Comprehensive functional characterization of the Glycoside Hydrolase Family 3 enzymes from *Cellvibrio japonicus* reveals unique metabolic roles in biomass saccharification

Cassandra E. Nelson[a], Mohamed A. Attia[b,c], Artur Rogowski[d], Carl Morland[d], Harry Brumer[b,c,e,f], and Jeffrey G. Gardner[a#]

Running Title
Complex glucan utilization in *C. japonicus*

Keywords
β-glucosidase, *Cellvibrio japonicus*, lignocellulose, mixed-linkage beta-glucan, xyloglucan

Author Affiliations
[a] Department of Biological Sciences, University of Maryland – Baltimore County, Maryland, USA
[b] Michael Smith Laboratories, University of British Columbia, Vancouver , Canada
[c] Department of Chemistry, University of British Columbia, Vancouver, Canada.
[d] Institute for Cell and Molecular Biosciences, Newcastle University, Newcastle Upon Tyne, UK.
[e] Department of Biochemistry and Molecular Biology, University of British Columbia, Vancouver, Canada
[f] Department of Botany, University of British Columbia, Vancouver , Canada

[#]Correspondence
Jeffrey G. Gardner
Department of Biological Sciences
University of Maryland - Baltimore County
Email: jgardner@umbc.edu
Phone: 410-455-3613
Fax: 410-455-3875

Supplemental Tables

Table S1A. Growth statistics of *E. coli* GH3 heterologous expression strains grown in a defined glucose medium (corresponding to Figure 2A)[a]

Strain	Growth Rate (gen hr^{-1})	Lag Time (hrs)	Max OD_{600}
Empty Vector Control[bc]	0.24±0.001	5	1.03±0.01

[a] Experiments were performed in biological triplicate
[b] Time points used to calculate growth rate were T_i=6 and T_f=12
[c] All heterologous expression strains grew as the empty vector control

Table S1B. Growth statistics of *E. coli* GH3 heterologous expression strains grown in a defined sophorose medium (corresponding to Figure 2B)[a]

Strain	Growth Rate (gen hr^{-1})	Lag Time (hrs)	Max OD_{600}
Empty Vector Control	ND[b]	ND	0.13±0.003
K12pBBRMCS-5/*bgl3A*[c]	0.17±0.003	21	0.95±0.02
K12pBBRMCS-5/*bgl3B*	ND	ND	0.14±0.003
K12pBBRMCS-5/*bgl3C*[d]	0.13±0.02	33	0.80±0.02
K12pBBRMCS-5/*bgl3D*[e]	0.13±0.02	27	0.92±0.10

[a] Experiments were performed in biological triplicate
[b] Not Determined due to lack of growth
[c] Time points used to calculate growth rate were T_i=22 and T_f=28
[d] Time points used to calculate growth rate were T_i=30 and T_f=40
[e] Time points used to calculate growth rate were T_i=28 and T_f=34

Table S1C. Growth statistics of *E. coli* GH3 heterologous expression strains grown in a defined gentiobiose medium (corresponding to Figure 2C)[a]

Strain	Growth Rate (gen hr^{-1})	Lag Time (hrs)	Max OD_{600}
Empty Vector Control	ND[b]	ND	0.12±0.004
K12pBBRMCS-5/*bgl3A*[c]	0.06±0.002	10	0.70±0.01
K12pBBRMCS-5/*bgl3B*	ND	ND	0.14±0.004
K12pBBRMCS-5/*bgl3C*[d]	0.08±0.003	8	0.72±0.02
K12pBBRMCS-5/*bgl3D*[e]	0.07±0.0.001	8	0.77±0.01

[a] Experiments were performed in biological triplicate
[b] Not Determined due to lack of growth
[c] Time points used to calculate growth rate were T_i=22 and T_f=28
[d] Time points used to calculate growth rate were T_i=30 and T_f=40
[e] Time points used to calculate growth rate were T_i=28 and T_f=34

Table S1D. Growth statistics of *E. coli* GH3 heterologous expression strains grown in a defined laminaribiose medium (corresponding to Figure 2D)[a]

Strain	Growth Rate (gen hr^{-1})	Lag Time (hrs)	Max OD_{600}
Empty Vector Control[b]	0.07±0.002	22	0.61±0.002
K12pBBRMCS-5/*bgl3A*[c]	0.23±0.01	5	0.71±0.01
K12pBBRMCS-5/*bgl3B*[d]	0.09±0.0002	15	0.86±0.02
K12pBBRMCS-5/*bgl3C*[c]	0.12±0.01	5	0.68±0.06
K12pBBRMCS-5/*bgl3D*[c]	0.22±0.004	5	0.71±0.004

[a] Experiments were performed in biological triplicate
[b] Time points used to calculate growth rate were T_i=32 and T_f=44
[c] Time points used to calculate growth rate were T_i=6 and T_f=12
[d] Time points used to calculate growth rate were T_i=16 and T_f=22

Table S1E. Growth statistics of *C. japonicus* GH3 mutants grown in a defined sophorose medium(corresponding to Figure 3)[a]

Strain	Growth Rate (gen hr^{-1})	Lag Time (hrs)	Max OD_{600}
Wild Type[b]	0.29±0.01	4	1.24±0.16
Δ*bgl3A*[c]	0.19±0.005	8	1.05±0.003
Δ*bgl3B*[d]	0.24±0.004	5	1.13±0.003
Δ*bgl3C*[b]	0.28±0.003	4	1.11±0.01
Δ*bgl3D*[b]	0.30±0.004	4	1.13±0.003
Wild Type[e]	0.28±0.003	3	1.41±0.01
Δ*bgl3A* Δ*bgl3B*[f]	0.24±0.003	9	1.18±0.01
Δ*bgl3A* Δ*bgl3C*	ND[g]	ND	0.14±0.01
Δ*bgl3A* Δ*bgl3D*[h]	0.27±0.01	9	1.31±0.01
Δ*bgl3B* Δ*bgl3C*[b]	0.21±0.01	4	1.31±0.02
Δ*bgl3B* Δ*bgl3D*[i]	0.22±0.01	4	1.31±0.01
Δ*bgl3C* Δ*bgl3*[j]	0.26±0.003	3	1.34±0.01
Wild Type[k]	0.20±0.02	3	1.20±0.02
Δ*bgl3A* Δ*bgl3B* Δ*bgl3*C	ND	ND	0.13±0.01
Δ*bgl3A* Δ*bgl3B* Δ*bgl3D*	ND	ND	0.10±0.01
Δ*bgl3A* Δ*bgl3C* Δ*bgl3D*	ND	ND	0.10±0.01
Δ*bgl3B* Δ*bgl3C* Δ*bgl3D*[b]	0.15±0.02	4	1.19±0.02
Δ4βG	ND	ND	0.10±0.004

[a] Experiments were performed in biological triplicate
[b] Time points used to calculate growth rate were T_i=5 and T_f=10
[c] Time points used to calculate growth rate were T_i=9 and T_f=19
[d] Time points used to calculate growth rate were T_i=6 and T_f=14
[e] Time points used to calculate growth rate were T_i=4 and T_f=10

[f] Time points used to calculate growth rate were T_i=10 and T_f=17
[g] Not determined due to lack of growth
[h] Time points used to calculate growth rate were T_i=10 and T_f=14
[i] Time points used to calculate growth rate were T_i=5 and T_f=19
[j] Time points used to calculate growth rate were T_i=4 and T_f=9
[k] Time points used to calculate growth rate were T_i=4 and T_f=8

Table S1F. Growth statistics of *C. japonicus* GH3 mutants grown in a defined laminaribiose medium (corresponding to Figure 4A-C)[a]

Strain	Growth Rate (gen hr^{-1})	Lag Time (hrs)	Max OD_{600}
Wild Type[b]	0.29±0.01	3	1.14±0.02
Δ*bgl3A*[c]	0.21±0.002	5	1.05±0.01
Δ*bgl3B*[d]	0.40±0.02	5	1.14±0.01
Δ*bgl3C*[e]	0.34±0.01	3	1.19±0.03
Δ*bgl3D*[f]	0.30±0.02	3	1.08±0.01
Wild Type[g]	0.32±0.04	4	1.19±0.03
Δ*bgl3A* Δ*bgl3B*[h]	0.23±0.003	4	1.08±0.01
Δ*bgl3A* Δ*bgl3C*[i]	0.16±0.002	9	0.91±0.001
Δ*bgl3A* Δ*bgl3D*[j]	0.24±0.03	6	1.04±-.01
Δ*bgl3B* Δ*bgl3C*[k]	0.29±0.01	3	1.17±0.04
Δ*bgl3B* Δ*bgl3D*[k]	0.27±0.02	3	1.14±0.01
Δ*bgl3C* Δ*bgl3D*[l]	0.32±0.02	4	1.14±0.01
Wild Type[b]	0.29±0.01	3	1.14±0.02
Δ*bgl3A* Δ*bgl3B* Δ*bgl3*C[l]	0.09±0.01	10	0.54±0.06
Δ*bgl3A* Δ*bgl3B* Δ*bgl3D*[m]	0.20±0.01	6	1.05±0.04
Δ*bgl3A* Δ*bgl3C* Δ*bgl3D*[n]	0.09±0.01	11	0.32±0.02
Δ*bgl3B* Δ*bgl3C* Δ*bgl3D*[o]	0.33±0.11	5	1.05±0.23
Δ4βG	ND[p]	ND	0.10±0.001

[a] Experiments were performed in biological triplicate
[b] Time points used to calculate growth rate were T_i=4 and T_f=8
[c] Time points used to calculate growth rate were T_i=6 and T_f=12
[d] Time points used to calculate growth rate were T_i=6 and T_f=10
[e] Time points used to calculate growth rate were T_i=4 and T_f=7
[f] Time points used to calculate growth rate were T_i=4 and T_f=9
[g] Time points used to calculate growth rate were T_i=5 and T_f=10
[h] Time points used to calculate growth rate were T_i=5 and T_f=13
[i] Time points used to calculate growth rate were T_i=10 and T_f=17
[j] Time points used to calculate growth rate were T_i=7 and T_f=12
[k] Time points used to calculate growth rate were T_i=4 and T_f=11
[l] Time points used to calculate growth rate were T_i=11 and T_f=21
[m] Time points used to calculate growth rate were T_i=7 and T_f=16
[n] Time points used to calculate growth rate were T_i=12 and T_f=24
[o] Time points used to calculate growth rate were T_i=6 and T_f=9
[p] Not determined due to lack of growth

Table S1G. Growth statistics of *C. japonicus* GH3 mutants grown in a defined curdlan medium (corresponding to Figure 4D-F)[ab]

Strain	Growth Rate (gen hr^{-1})	Lag Time (hrs)	Max OD_{600}
Wild Type[c]	0.14±0.04	3	0.19±0.002
Δ*bgl3A*[d]	0.13±0.01	3	0.18±0.003
Δ*bgl3B*[e]	0.20±0.03	4	0.19±0.01
Δ*bgl3C*[f]	0.12±0.02	4	0.17±0.002
Δ*bgl3D*[f]	0.16±0.01	4	0.19±0.002
Wild Type[d]	0.10±0.005	3	0.17±0.001
Δ*bgl3A* Δ*bgl3B*[d]	0.10±0.01	3	0.17±0.003
Δ*bgl3A* Δ*bgl3C*[g]	0.06±0.01	7	0.18±0.02
Δ*bgl3A* Δ*bgl3D*[d]	0.11±0.01	3	0.18±0.001
Δ*bgl3B* Δ*bgl3C*[d]	0.11±0.01	3	0.16±0.01
Δ*bgl3B* Δ*bgl3D*[e]	0.17±0.03	4	0.18±0.01
Δ*bgl3C* Δ*bgl3D*[d]	0.10±0.01	3	0.16±0.004
Wild Type[c]	0.14±0.04	3	0.19±0.002
Δ*bgl3A* Δ*bgl3B* Δ*bgl3*C[h]	0.05±0.004	8	0.16±0.005
Δ*bgl3A* Δ*bgl3B* Δ*bgl3D*[e]	0.20±0.001	4	0.19±0.01
Δ*bgl3A* Δ*bgl3C* Δ*bgl3D*[i]	0.03±0.01	13	0.13±0.01
Δ*bgl3B* Δ*bgl3C* Δ*bgl3D*[f]	0.15±0.01	4	0.18±0.01
Δ4βG[j]	0.02±0.002	11	0.13±0.005

[a] Experiments were performed in biological triplicate
[b] Time points were taken every 15 minutes
[c] Time points used to calculate growth rate were T_i=4 and T_f=7
[d] Time points used to calculate growth rate were T_i=4 and T_f=6
[e] Time points used to calculate growth rate were T_i=5 and T_f=6
[f] Time points used to calculate growth rate were T_i=5 and T_f=7
[g] Time points used to calculate growth rate were T_i=8 and T_f=13
[h] Time points used to calculate growth rate were T_i=9 and T_f=13
[i] Time points used to calculate growth rate were T_i=14 and T_f=19
[j] Time points used to calculate growth rate were T_i=12 and T_f=22

Table S1H. Growth statistics of *C. japonicus* GH3 mutants grown in a defined mixed linkage glucan medium (corresponding to Figure 4G-I)[a]

Strain	Growth Rate (gen hr^{-1})	Lag Time (hrs)	Max OD_{600}
Wild Type[b]	0.31±0.03	6	1.00±0.05
Δ*bgl3A*[c]	0.21±0.02	4	1.01±0.04
Δ*bgl3B*[d]	0.33±0.01	5	1.04±0.06
Δ*bgl3C*[e]	0.25±0.02	5	0.99±0.08
Δ*bgl3D*[f]	0.26±0.04	4	0.97±0.07
Wild Type[g]	0.36±0.02	6	1.02±0.01
Δ*bgl3A* Δ*bgl3B*[h]	0.18±0.02	9	0.96±0.02
Δ*bgl3A* Δ*bgl3C*[i]	0.23±0.02	9	1.05±0.001
Δ*bgl3A* Δ*bgl3D*[j]	0.23±0.02	7	1.03±0.03
Δ*bgl3B* Δ*bgl3C*[d]	0.29±0.02	5	0.94±0.01
Δ*bgl3B* Δ*bgl3D*[k]	0.31±0.06	7	0.96.0.01
Δ*bgl3C* Δ*bgl3D*[c]	0.23±0.01	4	1.05±0.11
Wild Type[b]	0.31±0.03	6	1.00±0.05
Δ*bgl3A* Δ*bgl3B* Δ*bgl3*C[l]	0.17±0.02	14	0.58±0.04
Δ*bgl3A* Δ*bgl3B* Δ*bgl3D*[m]	0.20±0.05	11	1.01±0.03
Δ*bgl3A* Δ*bgl3C* Δ*bgl3D*[n]	0.21±0.01	8	0.92±0.03
Δ*bgl3B* Δ*bgl3C* Δ*bgl3D*[o]	0.39±0.08	5	1.03±0.04
Δ4βG[p]	0.10±0.02	15	0.34±0.02

[a] Experiments were performed in biological triplicate
[b] Time points used to calculate growth rate were T_i=7 and T_f=10
[c] Time points used to calculate growth rate were T_i=5 and T_f=13
[d] Time points used to calculate growth rate were T_i=6 and T_f=11
[e] Time points used to calculate growth rate were T_i=6 and T_f=12
[f] Time points used to calculate growth rate were T_i=5 and T_f=9
[g] Time points used to calculate growth rate were T_i=7 and T_f=11
[h] Time points used to calculate growth rate were T_i=10 and T_f=15
[i] Time points used to calculate growth rate were T_i=10 and T_f=14
[j] Time points used to calculate growth rate were T_i=8 and T_f=13
[k] Time points used to calculate growth rate were T_i=6 and T_f=11
[l] Time points used to calculate growth rate were T_i=8 and T_f=11
[m] Time points used to calculate growth rate were T_i=15 and T_f=20
[n] Time points used to calculate growth rate were T_i=12 and T_f=16
[o] Time points used to calculate growth rate were T_i=9 and T_f=12
[p] Time points used to calculate growth rate were T_i=6 and T_f=10

Table S1I. Growth statistics of *C. japonicus* GH3 mutants grown in a defined xyloglucan medium (corresponding to Figure 5A-C)[a]

Strain	Growth Rate (gen hr^{-1})	Lag Time (hrs)	Max OD_{600}
Wild Type[b]	0.30±0.04	3	1.03±0.15
Δ*bgl3A*[b]	0.27±0.05	3	1.01±0.14
Δ*bgl3B*[c]	0.34±0.02	3	1.00±0.08
Δ*bgl3C*[d]	0.36±0.01	3	1.26±0.07
Δ*bgl3D*[e]	0.17±0.003	3	1.12±0.03
Wild Type[d]	0.36±0.01	3	0.89±0.04
Δ*bgl3A* Δ*bgl3B*[b]	0.27±0.02	3	0.91±0.003
Δ*bgl3A* Δ*bgl3C*[d]	0.35±0.02	3	0.91±0.01
Δ*bgl3A* Δ*bgl3D*[f]	0.12±0.02	5	0.98±0.07
Δ*bgl3B* Δ*bgl3C*[b]	0.29±0.03	3	0.5±0.03
Δ*bgl3B* Δ*bgl3D*[g]	0.16±0.02	2	0.83±0.03
Δ*bgl3C* Δ*bgl3D*[h]	0.13±0.03	6	0.98±0.06
Wild Type[i]	0.26±0.01	3	0.90±0.07
Δ*bgl3A* Δ*bgl3B* Δ*bgl3*C[b]	0.23±0.04	3	0.74±0.11
Δ*bgl3A* Δ*bgl3B* Δ*bgl3D*[j]	0.13±0.01	2	0.58±0.004
Δ*bgl3A* Δ*bgl3C* Δ*bgl3D*[k]	0.13±0.01	6	0.97±0.06
Δ*bgl3B* Δ*bgl3C* Δ*bgl3D*[g]	0.14±0.003	2	0.63±0.05
Δ4βG[l]	0.18±0.02	5	0.86±0.002

[a] Experiments were performed in biological triplicate
[b] Time points used to calculate growth rate were T_i=4 and T_f=9
[c] Time points used to calculate growth rate were T_i=4 and T_f=7
[d] Time points used to calculate growth rate were T_i=4 and T_f=8
[e] Time points used to calculate growth rate were T_i=4 and T_f=13
[f] Time points used to calculate growth rate were T_i=6 and T_f=16
[g] Time points used to calculate growth rate were T_i=3 and T_f=9
[h] Time points used to calculate growth rate were T_i=7 and T_f=13
[i] Time points used to calculate growth rate were T_i=4 and T_f=10
[j] Time points used to calculate growth rate were T_i=3 and T_f=10
[k] Time points used to calculate growth rate were T_i=7 and T_f=15
[l] Time points used to calculate growth rate were T_i=6 and T_f=10

Table S1J. Growth statistics of *C. japonicus* GH3 mutants grown in a defined xyloglucan oligosaccharide medium (corresponding to Figure 5D)[a]

Strain	Growth Rate (gen hr^{-1})	Lag Time (hrs)	Max OD_{600}
Wild Type[b]	0.26±0.01	8	1.04±0.01
Δ*bgl3A*[c]	0.27±0.01	8	1.02±0.01
Δ*bgl3B*[d]	0.21±0.004	8	1.00±0.01
Δ*bgl3C*[c]	0.16±0.01	8	1.03±0.01
Δ*bgl3D*[e]	0.23±0.01	10	0.76±0.01
Δ*bgl3B* Δ*bgl3D*[f]	0.14±0.004	8	0.71±0.01
Δ4βG[g]	0.15±0.01	8	0.67±0.01

[a] Experiments were performed in biological triplicate
[b] Time points used to calculate growth rate were T_i=9 and T_f=13
[c] Time points used to calculate growth rate were T_i=9 and T_f=14
[d] Time points used to calculate growth rate were T_i=9 and T_f=15
[e] Time points used to calculate growth rate were T_i=11 and T_f=14
[f] Time points used to calculate growth rate were T_i=9 and T_f=18
[g] Time points used to calculate growth rate were T_i=9 and T_f=17

Table S1K. Growth statistics of *C. japonicus* GH3 mutants grown in a defined gentiobiose medium (corresponding to Figure S4)[a]

Strain	Growth Rate (gen hr^{-1})	Lag Time (hrs)	Max OD_{600}
Wild Type[bc]	0.36±0.004	3	1.16±0.004

[a] Experiments were performed in biological triplicate
[b] All mutant strains grew as wild type
[c] Time points used to calculate growth rate were T_i=6 and T_f=10

Table S1L. Growth statistics of *C. japonicus* mutants grown in a defined xyloglucan medium (corresponding to Figure S6)[a]

Strain	Growth Rate (gen hr^{-1})	Lag Time (hrs)	Max OD_{600}
Wild Type[b]	0.24±0.01	2	1.05±0.04
Δ*xylA*[b]	0.25±0.04	2	0.96±0.06
Δ4βG[c]	0.15±0.01	3	0.99±0.06
Δ*xylA*Δ4βG[d]	0.09±0.01	2	0.39±0.01
Δ*xyl31A*	ND[e]	ND	0.15±0.02

[a] Experiments were performed in biological triplicate
[b] Time points used to calculate growth rate were T_i=3 and T_f=9
[c] Time points used to calculate growth rate were T_i=4 and T_f=12
[d] Time points used to calculate growth rate were T_i=3 and T_f=11

[e] Not Determined due to lack of growth

Table S2. Strains, plasmids, and primers used in this study

Strain, plasmid, or primer	Genotype or Sequence	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 K12 / pBBRMCS-5	Gm^r	(Nelson *et al.*, 2017)
E. coli K12 / pBBRMCS-5-*bgl3A*	*bgl3A*$^+$;Gm^r	(Nelson *et al.*, 2017)
E. coli K12 / pBBRMCS-5-*bgl3B*	*bgl3B*$^+$;Gm^r	(Nelson *et al.*, 2017)
E. coli K12 / pBBRMCS-5-*bgl3C*	*bgl3C*$^+$;Gm^r	(Nelson *et al.*, 2017)
E. coli K12 / pBBRMCS-5-*bgl3D*	*bgl3D*$^+$;Gm^r	(Nelson *et al.*, 2017)
C. japonicus Ueda 107	Wild Type	Laboratory collection
C. japonicus Δ*bgl3A*	Ueda 107 Δ*bgl3A*[a]	(Nelson *et al.*, 2017)
C. japonicus Δ*bgl3B*	Ueda 107 Δ*bgl3B*[b]	(Nelson *et al.*, 2017)
C. japonicus Δ*bgl3C*	Ueda 107 Δ*bgl3C*[c]	(Nelson *et al.*, 2017)
C. japonicus Δ*bgl3D*	Ueda 107 Δ*bgl3D*[d]	(Nelson *et al.*, 2017)
C. japonicus Δ*bgl3A*Δ*bgl3B*	Ueda 107 Δ*bgl3A*Δ*bgl3B*	(Nelson *et al.*, 2017)
C. japonicus Δ*bgl3A*Δ*bgl3C*	Ueda 107 Δ*bgl3A*Δ*bgl3C*	(Nelson *et al.*, 2017)
C. japonicus Δ*bgl3A*Δ*bgl3D*	Ueda 107 Δ*bgl3A*Δ*bgl3D*	(Nelson *et al.*, 2017)
C. japonicus Δ*bgl3B*Δ*bgl3C*	Ueda 107 Δ*bgl3B*Δ*bgl3C*	(Nelson *et al.*, 2017)
C. japonicus Δ*bgl3B*Δ*bgl3D*	Ueda 107 Δ*bgl3B*Δ*bgl3D*	(Nelson *et al.*, 2017)
C. japonicus Δ*bgl3C*Δ*bgl3D*	Ueda 107 Δ*bgl3C*Δ*bgl3D*	(Nelson *et al.*, 2017)
C. japonicus Δ*bgl3A*Δ*bgl3B*Δ*bgl3C*	Ueda 107 Δ*bgl3A*Δ*bgl3B*Δ*bgl3C*	(Nelson *et al.*, 2017)
C. japonicus Δ*bgl3A*Δ*bgl3B*Δ*bgl3D*	Ueda 107 Δ*bgl3A*Δ*bgl3B*Δ*bgl3D*	(Nelson *et al.*, 2017)
C. japonicus Δ*bgl3A*Δ*bgl3C*Δ*bgl3D*	Ueda 107 Δ*bgl3A*Δ*bgl3C*Δ*bgl3D*	(Nelson *et al.*, 2017)
C. japonicus Δ*bgl3B*Δ*bgl3C*Δ*bgl3D*	Ueda 107 Δ*bgl3B*Δ*bgl3C*Δ*bgl3D*	(Nelson *et al.*, 2017)
C. japonicus Δ4βG	Ueda 107 Δ*bgl3A*Δ*bgl3B*Δ*bgl3C*Δ*bgl3D*	(Nelson *et al.*, 2017)
C. japonicus Δ*xylA*	Ueda 107 Δ*xylA*[e]	(Nelson *et al.*, 2016)
C. japonicus Δ4βG Δ*xylA*	Ueda 107 Δ*bgl3A*Δ*bgl3B*Δ*bgl3C*Δ*bgl3D*Δ*xylA*	This study
C. japonicus Δ*xyl31A*	Ueda 107 Δ*xyl31A*[f]	(Larsbrink *et al.*, 2014)
Plasmids		

pRK2013	ColE1 RK2-Mob$^+$ RK2-Tra$^+$; Km^r	(Figurski & Helinski, 1979)
pK18*mobsacB*	pMB1 *ori mob*$^+$ *sacB*$^+$; Km^r	(Schafer *et al.*, 1994)
pK18Δ*xylA*	Contains 500bp upstream and downstream of *xylA* cloned into pK18*mobsacB*; Km^r	(Nelson *et al.*, 2016)
Primers		
Δ*xylA* CONF (5')	AGGTTTGTTCCATC	(Nelson *et al.*, 2016)
Δ*xylA* CONF (3')	GAACTTGAAASCTGCCTG	(Nelson *et al.*, 2016)
xylA INT (5')	GAATTCGCATCGGCAAAA	(Nelson *et al.*, 2016)
xylA INT (3')	TCTAGAACCGCCCCAGAA	(Nelson *et al.*, 2016)

[a] Gene locus CJA_0204
[b] Gene locus CJA_1497
[c] Gene locus CJA_0223
[d] Gene locus CJA_1140
[e] Gene locus CJA_3061
[f] Gene locus CJA_2706

References

Bradford, M.M., (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* **72**: 248-254.

Figurski, D.H. & D.R. Helinski, (1979) Replication of an origin-containing derivative of plasmid RK2 dependent on a plasmid function provided in *trans*. *Proceeding of the National Academy of Sciences* **76**: 1684-1652.

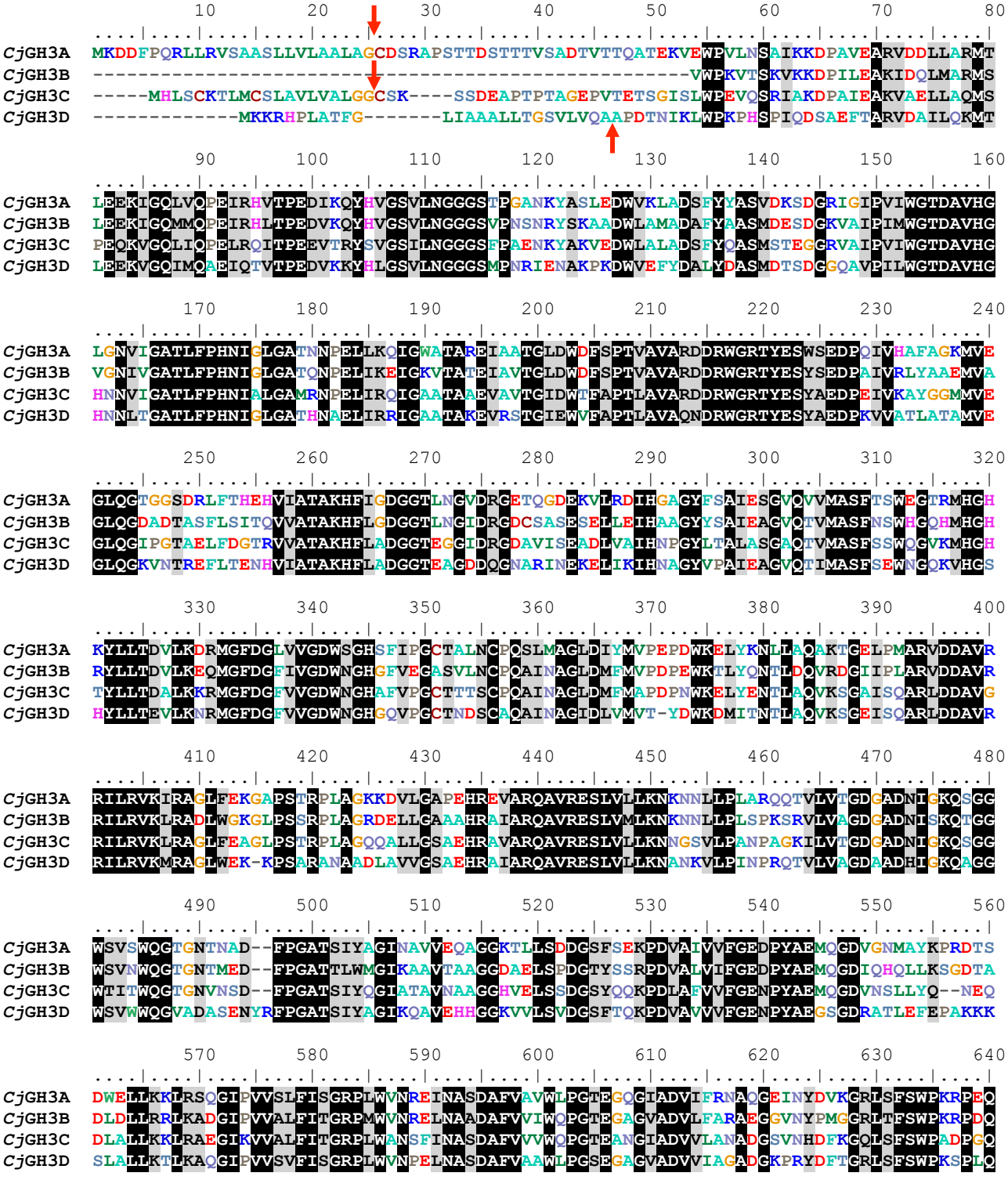
Larsbrink, J., A.J. Thompson, M. Lundqvist, J.G. Gardner, G.J. Davies & H. Brumer, (2014) A complex gene locus enables xyloglucan utilization in the model saprophyte Cellvibrio japonicus. *Molecular Microbiology* **94**: 418-433.

Nelson, C.E., N.R. Beri & J.G. Gardner, (2016) Custom fabrication of biomass containment devices using 3-D printing enables bacterial growth analyses with complex insoluble substrates. *Journal of microbiological methods* **130**: 136-143.

Nelson, C.E., A. Rogowski, C. Morland, J.A. Wilhide, H.J. Gilbert & J.G. Gardner, (2017) Systems analysis in Cellvibrio japonicus resolves predicted redundancy of beta-glucosidases and determines essential physiological functions. *Molecular Microbiology* **104**: 294-305.

Schafer, A., A. Tauch, W. Jager, J. Kalinowski, G. Theirbach & A. Puhler, (1994) Small mobilization multi-purpose cloning vectors derived from the Escherichia coli plasmids pK18 and pK19: selection of defined deletions in the chromosome of Corynebacterium glutamicum. *Gene* **145**: 69-73.

Supplemental Figures



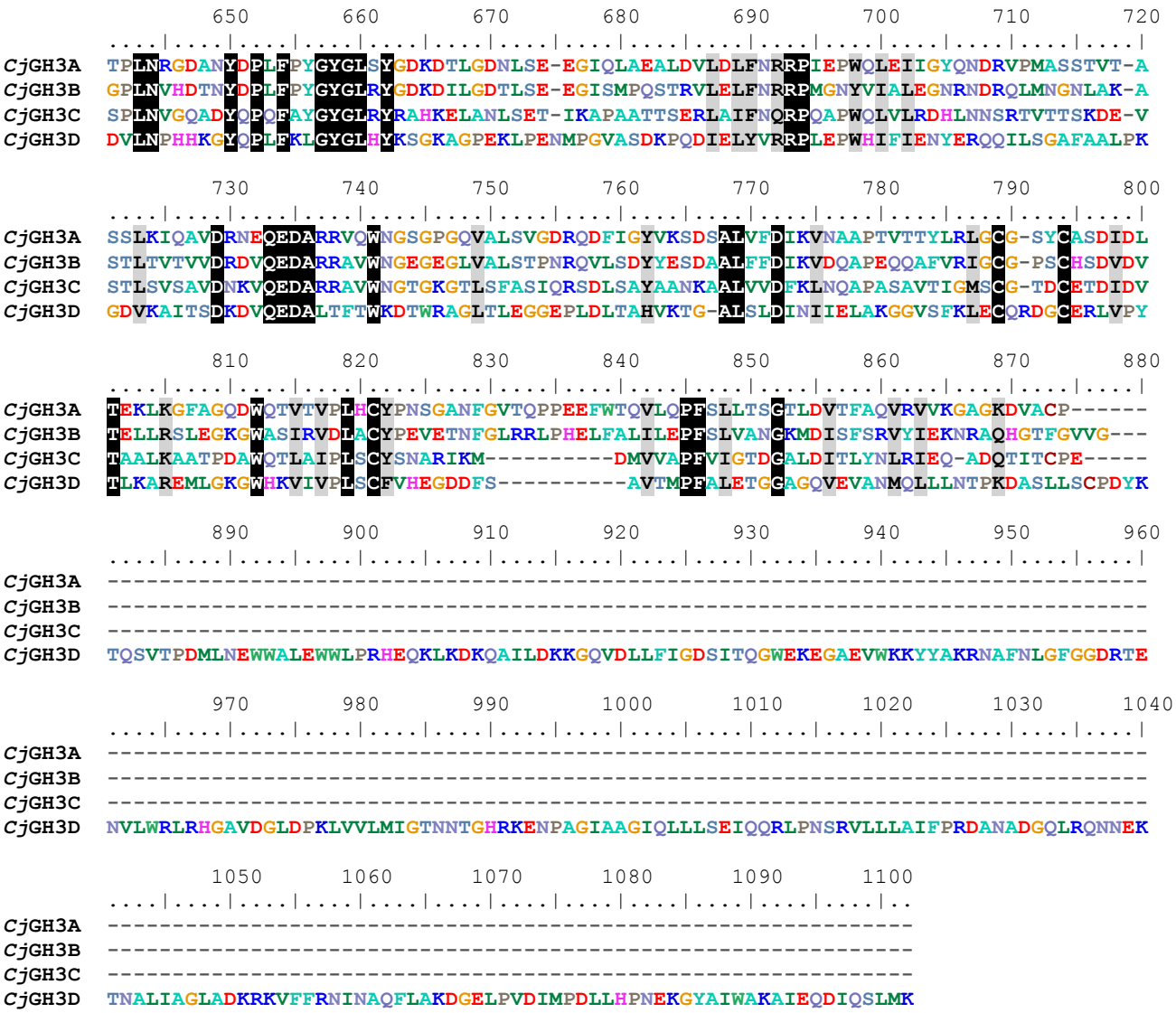


Fig S1. Amino acid sequence alignment of the *Cj*GH3 enzymes. Signal peptide cleavage sites are indicated by red arrows. The cleavable signal peptide is removed by the action of signal peptidase II in case of Bgl3A and Bgl3C, and signal peptidase I in case of Bgl3D.

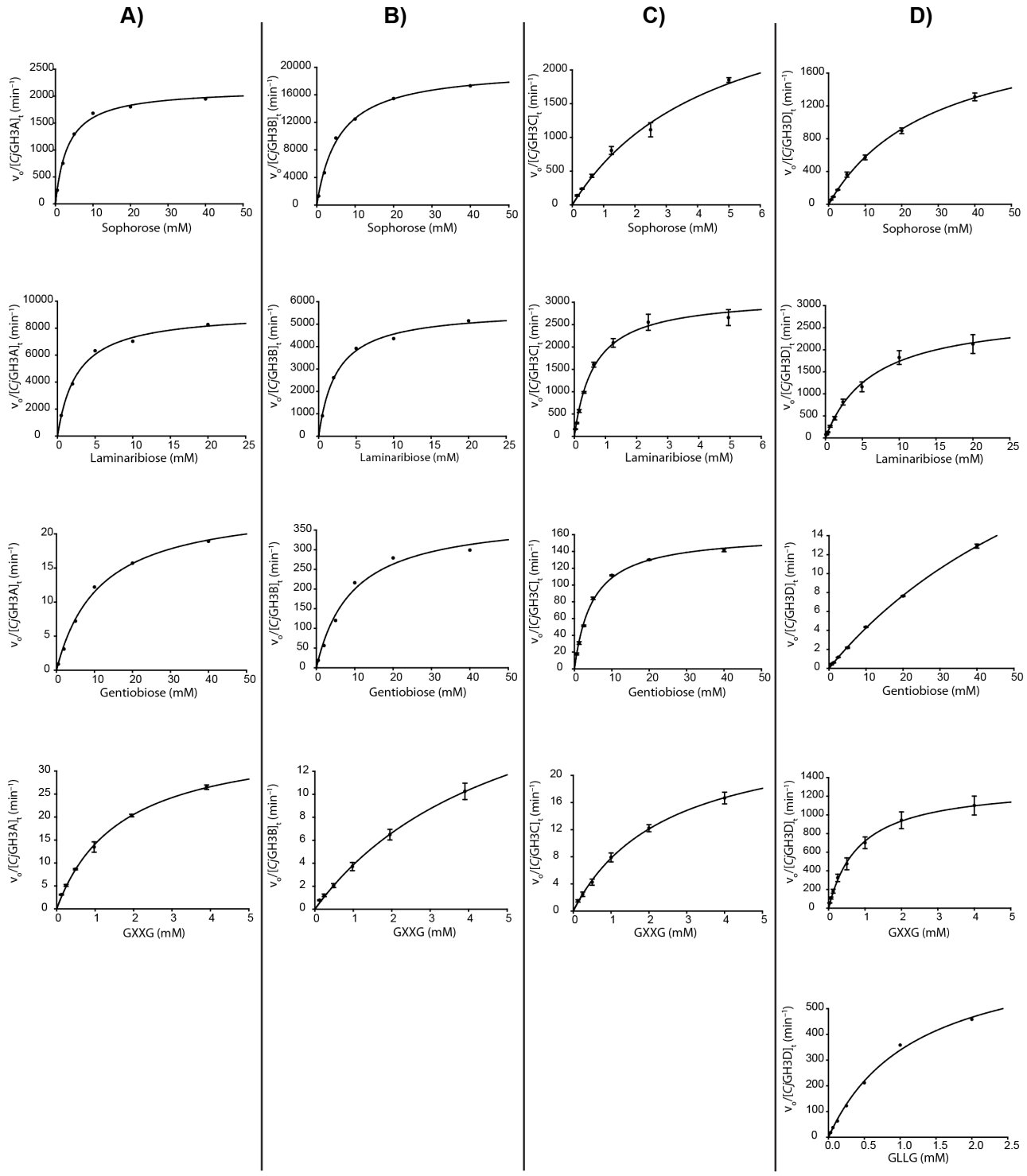


Fig S2. Kinetic parameters of GH3 enzymes against different gluco-disaccharides and xyloglucan-based oligosaccharides. **(A)** Bgl3A. **(B)** Bgl3B. **(C)** Bgl3C. **(D)** Bgl3D. These data are summarized in **Table 1**.

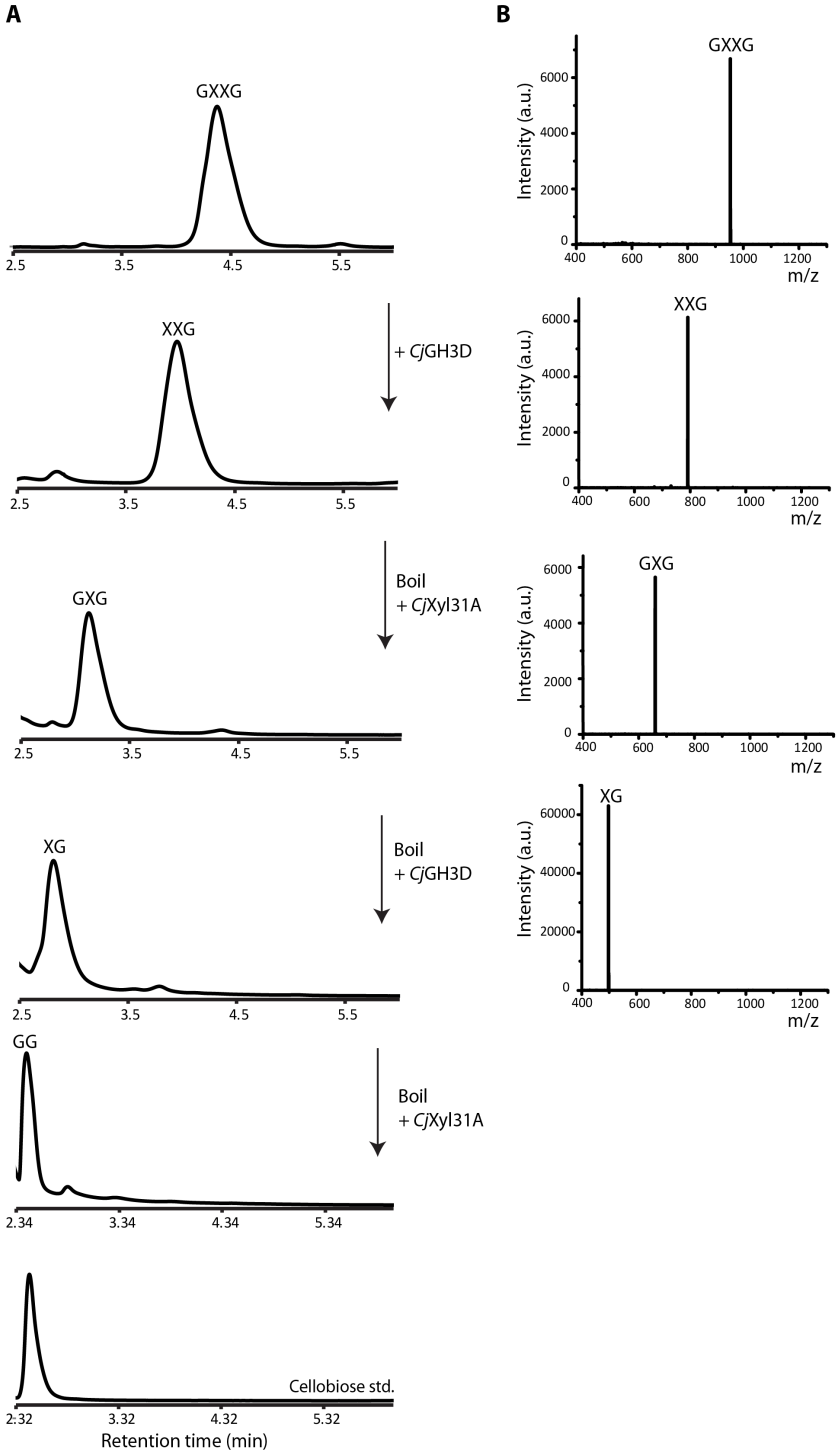


Fig S3. Sequential digestion of GXXG by *Cj*GH3D and *Cj*Xyl31A with a heat inactivation step between each treatment. (A) HPAEC-PAD analysis of GXXG and the product of each enzymatic step. **(B)** MALDI-TOF analysis of the same sample: GXXG ($[M+Na]^+$ calculated: 953.8, observed: 953.29), XXG ($[M+Na]^+$ calculated: 791.65, observed: 791.37), GXG ($[M+Na]^+$ calculated: 659.54, observed: 659.18), and XG ($[M+Na]^+$ calculated: 497.4, observed: 497.25).

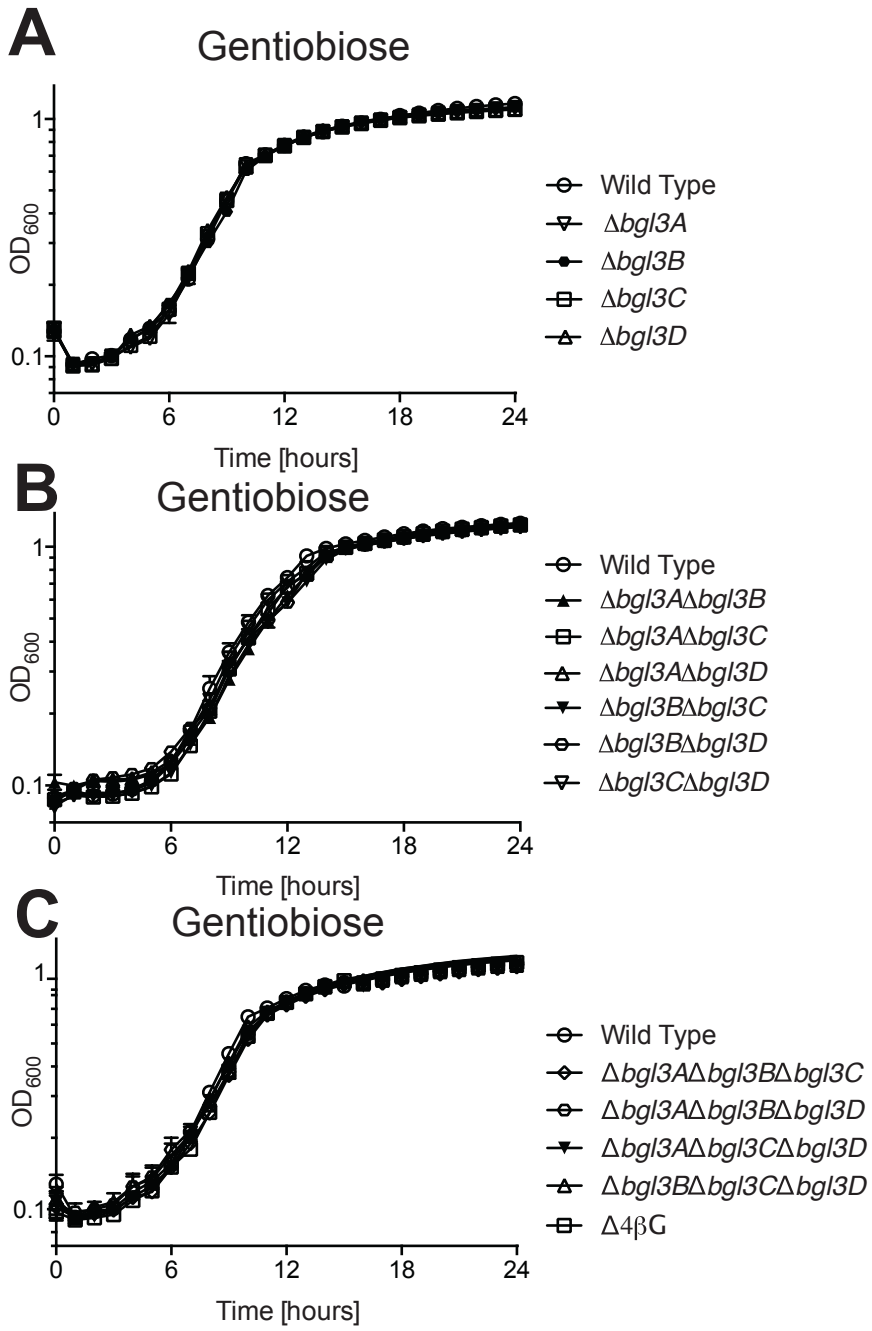


Fig S5. Growth analysis of GH3 mutants on gentiobiose. *C. japonicus* wild type and GH3 single **(A)**, double **(B)**, triple and quadruple **(C)** mutants were grown in defined media with 0.5% w:v gentiobiose as the sole carbon source for 24 hours at 30°C with a high level of aeration. Growth was monitored as optical density (OD) at 600 nm. Experiments were performed in biological triplicate with the error bars representing the standard deviation. Growth rates and maximum OD can be found in **Table S1**.

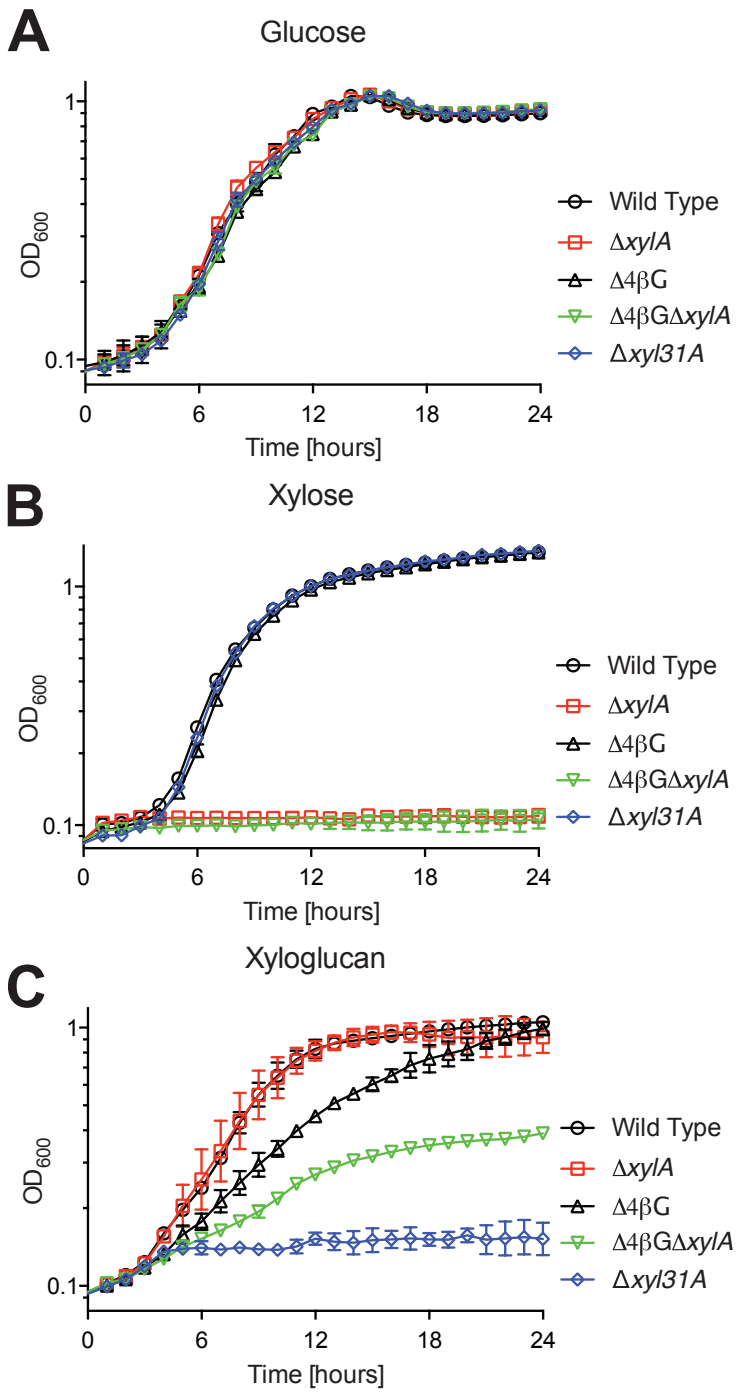


Fig S6. Importance of xylose consumption during xyloglucan degradation. *C. japonicus* wild type, the Δ4βG quadruple, Δ*xylA,* Δ*xyl31A*, and the Δ4βG Δ*xylA* quintuple mutants were grown with 0.5% w:v glucose **(A),** 0.5% w:v xylose **(B),** or 0.5% w:v xyloglucan **(C)** for 24 hours at 30°C with a high level of aeration. Growth was monitored as optical density (OD) at 600 nm. Experiments were performed in biological triplicate with the error bars representing the standard deviation. Growth rates and maximum OD can be found in **Table S1**.