## Supplementary Information

**Table S1: Primers used for cloning, qPCR, and mutant strain confirmation**

| **Primers for cloning** | **Sequence** |
|---|---|
| Bgl35A_TOPO_F | CACCGCCGCACCACTTCCTGAG |
| Bgl35A_TOPO_R | CTGAACGCTATAGCTGGC |
| Afc955A_TOPO_F | CACCATGAGCGCAGAGGTG |
| Afc955A_TOPO_R | CTTCTGCATACTCAAGAGATACTC |
| Bgl35A_Lic-F | CATCACCACCACCACGCCGCACCACTTCCTGAGTTGTTATCC |
| Bgl35A_Lic-R | TGAGGAGAAGGCGCGTTACTGAACGCTATAGCTGGCCATTTTGACACG |

| **Primers for qPCR** | |
|---|---|
| Xyl31A_F | CCAAGGGTAATGTGCTGCTACAAG |
| Xyl31A_R | CCTGGCGCAACGCATAAGAATCG |
| Bgl35A_F | GGAACAAATTGAGCCAGTAGAGGG |
| Bgl35A_R | CCACAGCAACACCAACCTTACCTTG |
| TBDR_F | GCTCGTTTTGATGCGGAATATGATTTCG |
| TBDR_R | CCAGTGTTAATATTATCGGCTTCTTTCTCG |
| Afc95A_F | CAGCTGTTGGCACGCCATACG |
| Afc95A_R | CCAATAATGCTGGTGTTGCCAGTG |
| Bgl2A_F | CCAACAACTCCAGGTGGTACAGC |
| Bgl2A_R | GATTTTCACTATCGCTTCCGGCATTC |
| Bgl2B_F | CGCAATGTCTGGCTCACCAAAACC |
| Bgl2B_R | CATCCACCGCAATACGGGCAG |
| Bgl2C_F | GAATACCGTTTGCGTCGCTTGAAGG |
| Bgl2C_R | CCATGCGATCAGCCATATCCAGTAC |
| Afc95B_F | GCTGTGCCAGCATATCTGGTATCAC |
| Afc95B_R | GACAAAAAAGCGCGAGGCCTCG |

**Primers used for confirming the *bgl35A* and *xyl31A* strain knock-out mutations**

| | |
|---|---|
| *bgl35A*_P1 | ATGCCACACCGATAATA |
| *bgl35A*_P2 | CAATGACGTTCAAATTGA |
| *bgl35A*_P3 | CAGGCGCTCGTAGAC |
| *bgl35A*_P4 | ATGCCACACCGATAATA |
| *bgl35A*_P5 | GTGTGGAATTGTGAGCG |
| *bgl35A*_P6 | CAATGACGTTCAAATTGA |
| *bgl35A*_P7 | GACAGTTTGATTTCTCCT |
| *bgl35A*_P8 | TTAATAGCTTTACCCGC |
| *xyl31A*_P1 | ACGGCTTATAAGGCA |
| *xyl31A*_P2 | ATTTCGGTGGTAGCT |
| *xyl31A*_P3 | CAGGCGCTCGTAGAC |
| *xyl31A*_P4 | ACGGCTTATAAGGCA |
| *xyl31A*_P5 | GTGTGGAATTGTGAGCG |
| *xyl31A*_P6 | ATTTCGGTGGTAGCT |
| *xyl31A*_P7 | TGAGCTTCCGTGATG |
| *xyl31A*_P8 | GCATAACCGGAGTTATAC |

**Table S2: Data collection and refinement statistics for *Cj*Bgl35A native and 1-deoxygalactonojirimycin (DGJ) ligand complex structures.**

| | *Cj*Bgl35A (PDB 4D1I) | *Cj*Bgl35A-GDJ (PDB 4D1J) |
|---|---|---|
| **Data collection** | | |
| Space group | P1 | P1 |
| Cell dimensions | | |
| *a*, *b*, *c* (Å) | 98.9, 115.8, 116.0 | 99.3, 116.1, 116.1 |
| $\alpha, \beta, \gamma$ (°) | 90.2, 90.3, 90.4 | 90.0, 90.1, 90.0 |
| Resolution (Å) | 46.0 – 1.80 (1.83 – 1.80) | 46.0 – 1.80 (1.83 – 1.80) |
| $R_{sym}$ or $R_{merge}$ | 0.063 (0.63) | 0.051 (0.57) |
| $I/\sigma I$ | 8.7 (1.1) | 9.8 (1.4) |
| Completeness (%) | 96.4 (95.1) | 97.1 (95.8) |
| Redundancy | 1.8 (1.8) | 2.2 (2.1) |
| | | |
| **Refinement** | | |
| Resolution (Å) | 1.80 | 1.80 |
| No. (unique) reflections | 459893 | 466658 |
| $R_{work}$ / $R_{free}$ | 18.5 / 21.2 | 17.2 / 19.5 |
| No. atoms | | |
| Protein | 33680 | 33647 |
| Ligand | n/a | 88 |
| Solvent/ions | 3622 | 4061 |
| B-factors | | |
| Protein | 30.4 | 28.9 |
| Ligand | n/a | 20.3 |
| Solvent/ions | 36.2 | 37.3 |
| R.m.s deviations | | |
| Bond lengths (Å) | 0.011 | 0.011 |
| Bond angles (°) | 1.48 | 1.47 |

*Values in parentheses denote highest resolution shell.

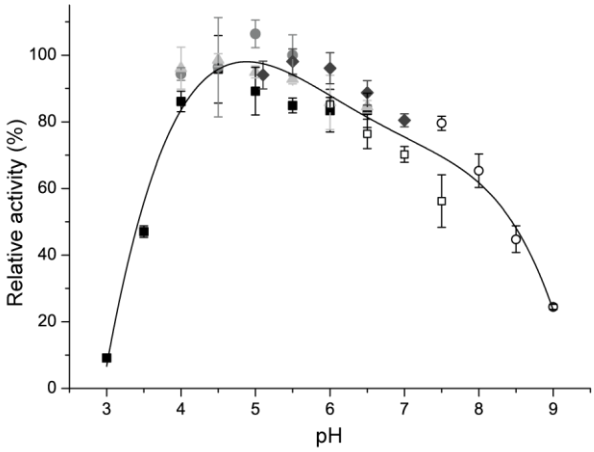


**Figure S1: pH-rate profile of *Cj*Bgl35A.** The following buffers were used at 50 mM: citrate (filled squares), acetate (filled circles), succinate (triangles), MES (diamonds), phosphate (empty squares), and glycylglycine (empty circles). The line is an arbitrary smooth curve through the data to guide the eye.

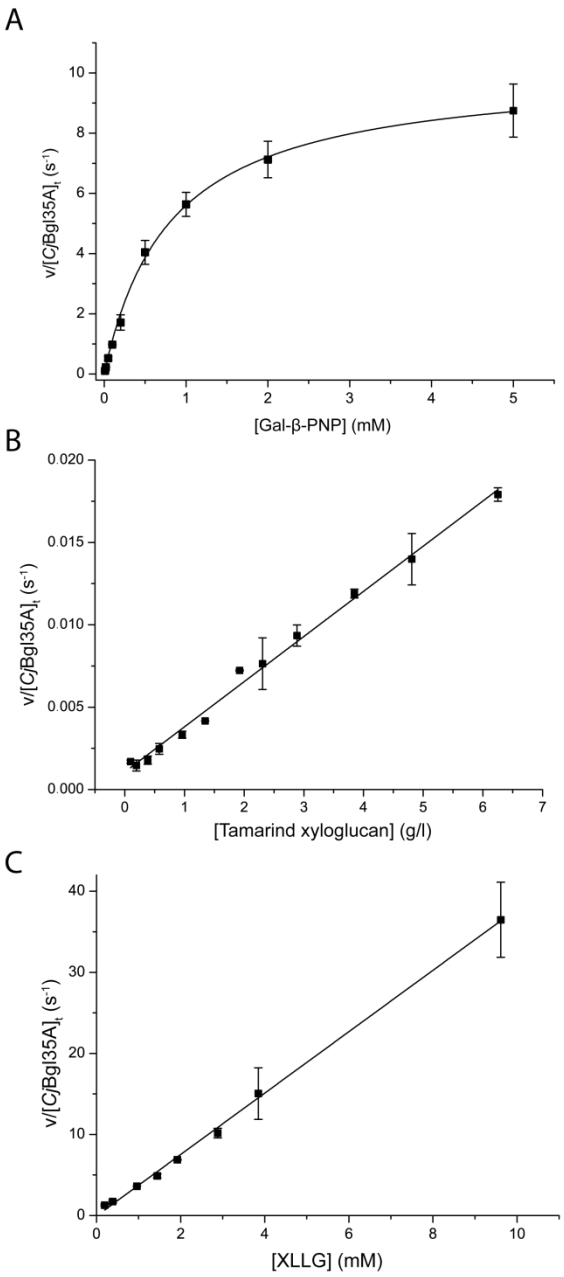


**Figure S2: Kinetic analysis of the hydrolysis of galactosides by *Cj*Bgl35A.** (A) Gal-β-PNP, (B) tamarind xyloglucan, and (C) XLLG. The amount of available galactose, used for calculating the velocity and the $k_{cat}/K_m$ value for tamarind xyloglucan, was calculated from the ratio of galactose present in the xyloglucan preparation (16 %, as reported by the manufacturer).

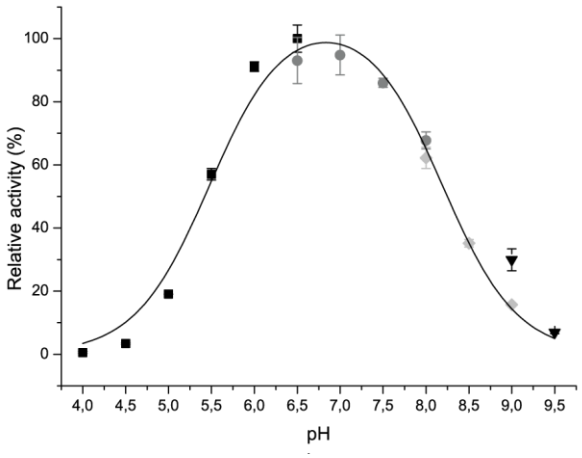


**Figure S3: pH-rate profile of *Cj*Afc95A.** The following buffers were used at 50 mM: citrate (squares), circles (sodium phosphate), glycylglycine (diamonds) and glycine (triangles).

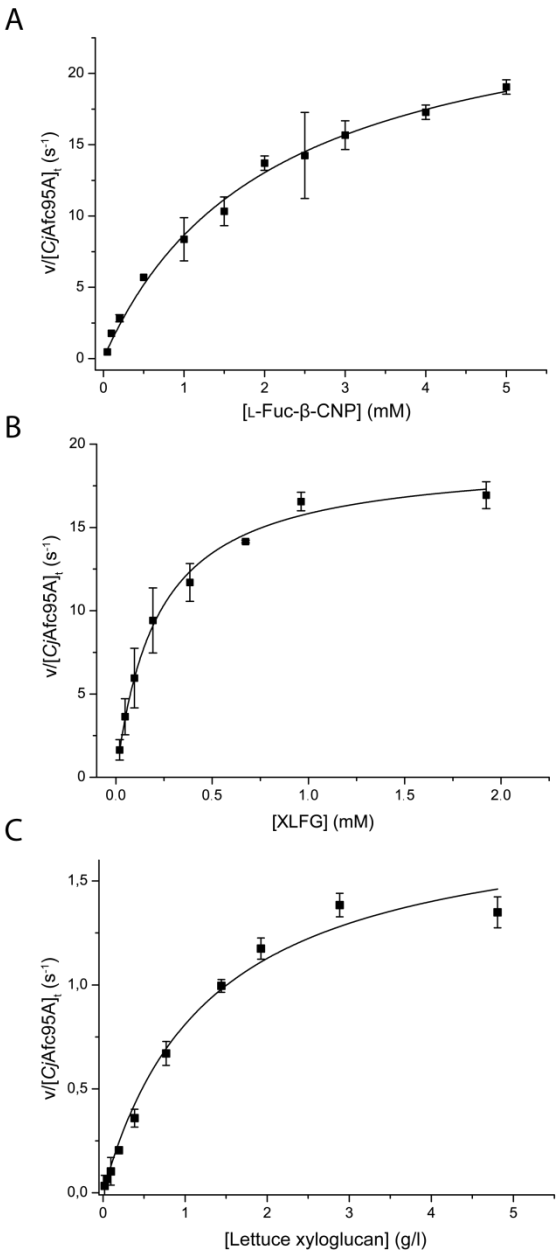


**Figure S4: Kinetic analysis of the hydrolysis of fucosides by *Cj*Afc95A.** (A) L-Fuc-α-CNP, (B) XLFG, and (C) lettuce XyG.

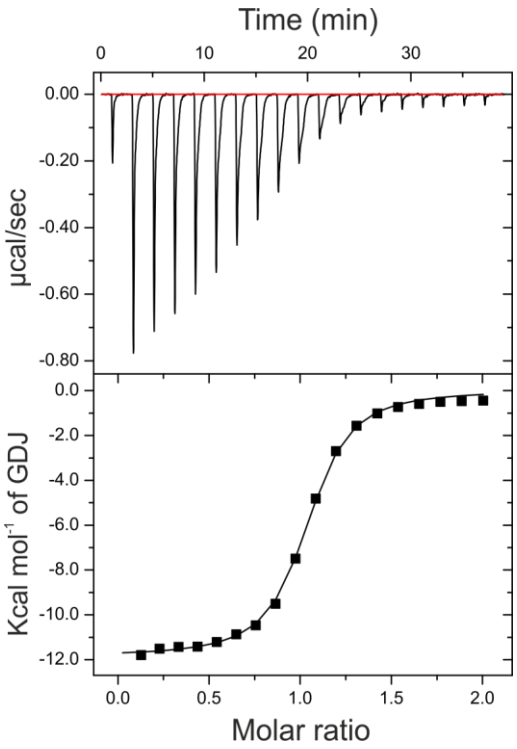


**Figure S5: ITC thermogram of GDJ binding to Bgl35A.** GDJ (390 µM) binds to Bgl35A (38.5 µM) with an average molar ratio (N) of 1.01 ± 0.0054 and average $K_d$ of 485 ± 42 nM.

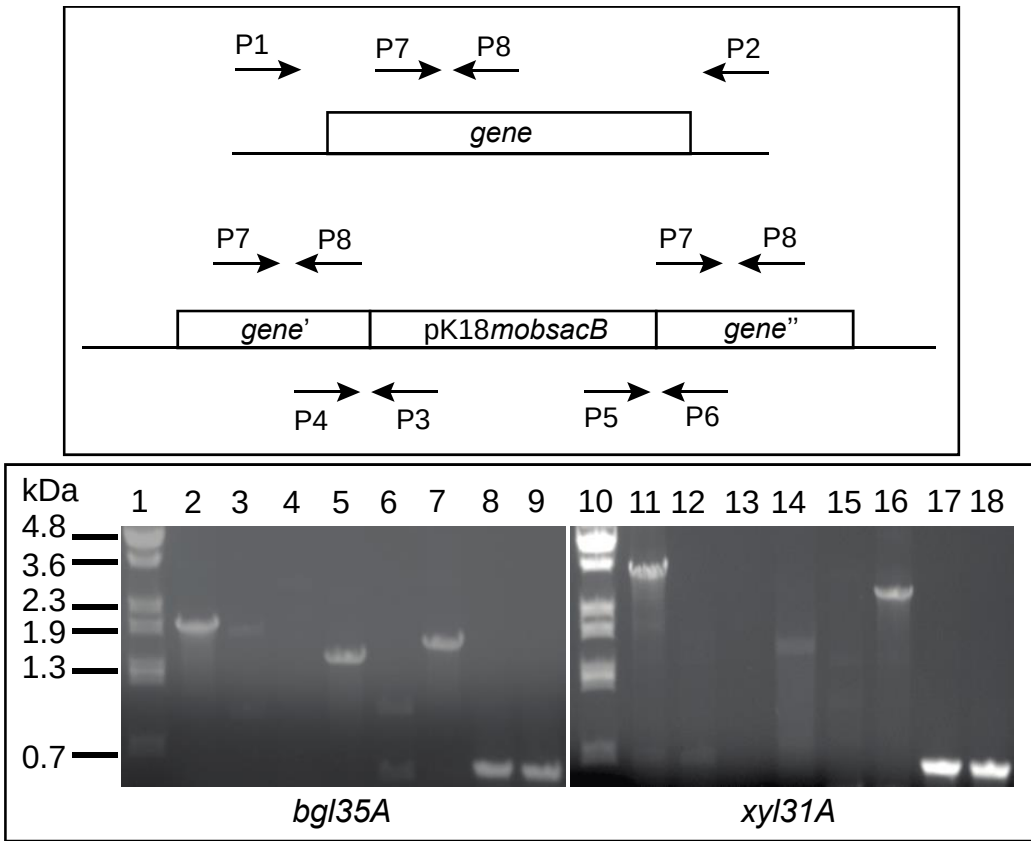


**Figure S6: PCR confirmation of *bgl35A* and *xyl31A* mutations in *C. japonicus*.** Overnight cultures of wild type and mutant strains were used as template for PCR, which were then run on 1% agarose gels, stained with ethidium bromide, and visualized on a BioRad GelDoc XR+ system. Top panel: Schematic for suicide plasmid pK18*mobsacB* insertion into a target gene and locations of the confirmatory primers used in the PCR series. Bottom panel: Gel photo confirming insertional inactivation of the *bgl35A* (left) and *xyl31A* (right) genes. In each case, the primers (P1, P2, etc.) are specific to either *bgl35A* or *xyl35A*, as indicated in Supplemental Table S1. **Lane 1**, λ *Bst*EII DNA ladder; **Lane 2**, PCR of *bgl35A* region from wild type *C. japonicus* with primers P1 and P2; **Lane 3** PCR of *bgl35A* region from *bgl35A* mutant with primers P1 and P2; **Lane 4**, PCR from wild type with primers P3 and P4; **Lane 5**, PCR from the *bgl35A* mutant with primers P3 and P4; **Lane 6**, PCR from wild type with primers P5 and P6; **Lane 7**, PCR from the *bgl35A* mutant with primers P5 and P6; **Lane 8**, PCR of internal *bgl35A* region from wild type with primers P7 and P8; **Lane 9**, PCR of internal *bgl35A* region from the *bgl35A* mutant with primers P7 and P8; **Lane 10**, λ *Bst*EII DNA ladder; **Lane 11**, PCR of *xyl31A* region from wild type *C. japonicus* with primers P1 and P2; **Lane 12,** PCR of *xyl31A* region from *xyl31A* mutant with primers P1 and P2; **Lane 13**, PCR from wild type with primers P3 and P4; **Lane 14**, PCR from the *xyl31A* mutant with primers P3 and P4; **Lane 15**, PCR from wild type with primers P5 and P6; **Lane 16**, PCR from the *xyl31A* mutant with primers P5 and P6; **Lane 17**, PCR of internal *xyl31A* region from wild type with primers P7 and P8; **Lane 18**, PCR of internal *xyl31A* region from the *xyl31A* mutant with primers P7 and P8.