2.3	2.2	β -Glucanase	CJA_0225
Arabinan	<i>gly43G</i>	2.2	2.2	α -Arabinofuranidase	CJA_3070
Arabinan	<i>abf43M</i>	2.2	2.4	α -Arabinofuranidase	CJA_0819
Arabinan	<i>gly43J</i>	2.2	2.0	α -Arabinofuranidase	CJA_3067
Mannan	<i>man5C</i>	2.1	3.5	Endomannanase	CJA_3470
Chitin	<i>hex20A</i>	2.1	2.8	Hexosaminidase	CJA_0350
Chitin	<i>chi19A</i>	2.0	2.7	Chitinase	CJA_0996
Arabinan	<i>afc95A</i>	2.0	2.0	α -fucosidase	CJA_2710
Arabinan	<i>abf51A</i>	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	<i>pel10B</i>	2.0	2.2	Pectate lyase	CJA_2040
Pectin	<i>pel1D</i>	2.0	2.3	Pectate lyase	CJA_2040
Polysaccharide deacetylase	<i>pda4C</i>	1.9	4.1	Deacetylase	CJA_3428
Cellulose	<i>cel5D</i>	1.9	2.7	Cellulase	CJA_3010
Xylan	<i>abf62A</i>	1.9	2.5	α -Arabinofuranidase	CJA_3281
Cellulose	<i>cbp2D</i>	1.9	3.4	Predicted redox	CJA_2616
Xylan	<i>gla67A</i>	1.9	2.8	α -Glucuronidase	CJA_2887
Xylan	<i>cbp35B</i>	1.8	2.7	Carbohydrate biding protein	CJA_0559
Pectin	<i>bgl35A</i>	1.7	2.2	β -Galactosidase	CJA_2707
Cellulose	<i>cel6A</i>	1.6	2.7	Cellulase	CJA_2473
Starch	<i>amy13B</i>	1.5	3.3	α -Amylase	CJA_1522
Mannan	<i>man5D</i>	1.4	2.2	Endomannanase	CJA_0244
Mannan	<i>man5B</i>	1.3	2.5	Endomannanase	CJA_2475
Carbohydrate Biding Protein	<i>cbp35C</i>	1.1	2.3	Carbohydrate binding protein	CJA_0494
Cellulose	<i>cel3B</i>	1.0	2.7	β -Glucosidase	CJA_1497

145 **Table S4. Product ratios after degradation of α -chitin and (GlcNAc)₆**
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Chitinase	Chitin degradation	(GlcNAc) ₆ degradation
	(GlcNAc) ₂ /GlcNAc ratio, 12 h	(GlcNAc) ₂ /(GlcNAc) ₄ ratio, 2 min
CjChi18A _{cat}	0.2 ± 0.002	1.8 ± 0.048
CjChi18B _{cat}	7.0 ± 0.409	13.4 ± 0.692
CjChi18C _{cat}	7.3 ± 0.148	1.5 ± 0.067
CjChi18D _{cat}	2.8 ± 0.031	1.1 ± 0.048
CjChi18B _{cat+CBM5}	19.1 ± 0.034	Not determined
CjChi18D _{cat+CBM5}	3.4 ± 0.109	Not determined

147

148 **Table S5. Strains, plasmids and primers used in this study**
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Strains, plasmid or primer	Genotype	Source or Reference
Strains		
<i>E. coli</i> DH5 α	λ - Φ 80dlacZ Δ M15 Δ (lacZYA-argF)U169 recA1 endA1 hsdR17(rk-mk) supE44 thi-1gyrA relA1	Laboratory collection
<i>E. coli</i> S17 λ pir	Tpr Smr recA thi pro hsdR hsdM+ RP4-2-TC::Mu::Km Tn7 λ pir	Laboratory collection
<i>C. japonicus</i> Ueda 107	Wild Type	Laboratory collection
<i>C. japonicus</i> Δ gsp	Ueda 107 Δ gsp	(8)
<i>C. japonicus</i> Δ chi18A	Ueda 107 Δ chi18A ^a	This study
<i>C. japonicus</i> Δ chi18B	Ueda 107 Δ chi18B ^b	This study
<i>C. japonicus</i> Δ chi18C	Ueda 107 Δ chi18C ^c	This study
<i>C. japonicus</i> Δ chi18D	Ueda 107 Δ chi18D ^d	This study
<i>C. japonicus</i> Δ chi18A Δ chi18B	Ueda 107 Δ chi18A Δ chi18B	This study
<i>C. japonicus</i> Δ chi18A Δ chi18C	Ueda 107 Δ chi18A Δ chi18C	This study
<i>C. japonicus</i> Δ chi18B Δ chi18C	Ueda 107 Δ chi18B Δ chi18C	This study
<i>C. japonicus</i> 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 <i>chi18A</i> cloned into pK18mobsacB; Km ^r	This study
pK18/ Δ chi18B	Contains 500bp upstream and downstream of <i>chi18B</i> cloned into pK18mobsacB; Km ^r	This study
pK18/ Δ chi18C	Contains 500bp upstream and downstream of <i>chi18C</i> cloned into pK18mobsacB; Km ^r	This study
pK18/ Δ chi18D	Contains 1000bp upstream and downstream of <i>chi18D</i> cloned into pK18mobsacB; Km ^r	This study
Primers		
(5') to amplify 750 bp upstream of <i>chi18D</i>	GCTATGACATGATTACGGGTGGTTATACGCGTAATA ACCTTC	This study
(3') to amplify 750 bp upstream of <i>chi18D</i>	GAATTAGCGTTTCATAGTGTTTTCTCAACGTTTTTA TATAAATACG	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 <i>chi18D</i>	CACTATGAAACGCTAATTCATGATTACCGGAAGC	This study
(3') to amplify 750 bp downstream of <i>chi18D</i>	GCCTGCAGGTCGACTGGTGATATCGATATAGCTGG CGTTG	This study
Δ <i>chi18A</i> _CONF_(5')	ATCATGGGCAGCTTTC	This study
Δ <i>chi18A</i> _CONF_(3')	AGCAGGAGCCTGGTA	This study
Δ <i>chi18B</i> _CONF_(5')	CAATTGGAAATTGGTAATC	This study
Δ <i>chi18B</i> _CONF_(3')	ATATAGTCACGCCCTATTTTG	This study
Δ <i>chi18C</i> _CONF_(5')	AAGGGCATCTGGTTATT	This study
Δ <i>chi18C</i> _CONF_(3')	GTATTTCTATCTGCGTTCAC	This study
Δ <i>chi18D</i> _CONF_(5')	CTGATTGTCCCCTATCTGC	This study
Δ <i>chi18D</i> _CONF_(3')	ATTTCCCAGCGATTGTTAC	This study
<i>chi18A</i> INT_(5')	GGTGGTTCTAGAGCTTGTATCAGTGCG	This study
<i>chi18A</i> INT_(3')	GGTGGTGAATTCGAAGCATCCTTCACATC	This study
<i>chi18B</i> INT_(5')	GGTGGTGAATTCGCTATGTGGCGTTGA	This study
<i>chi18B</i> INT_(3')	GGTGGTTCTAGACTATGTCGTGCCAAATA	This study
<i>chi18C</i> INT_(5')	GGTGGTAAGCTTAGTTTGGGACAACCTG	This study
<i>chi18C</i> INT_(3')	GGTGGTTCTAGATGGAGTTATTCAGCG	This study
<i>chi18D</i> INT_(5')	GGTGGTAAGCTTCGACATCCTCTGTTG	This study
<i>chi18D</i> INT_(3')	GGTGGTTCTAGAATAGGCATCACCAATA	This study

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

153

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

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