Systems analysis in *Cellvibrio japonicus* resolves predicted redundancy of β-glucosidases and determines essential physiological functions

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Running Title
Functional analysis of *C. japonicus* β-glucosidases

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Table S1. Growth statistics of *C. japonicus* GH3 mutants grown in a defined cellobiose medium[a]

Strain	Growth Rate (gen hr^{-1})	Lag Time (hrs)	Max OD_{600}
Wild Type[b]	0.24±0.03	3	1.08±0.039
Δ*cel3A*[c]	0.27±0.01	3	1.06±0.016
Δ*cel3B*[d]	0.15±0.01	4	0.99±0.002
Δ*cel3C*[b]	0.21±0.02	3	1.07±0.023
Δ*cel3D*[b]	0.23±0.02	3	1.06±0.011
Wild Type[e]	0.35±0.01	5	1.02±0.015
Δ*cel3A* Δ*cel3B*[f]	0.09±0.01	10	0.82±0.005
Δ*cel3A* Δ*cel3C*[g]	0.30±0.01	5	0.99±0.005
Δ*cel3A* Δ*cel3D*[g]	0.29±0.01	5	0.98±0.019
Δ*cel3B* Δ*cel3C*[h]	0.22±0.01	7	1.05±0.005
Δ*cel3B* Δ*cel3D*[h]	0.17±0.02	7	1.05±0.024
Δ*cel3C* Δ*cel3D*[g]	0.28±0.03	5	1.05±0.012
Wild Type[i]	0.36±0.01	2	1.05±0.002
Δ*cel3A* Δ*cel3B* Δ*cel3C*[j]	0.24±0.03	13	0.55±0.050
Δ*cel3A* Δ*cel3B* Δ*cel3D*[k]	0.19±0.03	10	0.85±0.036
Δ*cel3A* Δ*cel3C* Δ*cel3D*[l]	0.22±0.01	2	1.03±0.020
Δ*cel3B* Δ*cel3C* Δ*cel3D*[m]	0.16±0.02	5	0.97±0.005
Δ4βG[n]	0.22±0.01	13	0.52±0.028

[a] Experiments were performed in biological triplicate; average and standard deviation reported in Table
[b] Time points used to calculate growth rate were T_i=4 and T_f=12
[c] Time points used to calculate growth rate were T_i=4 and T_f=8
[d] Time points used to calculate growth rate were T_i=10 and T_f=14
[e] Time points used to calculate growth rate were T_i=6 and T_f=9
[f] Time points used to calculate growth rate were T_i=12 and T_f=19
[g] Time points used to calculate growth rate were T_i=6 and T_f=9
[h] Time points used to calculate growth rate were T_i=7 and T_f=11
[i] Time points used to calculate growth rate were T_i=5 and T_f=9
[j] Time points used to calculate growth rate were T_i=18 and T_f=22
[k] Time points used to calculate growth rate were T_i=15 and T_f=20
[l] Time points used to calculate growth rate were T_i=5 and T_f=9
[m] Time points used to calculate growth rate were T_i=8 and T_f=15
[n] Time points used to calculate growth rate were T_i=18 and T_f=23

Table S2. Growth statistics of *C. japonicus* GH3 mutants grown in a defined insoluble cellulose medium[a]

Strain	Growth Rate (gen hr^{-1})	Lag Time (hrs)	Max OD_{600}
Wild Type[b]	0.16±0.01	24	0.50±0.070
Δ*cel3A*[c]	0.17±0.02	26	0.51±0.042
Δ*cel3B*[d]	0.08±0.02	38	0.23±0.026
Δ*cel3C*[e]	0.16±0.02	26	0.49±0.040
Δ*cel3D*[d]	0.16±0.02	26	0.48±0.024
Wild Type[f]	0.18±0.001	20	0.52±0.059
Δ*cel3A* Δ*cel3B*[g]	0.16±0.04	52	0.52±0.039
Δ*cel3A* Δ*cel3Cf*	0.16±0.02	20	0.50±0.073
Δ*cel3A* Δ*cel3D*[f]	0.18±0.18	20	0.50±0.073
Δ*cel3B* Δ*cel3C*[h]	0.06±0.004	48	0.22±0.036
Δ*cel3B* Δ*cel3D*[h]	0.05±0.007	48	0.20±0.007
Δ*cel3C* Δ*cel3D*[f]	0.22±0.03	20	0.48±0.019
Wild Type[b]	0.17±0.01	24	0.49±0.057
Δ*cel3A* Δ*cel3B* Δ*cel3C*[i]	0.05±0.01	52	0.12±0.025
Δ*cel3A* Δ*cel3B* Δ*cel3D*[j]	0.04±0.002	52	0.13±0.010
Δ*cel3A* Δ*cel3C* Δ*cel3D*[k]	0.15±0.05	28	0.48±0.020
Δ*cel3B* Δ*cel3C* Δ*cel3D*[l]	0.09±0.01	32	0.19±0.027
Δ4βG[m]	0.06±0.01	52	0.14±0.034

[a] Experiments were performed in biological triplicate; average and standard deviation reported in Table
[b] Time points used to calculate growth rate were T_i=24 and T_f=38
[c] Time points used to calculate growth rate were T_i=26 and T_f=34
[d] Time points used to calculate growth rate were T_i=38 and T_f=48
[e] Time points used to calculate growth rate were T_i=26 and T_f=36
[f] Time points used to calculate growth rate were T_i=20 and T_f=32
[g] Time points used to calculate growth rate were T_i=52 and T_f=64
[h] Time points used to calculate growth rate were T_i=48 and T_f=72
[i] Time points used to calculate growth rate were T_i=58 and T_f=88
[j] Time points used to calculate growth rate were T_i=64 and T_f=78
[k] Time points used to calculate growth rate were T_i=30 and T_f=38
[l] Time points used to calculate growth rate were T_i=38 and T_f=56
[m] Time points used to calculate growth rate were T_i=50 and T_f=80

Table S3. Genes up-regulated during exponential growth on cellobiose (compared to glucose) and growth statistics of the corresponding mutants when grown in a defined cellobiose medium[a]

Gene	Predicted Function[b]	Fold Change[c]	Growth Rate (gen hr^{-1})[d]	Maximum OD_{600}[e]
adg97B	α-glucosidase	1.5	0.23±0.01[k]	1.05±0.016
aga27A	α-glucosidase[f]	2.5	0.24±0.01[k]	1.07±0.019
amy13D	α-amylase	1.2	0.24±0.004[l]	1.07±0.012
axe2A	acetyl xylan esterase	3.3	0.21±0.019[k]	1.09±0.004
bgl35A	β-galactosidase[g]	2.0	0.20±0.003[k]	1.06±0.004
cel3A	β-glucosidase[h]	1.5	0.27±0.01[m]	1.04±0.007
cel5C	cellulase	2.3	0.21±0.02[n]	1.08±0.013
cel9B	cellulase	1.3	0.25±0.01[l]	1.08±0.010
man26C	endo-1, 4-β mannanase[i]	1.3	0.23±0.02[o]	1.07±0.025
xyl31A	α-xylosidase[j]	2.3	0.23±0.003[k]	1.08±0.010

[a] RNAseq sampling experiments were performed in biological triplicate. Growth experiments were also performed in biological triplicate; average and standard deviation reported in Table
[b] predicted by Deboy *et. al.* (DeBoy *et al.*, 2008)
[c] $\log_2$ scale in comparison to gene expression when grown in glucose
[d] compare to the growth rate of wild type of 0.203 calculated from T_i=4 and T_f=12
[e] compare to the maximum OD_{600} of 1.06 for wild type
[f] confirmed by Halsted *et al.* (Halstead *et al.*, 2000)
[g] confirmed by Larsbrink *et al.* (Larsbrink *et al., 2014*)
[h] confirmed by Rixon *et al.* 1992
[i] confirmed by Cartmel *et al.* (Cartmell *et al.*, 2008)
[j] confirmed by Larsbrink *et al.* (Larsbrink *et al., 2011*)
[k] calculated from T_i=4 and T_f=12
[l] calculated from T_i=4 and T_f=11
[m] calculated from T_i=4 and T_f=8
[n] calculated from T_i=4 and T_f=13
[o] calculated from T_i=4 and T_f=10

Table S4. Strains, plasmids, and primers used in this study

Strain, plasmid, or primer	Genotype	Source or Reference
Strains		
E. coli DH5α	λ^-Φ80d*lacZ*ΔM15 Δ(*lacZYA-argF*)*U169 recA1 endA1 hsdR17*($r_k^-m_k^-$) *supE44 thi-1 gyrA relA1*	Laboratory collection
E. coli S17 λ_{pir}	Tpr Smr *recA thi pro hsdR hsdM*$^+$ RP4-2-TC::Mu::Km Tn7 λ_{pri}	Laboratory collection
E. coli K12		Laboratory collection
E. coli strain BL21(DE3)	F^- *ompT gal dcm lon hsdS*$_B$($r_B^-m_B^-$) λ(DE3 [*lacI lacUV5-T7p07 ind1 sam7 nin5*]) [*malB*$^+$]$_{K\text{-}12}$(λ^S)	Merck
E. coli K12 pBBRMCS-5	Gm^r	This study
E. coli K12 pBBRMCS-5/*cel3A*	*cel3A*$^+$;Gm^r	This study
E. coli K12 pBBRMCS-5/*cel3B*	*cel3B*$^+$;Gm^r	This study
E. coli K12 pBBRMCS-5/*cel3C*	*cel3C*$^+$;Gm^r	This study
E. coli K12 pBBRMCS-5/*cel3D*	*cel3D*$^+$;Gm^r	This study
C. japonicus Ueda 107	Wild Type	Laboratory collection
C. japonicus Δ*gsp*	Ueda 107 Δ*gsp*	(Gardner & Keating, 2010)
C. japonicus Δ*cel3A*	Ueda 107 Δ*cel3A*[a]	This study
C. japonicus Δ*cel3B*	Ueda 107 Δ*cel3B*[b]	This study
C. japonicus Δ*cel3C*	Ueda 107 Δ*cel3C*[c]	This study
C. japonicus Δ*cel3D*	Ueda 107 Δ*cel3D*[d]	This study
C. japonicus Δ*cel3A*Δ*cel3B*	Ueda 107 Δ*cel3A*Δ*cel3B*	This study
C. japonicus Δ*cel3A*Δ*cel3C*	Ueda 107 Δ*cel3A*Δ*cel3C*	This study
C. japonicus Δ*cel3A*Δ*cel3D*	Ueda 107 Δ*cel3A*Δ*cel3D*	This study
C. japonicus Δ*cel3B*Δ*cel3C*	Ueda 107 Δ*cel3B*Δ*cel3C*	This study
C. japonicus Δ*cel3B*Δ*cel3D*	Ueda 107 Δ*cel3B*Δ*cel3D*	This study
C. japonicus Δ*cel3C*Δ*cel3D*	Ueda 107 Δ*cel3C*Δ*cel3D*	This study
C. japonicus Δ*cel3A*Δ*cel3B*Δ*cel3C*	Ueda 107 Δ*cel3A*Δ*cel3B*Δ*cel3C*	This study
C. japonicus Δ*cel3A*Δ*cel3B*Δ*cel3D*	Ueda 107 Δ*cel3A*Δ*cel3B*Δ*cel3D*	This study
C. japonicus Δ*cel3A*Δ*cel3C*Δ*cel3D*	Ueda 107 Δ*cel3A*Δ*cel3C*Δ*cel3D*	This study
C. japonicus Δ*cel3B*Δ*cel3C*Δ*cel3D*	Ueda 107 Δ*cel3B*Δ*cel3C*Δ*cel3D*	This study
C. japonicus Δ4βG	Ueda 107 Δ*cel3A*Δ*cel3B*Δ*cel3C*Δ*cel3D*	This study
C. japonicus Δ*adg97B*	Ueda 107 Δ*adg97B*[e]	This study
C. japonicus aga27A::pK18*aga27A*KO	Ueda 107 *aga27A*::pK18*mobsacB*[f]	This study
C. japonicus Δ*amy13D*	Ueda 107 Δ*amy13D*[g]	This study
C. japonicus axe2A::pk18*axe2A*KO	Ueda 107 *axe2A*::pk18*mobsacB*[h]	This study
C. japonicus Δ*bgl35A*	Ueda 107 Δ*bgl35A*[i]	This study
C. japonicus cel5C::pk18*cel5C*KO	Ueda 107 *cel5C*::pk18*mobsacB*[j]	This study
C. japonicus cel9B::pk18*cel9B*KO	Ueda 107 *cel9B*::pk18*mobsacB*[k]	This study
C. japonicus Δ*man26C*	Ueda 107 Δ*man26C*[l]	This study
C. japonicus Δ*xyl31A*	Ueda 107 Δ*xyl31A*[m]	This study

Plasmids

pRk2013	ColE1 RK2-Mob$^+$ RK2-Tra$^+$; Km^r	(Figurski & Helinski, 1979)
pK18*mobsacB*	pMB1 *ori mob*$^+$ *sacB*$^+$; Km^r	(Schafer *et al.*, 1994)
pET28b	ColE1 T7promoter:*lacO*-MCS-T7terminator; Km^r	Merck
pK18/Δ*cel3A*	Contains 500bp upstream and downstream of *cel3A* cloned into pK18*mobsacB*; Km^r	This study
pK18/Δ*cel3B*	Contains 500bp upstream and downstream of *cel3B* cloned into pK18*mobsacB*; Km^r	This study
pK18/Δ*cel3C*	Contains 500bp upstream and downstream of *cel3C* cloned into pK18*mobsacB*; Km^r	This study
pK18/Δ*cel3D*	Contains 500bp upstream and downstream of *cel3D* cloned into pK18*mobsacB*; Km^r	This study
pK18/Δ*adg97B*	Contains 500bp upstream and downstream of *adg97B* cloned into pK18*mobsacB*; Km^r	This study
pK18/*aga27A*KO	Contains 500bp internal *aga27A* cloned into pK18*mobsacB*; Km^r	This study
pK18Δ*amy13D*	Contains 500bp upstream and downstream of *amy13D* cloned into pK18*mobsacB*; Km^r	This study
pk18/*axe2A*KO	Contains 500bp internal *axe2A* cloned into pk18*mobsacB*; Km^r	This study
pK18/Δ*bgl35A*	Contains 500bp upstream and downstream of *bgl35A* cloned into pK18*mobsacB*; Km^r	This study
pk18/*cel5C*KO	Contains 500bp internal *cel5C* cloned into pk18*mobsacB*; Km^r	This study
pk18/*cel9B*KO	Contains 500bp internal *cel9B* cloned into pk18*mobsacB*; Km^r	This study
pK18/Δ*man26C*	Contains 500bp upstream and downstream of *man26C* cloned into pK18*mobsacB*; Km^r	This study
pK18/Δ*xyl31A*	Contains 500bp upstream and downstream of *xyl31A* cloned into pK18*mobsacB*; Km^r	This study
pBBRMCS-5	Gm^r	(Elzer *et al.*, 1994)
p*cel3A*	Contains *cel3A* cloned into pBBRMCS-5; Gm^r	This study
p*cel3B*	Contains *cel3B* cloned into pBBRMCS-5; Gm^r	This study
p*cel3C*	Contains *cel3C* cloned into pBBRMCS-5; Gm^r	This study
p*cel3D*	Contains *cel3D* cloned into pBBRMCS-5; Gm^r	This study
pET28b:*cel3A*	Contains *cel3A* cloned into pET28b; Kan^r	This study
pET28b:*cel3B*	Contains *cel3B* cloned into pET28b; Kan^r	This study

Primers

Δ*cel3A* CONF (5')	GAT TTA CCA GGGTGTTC	This study
Δ*cel3A* CONF (3')	TTCGCTATTTGAAAGGTA	This study

cel3A INT (5')	GGTGGTGGATCCCTGCCGATACTGT	This study
cel3A INT (3')	GGTGGTTCTAGAGCCAATAAAGTGCT	This study
Δ*cel3B* CONF (5')	TGATCATAGTGGCCAT	This study
Δ*cel3B* CONF (3')	TACTCATAGCAGCCG	This study
cel3B INT (5')	GGTGGTGGATCCATGGATGAGAGCGA	This study
cel3B INT (3')	GGTGGTTCTAGACAGTCAACAAATAAC	This study
Δ*cel3C* CONF (5')	CAGTCGCCCTTATGT	This study
Δ*cel3C* CONF (3')	ATTCACAATTCCTGGTGT	This study
cel3C INT (5')	GGTGGTGAATTCCTGAAACATCCGGC	This study
cel3C INT (3')	GGTGGTTCTAGAGTTTAGCCGTGGCA	This study
Δ*cel3D* CONF (5')	TGGGTAATACATTGCTTG	This study
Δ*cel3D* CONF (3')	TGCCAGGTATGTGGA	This study
cel3D INT (5')	GGTGGTGAATTCGCTGACTGAGGTAT	This study
cel3D INT (3')	GGTGGTTCTAGACAACAACAGCAACA	This study
cel3A EXP (5')	GCGCGCCATATGTGTGATTCCCGCGCTCCC	This study
cel3A EXP (3')	GCGCGCGAGCTCGGGGCAGGCGACGTCTTT	This study
cel3B EXP (5')	GCGCGCCATATGCTGTGGCCAAAAGTCACC	This study
cel3B EXP (3')	GCGCGCGAGCTCACCAACAACACCAAATGT	This study
Δ*adg97B* CONF (5')	ATGATGGTGCGATTCG	This study
Δ*adg97B* CONF (3')	TAATCCTTGACCGCAT	This study
adg97B UP (5')	ACAGCTATGACATGATTACGTCCGAAGTTTTATTAAGCAGC	This study
adg97B UP (3')	ATTGGCAATTACATCATATTCCCCTGACATATCTCGGT	This study
adg97B DOWN (5')	GAATATGATGTAATTGCCAATACCCAAGCCGTTG	This study
adg97B DOWN (3')	TGCATGCCTGCAGGTCGACTCTGTAATCCTTGACCGCATAG	This study
adg97B INT (5')	TAT GCG TCT ATG ACA CTG	This study
adg97B INT (3')	TCA ATT CGC TTG GCT TTG	This study
aga27A KO (5')	GGTGGTGAATTCACATCCGGTATGAA	This study
aga27A KO (3')	GGTGGTTCTAGATGTTTGGTCGAGGATG	This study
aga27A CONF (5')	GGTGGTGAATTCGTACCCAACATCACC	This study
aga27A CONF (3')	GTGGTTCTAGATAAAAAAACGCCGC	This study
Δ*amy13D* CONF (5')	TCAAATGGCCGCTGA	This study
Δ*amy13D* CONF (3')	TGACAGTCAGGAATGC	This study
amy13D UP (5')	GCTATGACATGATTACGAATTCTTCAAATGGCCGCTGACCAA	This study
amy13D UP (3')	AGCCTTGACATCAAATCCCCCATTGTTGACCGTTTTG	This study
amy13D DOWN (5')	GATTTGATCTGAAGGCTTTGGTATTGATGACTGAAC	This study
amy13D DOWN (3')	GCCTGCAGGTCGACTCTAGACCCCTTTGACAGTCACGAATGC	This study
amy13D INT (5')	GGTGGTGGATCCGATCTTGCGACCGATC	This study
amy13D INT (3')	GGTGGTTCTAGACGGAAAGGATCAACAC	This study

axe2A KO (5')	GGTGGTAAGCTTTGGTCGGGATTGTTC	This study
axe2A KO (3')	GGTGGTTCTAGATGAAACCGCCTTTAA	This study
axe2A CONF (5')	GGTGGTAAGCTTATTGCGGATATGCAG	This study
axe2A CONF (3')	GGTGGTTCTAGACAAAAGGCCTGCAA	This study
Δ*bgl35A* CONF (5')	CGTAGCAAGTACCTGAT	This study
Δ*bgl35A* CONF (3')	GTACCACGGTTTTCCTC	This study
bgl35A INT (5')	GGTGGTTCTAGATTGCCACACCGATA	This study
bgl35A INT (3')	GGTGGTGGATCCCAATGACGTTCAAAT	This study
cel5C KO (5')	GGTGGTGAATTCAGCGTGGCGTGAA	This study
cel5C KO (3')	GGTGGTTCTAGAGATATGCGCCCCTT	This study
cel5C CONF (5')	GGTGGTGAATTCCAGCGGCTGTTATC	This study
cel5C CONF (3')	GGTGGTTCTAGAGTTCCAGGCAATGC	This study
cel9B KO (5')	GGTGGTGAATTCGCAGGTTGTGGCA	This study
cel9B KO (3')	GGTGGTTCTAGAGTTGTAGTCACCCG	This study
cel9B CONF (5')	GGTGGTGAATTCCGGGTCCGATAAG	This study
cel9B CONF (3')	GGTGGTTCTAGACATACCCTGCCGTA	This study
Δ*man26C* CONF (5')	GTGGCAAATACGCCCA	This study
Δ*man26C* CONF (3')	CAGCGACTTGTCCAG	This study
man26C INT (5')	GGTGGTTCTAGAACCAGTCGCCGTTAT	This study
man26C INT (3')	GGTGGTGGATCCCTCCCCTGATTGATAC	This study
Δ*xyl31A* CONF (5')	TGTAGCTGAGCCATTG	This study
Δ*xyl31A* CONF (3')	TACCAGCCCTACCTG	This study
xyl31A INT (5')	GGTGGTGAATTCTGAGCTTCCGTGATG	This study
xyl31A INT (3')	GGTGGTTCTAGAATTTCGGTGGTAGCT	This study
P#4 (GENERAL KO PRIMER) (3')	CAGGCGCTCGTAGAC	(Gardner & Keating, 2010)
P#5 (GENERAL KO PRIMER) (5')	GTGTGGAATTGTGAGCG	(Gardner & Keating, 2010)

[a] BioCyc accession number CJA_0204
[b] BioCyc accession number CJA_1497
[c] BioCyc accession number CJA_0223
[d] BioCyc accession number CJA_1140
[e] BioCyc accession number CJA_0736
[f] BioCyc accession number CJA_0246
[g] BioCyc accession number CJA_0737
[h] BioCyc accession number CJA_3103
[i] BioCyc accession number CJA_2707
[j] BioCyc accession number CJA_3369
[k] BioCyc accession number CJA_1633
[l] BioCyc accession number CJA_0236
[m] BioCyc accession number CJA_2706

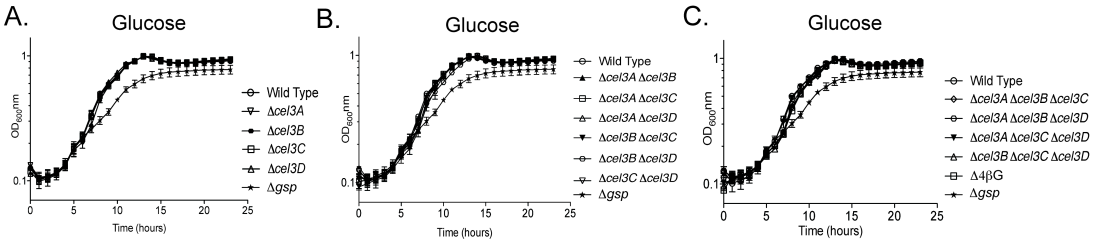

Fig. S1. All β-glucosidase mutants can grow as wild type on glucose. Single **(A),** double **(B),** triple, and quadruple **(C)** deletions were grown with (0.25%) glucose as the sole carbon source. Experiments were performed in biological triplicate and error bars represent standard deviation. These growth experiments were performed simultaneously, but are separated into multiple panels for clarity. As a consequence, the control strains (wild type and Δ*gsp*) are repeated in each panel.

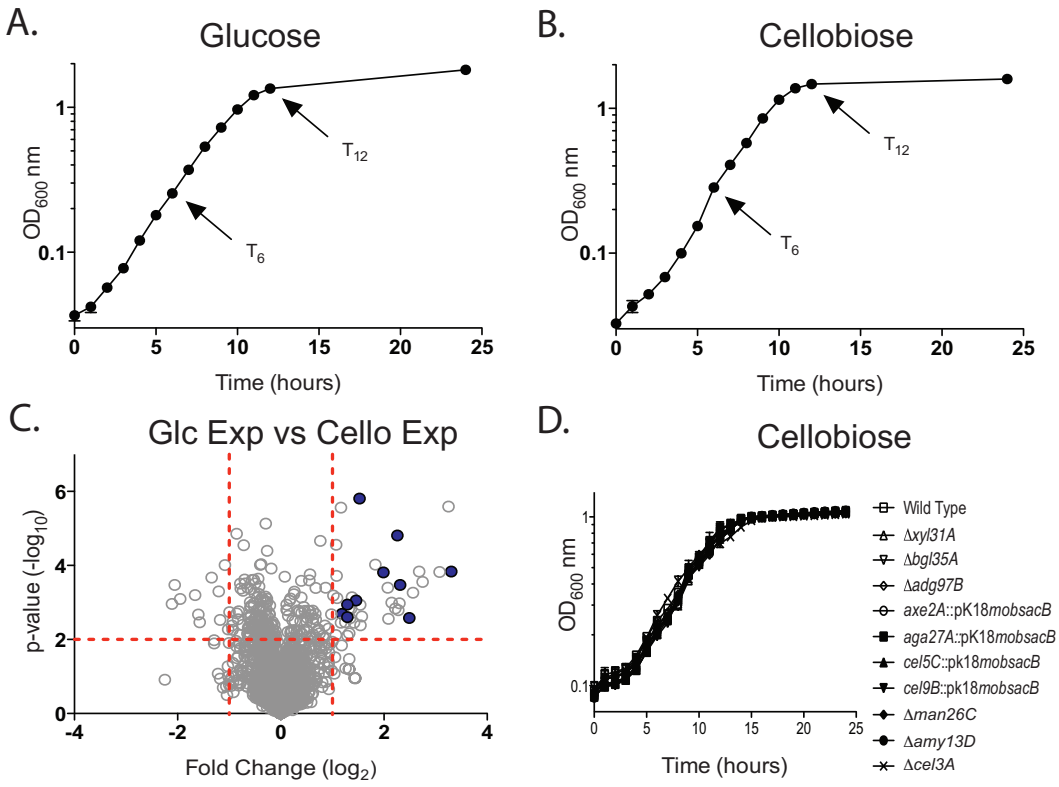

Fig. S2. *C. japonicus* CAZyme genes identified as up-regulated during exponential growth on cellobiose when compared to growth on glucose displayed no growth defects on cellobiose when deleted individually. Samples for RNAseq were taken during growth on 0.5% glucose **(A)** and 0.25% cellobiose **(B)** during exponential growth (Exp) and stationary phase (Sta) as indicated by the arrows. Genes up-regulated during exponential growth on cellobiose compared to exponential growth on glucose were identified using a volcano plot **(C)**. The fold change (log_2 scale) is plotted on the x-axis and the p-value ($-log_{10}$ scale) is plotted on the y-axis. The red dashed lines indicate the significance cut-off values. Each gray circle represents a gene, and the blue-filed circles represent an up-regulated CAZyme gene. Single mutants were made of each of the up-regulated CAZyme genes and growth analysis was performed on 0.5% cellobiose **(D)**. These growth experiments were performed in biological triplicate and error bars represent standard deviation, but in most cases are too small to be observed.

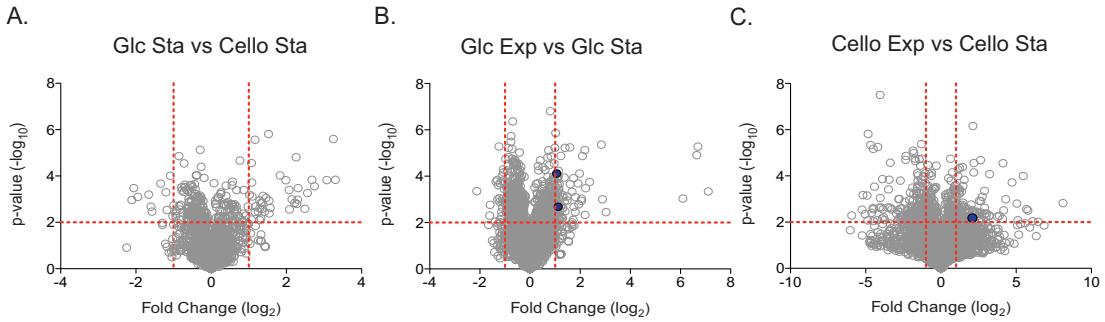

Fig. S3. Control RNAseq experiments identify CAZyme genes regulated by growth phase. RNAseq expression data from growth during stationary phase (Sta) on (0.5%) glucose was compared to stationary phase on (0.25%) cellobiose **(A)**. Expression data from exponential growth on (0.5%) glucose was compared to stationary phase on (0.5%) glucose. Both *amy13J* and *gly57A* genes were significantly up-regulated, and represented as blue closed circles **(B).** Expression data from exponential phase during growth on (0.25%) cellobiose was compared to stationary phase on (0.25%) cellobiose. The up-regulated CAZyme gene, as indicated by a blue circle, was *cel3B* **(C)**. The fold change (log_2 scale) is plotted on the x-axis and the p-value ($-log_{10}$ scale) is plotted on the y-axis. The red dashed lines indicate the significance cut-off values. Each gray circle represents a single *C. japonicus* gene.

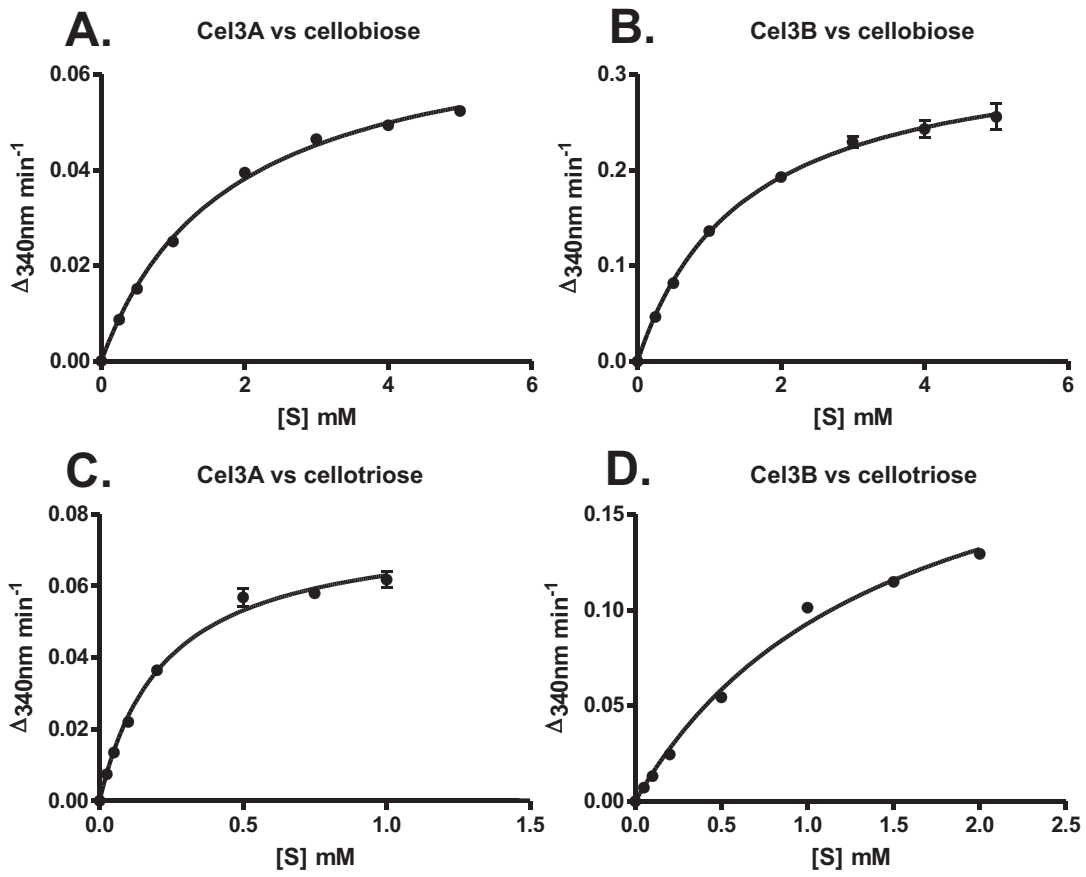

Fig. S4. Example of kinetic graphs for Cel3A and Cel3B. The enzymes were produced in recombinant form from *E. coli* and purified by electrophoretic homogeneity by IMAC. The assays were carried out using the Megazyme International Glucose Detection Kit. Assays were carried in 20 mM sodium phosphate pH 7.5 at 37 °C. The concentrations of enzyme used in the assays were as follows: Cel3A was at 50 nM and 10 nM against cellobiose and cellotriose, respectively; Cel3B was at 5 nM and 50 nM against cellobiose and cellotriose, respectively. The y-axes report the mM concentration of glucose produced per min. The assays used three technical replicates. Error bars represent the standard error of the mean, though in some cases are too small to be seen on the graph. Additional details on the kinetic experiments can be found in Table 2.

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