

1.0 α -Mannan in fungi

It is possible that the *Bt* mannan PULs target edible fungi cell walls in addition to yeast α -mannans. To test this hypothesis polysaccharides were extracted by a hot water extraction method from the common white button mushroom, *Agaricus bisporus*. The growth of the Δ PUL-Man1/2/3 mutant was not impaired compared to wild type *Bt* when grown on a minimal media with the mushroom extracted polysaccharides as a sole carbon source. This would suggest that PUL-Man1/2/3 are not required for growth and therefore the extracted mushroom glycans are not α -mannan. Also BT3774 and BT3792 were unable to release mannose or manno-oligosaccharides from the mushroom extracted polysaccharides confirming the absence of α -mannan. In addition a previous study⁴⁴ found N-glycans in *A. bisporus* to be of the high mannose N-glycan variety lacking the elongated α 1,6 mannose backbone found in yeast mannan, consistent with the observation that genes encoding GT62 glycosyltransferases, the enzymes required to generate the extended α 1,6-Man backbone of yeast mannan, are absent from the *A. bisporus* genome back. Therefore *A. bisporus* is not a source of α -mannan utilised by Pul-Man 1/2/3 in the human diet. It is acknowledged, however, that other fungi may synthesise α -mannans. For example, the outer chain of *Aspergillus oryzae* mannan consists of the canonical α -1,6 linked mannose backbone, capped with α -1,2 linked mannose residues. Appended to the non-reducing end of these mannose caps are β -1,2 linked galactose residues in a furanose configuration. Uncapped mannose moieties of the backbone are likewise substituted with β -1,2-linked galactofuranose residues⁴⁵. The mannan of *A. niger* also has an α -1,6 linked mannose backbone, substituted in the 2-position with α -1,2-mannose, and α -1,2-linked mannobiose or mannotriose sidechains. There are also β -1,5-linked galactofuranose side-chains with an average length of 4 units but it is unclear if these side-chains are appended to the mannopyranosyl units in the side-chains, main chain or both⁴⁶. Black truffles, although considered a delicacy and thus not likely to be extensively consumed by humans, contains several glycosyltransferases belonging to GT62, the family that contains enzymes that synthesise both the α -1,6-linked Man backbone and the α -1,2-linked Man side chains. It is possible, therefore, that these Basidiomycetes also produce yeast-like mannans⁴⁷.

2.0 Potential relevance of yeast mannan degradation to Crohn's disease (CD)

In a group of CD patients there is a disturbed humoral and cellular immunological response to yeast mannan leading to activation, rather than inhibition, of T-cells¹⁰. Furthermore, CD

patients often have antibodies (ASCA) that target the terminal Man- α 1,3-Man disaccharide of α -mannan^{48,49}, suggesting a relationship between the polysaccharide and CD. This is supported by the observation that the ASCA epitope inhibits the killing of bacteria by macrophages, suggesting and that the suppression of mucosal phagocyte function by α -mannan contributes to CD pathogenesis¹¹. It is potentially significant, therefore, that in addition to metabolising yeast mannan, *Bt* presents a surface side-chain cleaving enzyme upregulated by yeast mannan, the GH99 an endo- α 1,2-mannosidase²⁹, which is capable of degrading the ASCA epitope, and thus relieve the deleterious effects of the polysaccharide on CD patients. It is also potentially significant that in the microbiota of populations with CD there is a marked reduction (~35%) in the level of *Bt* compared to healthy individuals⁵⁰. This dysbiosis may impair the metabolism of yeast mannan, which could exacerbate the pathology of CD for reasons outlined above. A recent study showed that the microbiota of CD patients that went into remission contained high levels of *Bacteroides*, but low levels of this phylum in patients where there was a recurrence of the disease⁵¹. Indeed *Bt* is an FDA approved orphan drug for the treatment of paediatric Crohns disease.

3.0 Genomics and transcriptomics of mannan degradation

3.1 Expression of mannan PULs: The data presented in **Extended Data Fig. 10A** show that the three mannan PULs are actively transcribed in gnotobiotic mice fed a diet containing 50% bread, yeast mannan or a polysaccharide-free diet. One explanation for these results is that all of the diets contain contaminating levels of yeast that serve to activate expression *in vivo* and that *Bt* may be particularly sensitive to low levels of these signals. To test this hypothesis we performed a polysaccharide exposure experiment in which *Bt* was pre-grown in MM-glucose, washed in anaerobic medium containing no carbohydrate and exposed to various concentrations (0.01 to 50 μ g/ml) of either yeast mannan or maize amylopectin (starch) for 30 min (we established for both polysaccharides that this amount of time resulted in maximum expression when exposed to 5 mg/ml polysaccharide; data not shown). The data presented in **Extended Data Fig. 10B** show that PUL-Man1 and 2 are highly sensitive to limiting amounts of mannan and are more sensitive than the starch utilization system. However, expression of PUL-Man3 is less sensitive under these conditions. In contrast to these expression sensitivity measurements, when *Bt* was similarly exposed for 30 min to aqueous extracts from the base diets used in the animal experiment (glycan-free diet), and an extract from the high fibre diet (natural diet) that mice were fed prior to the start of the experiment, no expression of PUL-Man1 and PUL-Man2 was detected, although transcripts of PUL-Man3 were evident, but still well below what is observed *in vivo* (compare **Extended**

Data Fig. 10A and C). When the glycan-free diet was supplemented with mannan all three mannan PULs were expressed. Thus, despite the sensitivity of *Bt* to extracellular mannan, there was no evidence that contaminating yeast in the diet is the source of the high amount of mannan PUL expression observed *in vivo*. Collectively, these results suggest that, in addition to yeast mannan, a host signal also activates Pul-Man1/2/3, although the activating ligand is not derived from mammalian high mannose N-glycans (HMNG; **Fig. 1C**).

3.2 Survival of mutants of *Bt* lacking the mannan PUL *in vivo*: It is particularly surprising that the Δ Pul-Man1/2/3 mutant outcompeted wild type *Bt* in animals fed the glycan-free diet that lacked available mannan. While the evolutionary rationale for this observation is not entirely clear, it is possible that the activation of the mannan PULs by host glycans or another endogenous signal, in the absence of *bona fide* yeast mannan, imposes an energetic burden that is not offset by acquisition of usable mannose. Interestingly, competition between wild-type and mutant strains in mice fed the 50% bread diet was significantly shifted towards the wild-type strain (20% and 10% wild type population on the bread and glycan-free diets, respectively; **Fig. 2A**), suggesting that the presence of yeast in a real food product impacts upon *Bt* fitness in a manner that is dependent on the possessing of α -mannan. Although the improved fitness may be viewed as modest, it should be noted that deletion of the xyloglucan PUL from *B. ovatus* had no impact on the survival of the bacterium in gnotobiotic mice fed a “natural” diet rich in xyloglucan, despite there being a very high selection for this locus in the human microbiome²¹. Collectively, these results reinforce the idea that yeast mannan is an important and consistent nutrient in the human diet. If it were not, given the strong selection pressure against wild type *Bt* in mammals in the absence of dietary mannan, it would be expected that most *Bt* strains would lose these PULs in order to offset this competitive disadvantage. The results from human metagenomic data searches (**Extended Data Fig. 8**) and sequencing of human isolates of *Bt* reveal the opposite to be true: at least 60% of human gut metagenomes harbour bacteria with at least one of these mannan PULs and all 11 currently sequenced *Bt* strains contain copies of PUL-Man 2 and 3 (only the sequenced type strain of *Bt* harbors PUL-Man 1, which is contained within a integrative chromosomal element).

3.3 Mannan PULs in the genomes of *Bacteroidetes*: As shown in **Fig. 1D** the capacity to utilize yeast mannan as a nutrient is limited to a small number of *Bacteroidetes*, including *Bt*, isolated from the human gut. Genome analysis of other *Bacteroidetes* isolated from mammalian guts, however, have shown that a proportion of these organisms contain elements of the three *Bt* mannan PULs (not shown) and, consistent with this genomic data, strains of *B. ovatus* and *B. xylanisolvens* are able to grow on variants of *S. cerevisiae*

mannan that contain reduced or no side chains, structures illustrated in **Extended Data Fig. 5**, but not on the polysaccharide produced by the wild type yeast. Indeed, several unsequenced *B. ovatus* and *B. salyersiae* strains possess similar or better ability to grow on native *S. cerevisiae* mannan as strains of *Bt* (**Fig. 1D**). Future genomic/transcriptomic analysis of these strains will be required to locate their active mannan-degrading PULs and determine if they share similar or divergent strategies with *Bt*. Additional genome analysis of five strains of *B. xylanisolvens* isolated from the large bowel of pigs fed distillers grain (which contain yeast mannan; **Extended Data Fig. 9C**) showed that these bacteria had inherited PUL-Man1, but not other components of the mannan degrading apparatus from *Bt*, including PUL-Man2, which shows substantial synteny with PUL-Man1. It should be emphasised that the yeast mannan present in the distillers grain retained its side chains, **Extended Data Fig. 9C**, and thus the debranching of the polysaccharide would need to be mediated by other organisms in the pig gut microbiota. The identity of these bacteria are unclear, although, as shown in **Fig. 4**, the organisms that act in synergy with *B. xylanisolvens* to degrade yeast mannan does not include *Bt*, which metabolizes the polysaccharide through a selfish mechanism. Other than the human type strain of *Bt* and these five porcine *B. xylanisolvens* isolates, the cTn, harbouring PUL-Man1, has not been observed in any other sequenced bacterium, including strains of *B. xylanisolvens* and other species isolated from the same USDA facility and other animals (goats, cows) that were not fed diets augmented with distillers grains. This implies that transposon-mediated lateral gene transfer of PUL-Man1 is a rare event. Thus, the increased presence of yeast in some dietary formulations may select for microorganisms that possess PULs that orchestrate the degradation of α -mannan (or variants of the glycan).

4.0 Enzymology of yeast mannan and HMNG degradation by *Bt*

In addition to the GH38 α -mannosidase BT3774, described in the main body of the paper, the periplasmic removal of the side chains of yeast mannan also require the synergistic action of two phosphatases (BT2630 and BT3783) and two GH92 α -mannosidases, (BT2629 and BT3784), **Fig. 2D and 2E, Supplementary Tables 2 and 3**. BT2629, BT3784 and BT3774 display a preference for α 1,2-Man linkages but in different contexts. Elucidating the contribution of the GH92 and GH38 mannosidases to α -mannan degradation was facilitated through the use of mutants of *S. cerevisiae* that synthesise α -mannan variants lacking the terminal α 1,3-Man linkages (mnn1)⁵², containing single α 1,2-Man units linked to the backbone (mnn5) or comprising only the α 1,6-Man backbone (mnn2), **Extended Data**

Fig. 5¹⁵. The activity of these enzymes against the α -mannan variants showed that after removal of the capping α 1,3-Man linkage by BT3774, the exposed α 1,2-Man is hydrolyzed primarily by the GH92 enzymes BT2629 and BT3784, while the α 1,2-Man linked directly to the α -mannan backbone is cleaved by BT3774, **Fig. 3**.

Removal of the phosphorylated side chains is initiated by the GH92 enzyme BT2629, which cleaves the terminal mannose of Man- α 1,2-Man-1-PO₄-6-Man- α 1,2-ManB (ManB is a component of the α 1,6-mannan backbone), to generate Man-1-PO₄-6-Man- α 1,2-Man-B, which is then hydrolyzed by BT3774 to PO₄-6-Man- α 1,2-ManB, **Fig. 2D**. The exposed Man-6-phosphate linkage was removed by either of the two phosphatases to generate Man- α 1,2-ManB, which was again hydrolyzed by BT3774 to complete side chain removal. The biochemical properties of the two phosphatases (BT2630 and BT3783) are presented in **Supplementary Table 3**, and the structure of BT3783, **Extended Data Fig. 3GH**, reveal why these enzymes are able to dephosphorylate yeast mannan in which the O6-linked phosphate groups have been uncapped (see **Supplementary Information Section 5.4** for a detailed description).

In contrast to *S. cerevisiae* α -mannan the α 1,6-Man backbone in *Schizosaccharomyces pombe* mannan is decorated primarily with single α -Gal residues. These side chains prevent access to *Bt* endo- α 1,6-mannanases, **Extended Data Fig. 1E**. BT2620, a member of GH97, was shown to hydrolyze 4-nitrophenyl- α -D-galactopyranoside but not 4-nitrophenyl- α -D-glucopyranoside (data not shown), demonstrating that the enzyme is a α -galactosidase. Consistent with this activity, BT2620 was able to release galactose from *S. pombe* mannan but was not active on *S. cerevisiae* α -mannan. Furthermore, *S. pombe* mannan treated with BT2620 was degraded by endo- α 1,6-mannanases, **Extended Data Fig. 1E**, demonstrating that the α -galactosidase plays a key role in deconstructing the polysaccharide by removing steric constraints imposed by the α -Gal side chains. Indeed the greatly reduced ability of the Δ PUL-Man1 mutant to grow on *S. pombe* mannan, **Extended Data Fig. 1B**, reflects the loss of the α -galactosidase as all the other components required to degrade the mannan backbone are encoded by PUL-Man2.

4.1 Non-mannan PUL α -mannosidases: To identify the putative surface enzyme(s) that removes the key α 1,2-Man at the base of the side chains, the capacity of the *Bt* α -mannosidases, which are not encoded by the mannan PULs, to cleave this linkage was assessed (19 GH92 and one GH38 enzymes). Only one GH92 enzyme, BT2199, which contains a typical lipoprotein box and is thus likely to be presented on the bacterial surface,

could facilitate GH76 *endo*-mannanase attack of yeast α -mannan, **Extended Data Fig. 4A**. It should be noted, however, that BT2199 is ~100-fold less active than the PUL-Man2 encoded periplasmic GH38 α -mannosidase BT3774 against this linkage, and thus the enzyme mediates only very limited extracellular removal of side chains and exposure of the mannan backbone. The BT2199 gene is a component of a PUL that is controlled by a SARP/OmpR family regulator, and encode encodes a pair of SusC/Dlike proteins and two other proteins of unknown function. The ligand(s) that activates the PUL is unknown, however, the locus is not induced by yeast mannan.

4.2 BT3774 (GH38-CBM32): To determine if the family 32 non-catalytic carbohydrate binding module (CBM32) appended to the enzyme contributes to substrate binding, the three highly conserved active site residues of the catalytic module, His38, Asp40 and Trp43 were substituted with alanine and the capacity of the enzyme to mediate catalysis and binding mannan were evaluated. The mutant was inactive and did not bind to yeast mannan (data not shown). This suggests that the CBM32 plays a structural rather than a binding role in the action of BT3774. This is supported by the observation that neither the GH38 catalytic module nor the CBM32 module could be expressed as individual entities in soluble form in *E. coli*.

4.3 Degradation of HMNG: HMNG that enters the periplasm is degraded by three GH92 enzymes encoded by PUL-HMNG to generate Man- α 1,6-Man- β 1,4-GlcNAc. To explore whether the trisaccharide is degraded further, a culture of *Bt* grown to stationary phase on HMNG was analyzed by HPAEC. While a low amount of mannose was observed, the trisaccharide was not evident showing that the bacterium completely degraded the N-glycan. The enzymes that could degrade the trisaccharide include the GH125 α 1,6-mannosidases and a GH2 β 1,4-mannosidase that was shown previously to cleave Man- β 1,4-GlcNAc⁵³. As the expression of these two enzymes is not induced by HMNG, the hydrolysis of the trisaccharide is likely the rate limiting step in the metabolism of the glycan. This view is supported by the appearance of the trisaccharide when HMNG was exposed to a *Bt* sonicated extract for a short period of time (1 h), while the trisaccharide was not evident after the reaction was continued for 16 h. The other intermediates (i.e. Man₈GlcNAc to Man₃GlcNAc) were not observed after 1 h (data not shown).

4.4 Biochemical properties of BT3791: The predicted outer surface proteins encoded by the three mannan PULs were screened for binding to wild type *S. cerevisiae*, *C. albicans* and *S. pombe* mannans and variants of the *S. cerevisiae* polysaccharide lacking some or all of the side chain linkages. The SusD-like proteins that could be produced recombinantly

(BT2625 and BT3855), albeit at low yields, did not bind to any of the mannans tested, suggesting that either they do not recognize the polysaccharide *in vivo*, or their interaction with glycans requires the presence of the cognate SusC-like protein. While BT3791 did not recognise any of the branched mannans, the protein did bind to the fully debranched mannan produced by the *S. cerevisiae* variant *mnn2*, with a K_A of $\sim 10^3 \text{ M}^{-1}$ (data not shown). The biological rationale for this specificity is not obvious as no significant debranched mannan is generated on the surface by *Bt*. It is possible that the role of BT3791 is to capture mannan that has been debranched by other organisms in the human microbiota.

Section 5 Structural analysis of the degradative enzymes

5.1 GH125 α 1,6-mannosidases: The inability of the enzyme to attack intact yeast mannan indicates that the enzymes are unable to accommodate α -1,6-linked Man residues decorated at O2 (side chains are generally appended α -1,2 to the mannan backbone) in the active site (-1 subsite) and/or the +1 subsite. The crystal structure of BT3781 (pdb code 2P0V), encoded by PUL-Man2, overlayed with the GH125 enzyme *CpGH125*¹⁶ showed a high level of structural similarity with a rmsd of 1.5 Å, consistent with the $\sim 40\%$ amino acid sequence identity between the two proteins. The specificity of BT3781 for mannobiose is entirely consistent with the structure of *CpGH125* in complex with ligands, which revealed only two sugar binding subsites, -1 and +1. The structural data also show that the O2 and O3 of Man bound at -1 and +1 of *CpGH125*, and by analogy BT3781, are buried in the binding sites, explaining why the *Bacteroides* enzyme is unable to accommodate side chains, **Extended Data Fig. 3E**.

5.2 GH76 endo- α 1,6-mannanase: NMR analysis of BT3792 catalyzed reactions showed that the enzyme cleaved mannosidic linkages with retention of anomeric configuration, **Extended Data Fig. 4E**. These data indicate that BT3792, and by inference all members of GH76, hydrolyze glycosidic linkages through an acid-base assistant double displacement (retaining) mechanism, which is entirely consistent with the transglycosylating activity observed for the fungal GH76 enzymes⁵⁴. The crystal structure of BT3792 showed that the enzyme displays an α_6/α_6 -fold, **Extended Data Fig. 3A**. Perpendicular to the orientation of the α -helices is a highly curved cleft-like structure, which would support the conformation adopted by α -mannan. In the centre of the cleft are two highly conserved aspartates within the GH76 family, Asp258 and Asp259, that are essential for catalytic activity and are separated by $\sim 5 \text{ Å}$, **Extended Data Fig. 3A** and **Supplementary Table 2**, which is optimal for the catalytic carboxylate residues of retaining glycoside hydrolases⁵⁵. These data indicate

that the highly curved cleft, which is conserved in the structure of the two other GH76 proteins currently available, **Extended Data Fig. 3A**, comprises the substrate binding site in which Asp258 and Asp259 are the catalytic residues.

5.3 GH99 endo- α 1,2-mannosidase: GH99 endo- α 1,2-mannosidase BT3862²⁹, and the GH92 α -1,3-mannosidase BT3858¹⁷, display activities that could, potentially, contribute to side chain removal. Consistent with its mammalian homologs, BT3862 attacks the α 1,2-linkage in the sequence Glc- α 1,3-Man- α 1,2-Man-R, however, it is possible that the enzyme could also recognize Man- α 1,3-Man- α 1,2-Man-R, which decorates the mannan backbone. This hypothesis was supported by the observation that BT3862 was ~10-fold more active on methylumbeliferyl-Man- α 1,3-Man than methylumbeliferyl-Man- α 1,3-Glc, while the enzyme was able to hydrolyze yeast mannan side chains capped with α 1,3-Man (Man- α 1,3-Man- α 1,2-Man- α 1,2-Man-CH₃), **Supplementary Table 2**. Intriguingly, despite displaying a strong preference for Man- α 1,3-Man¹⁷, BT3858 did not attack wild type yeast mannan whose side chains are generally capped with α -1,3-linked Man³⁰, suggesting that steric constraints prevent access to the α -1,3-Man terminal sugars (BT3858 was active against Man- α 1,3-Man- α 1,2-Man- α 1,2-Man-CH₃). Thus, the likely role of the α 1,3-mannosidase activity displayed by BT3858 is to remove the disaccharide generated by BT3862. It is interesting to note that the capacity of BT3858 to hydrolyze the reaction product of the GH99, α -1,3-mannobiose, but not the intact substrate for the endo- α 1,2-mannosidase in *S. cerevisiae* mannan, shows how these two enzymes have evolved to function synergistically, rather than competing for the same substrate (side chains in which the terminal α 1,3-Man linkage has been removed are not substrates for BT3862). It is also, likely significant that the GH99 enzyme is catalytically slow, which is consistent with the emerging model in which the bulk of glycan degradation occurs in the periplasm; the strategy for polysaccharide deconstruction on the surface is to minimize sugar loss, while generating molecules that can be imported through the SusC porins.

In contrast to mammalian GH99 endo-mannosidases that remove Glc- α 1,3-Man from N-glycans, the bacterial orthologs, described above, display a preference for Man- α 1,3-Man, consistent with their target substrate, yeast mannan. To explore the mechanism for α 1,3-Man specificity at the -2 subsite, the structure of a close homolog to BT3862, BxGH99, was solved in complex with the transition state mimic Man- α 1,3-isofagamine bound at subsites -1 and -2, and α 1,2-mannobiose located at subsites +1 and +2. Man. The data, **Extended Data Fig. 3C**, show that polar and hydrophobic interactions with Man at the -2 subsite are

conserved in the two *Bacteroides* enzymes. The sugar preference at the -2 subsite, however, is not mediated through interactions with the axial O2 of Man, but through repulsion of the equatorial O2 of Glc by Trp126. Interestingly, in bacterial GH99 enzymes the amino acid at position 126 is mainly a Trp or Tyr, while in eukaryotic members of the family it is invariably a Tyr. The phenolic amino acid is predicted to H-bond with the O2 of a glucoside, conferring a preference for Glc at the -2 subsite, consistent with the role of eukaryotic endo- α 1,2-mannosidases in glycoprotein processing in the Golgi apparatus. At the +2 subsite the O1 and O6 of the bound Man bound is solvent exposed, explaining how the enzyme can attack substrate where the sugar at the distal positive subsite is a component of the α 1,6-linked mannan backbone.

5.4 Sugar 6-phosphatases: PUL-Man1 and PUL-Man2 contain homologous proteins, BT2630 and BT3783, respectively that display sequence similarity with proteins in the Exonuclease-Endonuclease-Phosphatase (EEP) domain superfamily⁵⁶. Consistent with this sequence similarity, both BT2630 and BT3783 were shown to be phosphatases active against Man-6-P and Glc-6-P, displaying similar catalytic efficiency for the two substrates, but were inactive against mannose-1-phosphate, glucose-1-phosphate and fructose-2-phosphate, **Supplementary Table 3**. It is apparent, therefore, that both BT3783 and BT2630 are sugar-6-phosphatases that are magnesium dependent; the two enzymes are inactive in the presence of EDTA and activity is only fully recovered by the addition of excess magnesium ions, **Supplementary Table 3**. The observed metal dependency is consistent with the crystal structure of BT3783, which shows a canonical metal binding site, occupied by magnesium, at the presumed active site, **Extended Data Fig. 3G**. In the context of yeast mannan, these enzymes are predicted to cleave the mannose-6-phosphate linkages, however, neither enzyme releases mannose from intact mannan where the phosphate makes a 1-PO₄-6 bridge between two Man residues. This is consistent with the observation that in a structural homolog of BT3783 (PDB 4GYZ; tyrosyl-DNA phosphodiesterase) the “free” O-groups of the phosphate are all making polar interactions with the enzyme and thus steric constraints would prevent the accommodation of a phosphodiester bond.

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Supplementary Table 1 Sequence identify of proteins encoded by core PUL-Man1 and core PUL-Man2

PUL-Man1 proteins	PUL-Man2 proteins	Sequence identity (%)
BT2623 (GH76 mannanase)	BT3792 (GH76 mannanase)	37
BT2625 (SusD homolog)	BT3789 (SusD homolog)	26
BT2626 (SusC homolog)	BT3788 (SusC homolog)	40
BT2628 (HTCS) ^a	BT3781 (HTCS)	81
BT2629 (GH92 mannosidase)	BT3784 (GH92 mannosidase)	91
BT2631 (GH76 mannanase)	BT3792 (GH76 mannanase)	87
BT2632 (GH125 mannosidase)	BT3781 (GH125 mannosidase)	92

^a: Hybrid two component system

Supplementary Table 2. Kinetic parameters of mannan degrading enzymes

Enzyme	CAZy family	Substrate	K_M (mM) ^a	k_{cat} (min ⁻¹)	k_{cat}/K_M (min ⁻¹ mM ⁻¹) ^a
BT2623	GH76	Mannan (mnn2) ^b	1.9±0.4	17820±1200	9378
BT2631	GH76	Mannan (mnn2)	nd	nd	44580±480
BT3782	GH76	Mannan (mnn2)	0.43±0.2	42300±1320	98372
BT3792	GH76	Mannan (mnn2)	2.4±1.1	75780±1262	31575
BT2623, BT2631, BT3782, BT3792	GH76	Mannans (wild type, mnn1 and mnn5) ^b	No activity detected		
BT3792:D167A	GH76	Mannan (mnn2)	No activity detected		
BT3792:D168A	GH76	Mannan (mnn2)	No activity detected		
BT2632	GH125	α 1,6-Mannobiose	0.43±0.04	1777±44	4133
BT3781	GH125	α 1,6-Mannobiose	0.34±0.03	1051±22	3091
BT2632	GH125	α 1,6-Mannotriose	0.53±0.07	2613±132	4930
BT3781	GH125	α 1,6-Mannotriose	0.59±0.05	2313±90	3920
BT2632	GH125	α 1,6-Mannotetraose	0.67±0.07	1260±60	1881
BT3781	GH125	α 1,6-Mannotetraose	0.42±0.06	1239±59	2960
BT3862	GH99	α 1,3- α 1,2- α 1,2-Man ₄ ^{ch}	2.6	180	70
BT3862	GH99	Glc- α 1,3-Man-Umbe ^{dh}	nd	nd	0.0042
BT3862	GH99	Man- α 1,3-Man-Umbe ^h	1.3	0.069	0.038
BT3858	GH92	α 1,3- α 1,2- α 1,2-Man ₄ ^h	nd ^g	nd	47
BT3858	GH92	α 1,3-Mannobiose ^h	nd	nd	53
BT3774	GH38	α 1,2-Mannobiose ^h	3.8±0.57	22.7±1.2	6.0
BT3774	GH38	α 1,3-Mannobiose ^h	8.8±4.5	24.0±5.0	2.7
BT3774	GH38	α 1,6-Mannobiose	13.8±3.8	10.6±1.4	0.8
BT2620	GH97	4-Nitrophenyl- α -Gal	nd	nd	56
BT3994	GH92	α 1,6-Mannobiose	No activity detected		
BT3994	GH92	α 1,6-Mannotriose	No activity detected		
BT3994	GH92	Man ₃ GlcNAc ₂ ^e	nd	nd	165
BT3994	GH92	Man ₅ GlcNAc ₂ ^f	nd	nd	391

^a The K_M for yeast mannan in both kinetic parameters is in mg ml⁻¹

^b Mannan (mnn2) is yeast mannan, isolated from a *S. cerevisiae* mutant, that comprises an undecorated α 1,6-Man-linked polysaccharide. The variants mnn1 and mnn5 contain truncated side chains ¹⁵.

^c α 1,3- α 1,2- α 1,2-Man₄ corresponds to Man- α 1,3-Man- α 1,2-Man- α 1,2-Man-1-CH₃.

^d Umbe corresponds to a 4-methyl-umbelliferyl

^e Man₃GlcNAc₂ is the core structure of N-glycans lacking α 1,3-Man and α 1,2-Man residues.

^f Man₅GlcNAc₂ is the core structure of N-glycans lacking α 1,2-Man residues

^g nd; not determined.

^h Kinetic parameters were determined from single non-linear (k_{cat} and K_M shown) or linear (k_{cat}/K_M only) regression plots due to limited substrate availability. All other experiments were technical triplicates.

Supplementary Table 3 Kinetic parameters of PUL-Man1 and PUL-Man2 encoded phosphatases

Phosphatase	Substrate	Metal ^c	K_M (mM)	k_{cat} (min ⁻¹)	k_{cat}/K_M (min ⁻¹ mM ⁻¹)
BT3783	Man-6-P	Mg ²⁺	41.3 ± 19.0	16314.0 ± 987.0	395.0
BT3783	Glu-6-P	Mg ²⁺	17.0 ± 2.4	1620.0 ± 78.0	95.3
BT2630	Man-6-P	Mg ²⁺	21.1 ± 2.6	35.0 ± 2.8	1.7
BT2630	Glu-6-P	Mg ²⁺	31.5 ± 6.3	28.1 ± 3.8	1.0
BT3783 or BT2630	Man-1-P, Glu-1-P, Fru-6-P	Mg ²⁺	No activity detected		
Phosphatase ^a	Substrate ^b	EDTA-treated enzyme in excess of metal ^c		Relative specific activity (μmol product/μmol enzyme/min) ^d	
BT3783	Man-6-P	Mg ²⁺		1 (0.32)	
BT3783	Man-6-P	Co ²⁺		0.091 (0.031)	
BT3783	Man-6-P	Zn ²⁺		0.0091 (0.0030)	
BT3783	Man-6-P	Mn ²⁺		0.059 (0.019)	
BT3783	Man-6-P	Ni ²⁺		0.056 (0.18)	
BT3783	Man-6-P	Ca ²⁺		0.025 (0.0080)	
BT3783	Man-6-P	No metal added back			
BT3783	Man-6-P	Cs ²⁺ , Cu ²⁺ , Li ²⁺ , Cd ²⁺		No activity detected	
BT3783	Glu-6-P	Mg ²⁺		1.0 (0.151)	
BT3783	Glu-6-P	Co ²⁺		0.041 (0.0062)	
BT3783	Glu-6-P	Zn ²⁺		0.041 (0.0062)	
BT3783	Glu-6-P	Mn ²⁺		0.020 (0.0034)	
BT3783	Glu-6-P	Ni ²⁺		0.18 (0.026)	
BT3783	Glu-6-P	Ca ²⁺		0.057 (0.0084)	
BT3783	Glu-6-P	No metal added back			
BT3783	Glu-6-P	Cs ²⁺ , Cu ²⁺ , Li ²⁺ , Cd ²⁺		No activity detected	
BT2630	Man-6-P	Mg ²⁺		1 (1.675)	
BT2630	Man-6-P	Co ²⁺		0.55 (0.92)	
BT2630	Man-6-P	Mn ²⁺		0.27 (0.46)	
BT2630	Man-6-P	Ca ²⁺		0.059 (0.10)	
BT2630	Man-6-P	No metal added back			
BT2630	Man-6-P	Zn ²⁺ , Ni ²⁺ , Cs ²⁺ , Cu ²⁺ , Li ²⁺ , Cd ²⁺		No activity detected	
BT2630	Glu-6-P	Mg ²⁺		1 (11.85)	
BT2630	Glu-6-P	Co ²⁺		0.0081 (0.10)	
BT2630	Glu-6-P	Ni ²⁺		0.0042 (0.05)	
BT2630	Glu-6-P	No metal added back			
BT2630	Glu-6-P	Zn ²⁺ , Mn ²⁺ , Ca ²⁺ , Cs ²⁺ , Cu ²⁺ , Li ²⁺ , Cd ²⁺		No activity detected	

^aEnzyme was treated with 5 mM EDTA and then purified from the chelating agent by gel filtration using a PD10 column.

^bThe activity of BT3783 was determined with Man-6-P at 20 mM or Glc-6-P at 4 mM. BT2630 was assayed using Man-6-P at 9 mM or Glc-6-P at 25 mM

^cThe metals were at 5 mM except Zn²⁺ that was at 0.25 mM.

^dThe values in *parenthesis* are relative to the activity of the enzyme in the presence of Mg^{2+} , which was assigned a value of 1.0. These data were from single assays. The assays used to determine kinetic parameters were technical triplicates.

Supplementary Table 4. Activity of GH76 mannanases against manno oligosaccharides

Oligosaccharide d.p.	k_{cat}/K_m ($M^{-1} \text{ min}^{-1}$)			
	BT2623s	BT2631p	BT3792s	BT3782p
3 ^a	No Activity	$1.0 (0.10) \times 10^3$	No Activity	$5.6 (0.20) \times 10^3$
4 ^a	$2.2 (0.1) \times 10^2$	$1.3 (0.002) \times 10^5$	$1.8 (0.03) \times 10^3$	$5.8 (0.30) \times 10^5$
5 ^a	$7.2 (0.1) \times 10^2$	$1.3 (0.07) \times 10^6$	$2.4 (0.05) \times 10^3$	$3.9 (0.18) \times 10^6$
6 ^b	$1.8 (0.13) \times 10^4$	$1.2 (0.05) \times 10^6$	$1.1 (0.06) \times 10^5$	$6.1 (0.16) \times 10^6$
7 ^b	$5.0 (0.37) \times 10^4$	$1.7 (0.18) \times 10^6$	$1.3 (0.10) \times 10^6$	$4.5 (0.23) \times 10^6$
8 ^b	$1.5 (0.06) \times 10^5$	$1.9 (0.13) \times 10^6$	$1.5 (0.13) \times 10^6$	$1.0 (0.06) \times 10^7$

The surface (s) or periplasmic (p) location of the enzymes is indicated.

^a The mean of duplicate technical replicates with standard error of the mean in *parenthesis*.

^b Single assays. Errors in *parenthesis* are derived directly from the fit to the linear regression line.

Supplementary Table 5: Affinities of BT3984 and BT3986 for HMNG-derived ligands

Ligand	$K_a \times 10^3$ (M^{-1})	ΔH (kcal mol^{-1})	$T\Delta S$ (kcal mol^{-1})
BT3984			
GlcNAc	0.85 ± 0.07	-11.29 ± 0.43	-7.78
GlcNAc ₂	0.93 ± 0.05	-10.56 ± 0.39	-6.71
LacNAc	1.41 ± 0.10	-7.59 ± 0.26	-3.28
GlcNAc + EDTA	1.15 ± 0.08	-9.02 ± 0.31	-4.86
BT3986			
D-Mannose	0.81 ± 0.07	-11.97 ± 0.59	-8.25
D-Mannose + EDTA	0.87 ± 0.04	-8.75 ± 0.19	-4.74
α -1,2-mannobiose	1.15 ± 0.04	-11.97 ± 0.21	-7.81
α -1,3-mannobiose	0.76 ± 0.13	-9.30 ± 0.72	-5.75
α -1,6-mannobiose	0.91 ± 0.05	-12.52 ± 0.61	-8.73
Man3(A)	3.04 ± 0.15	-7.95 ± 0.20	-3.19
Man3(B)	2.54 ± 0.17	-11.09 ± 3.31	-6.44

The ITC data were fitted to a single site binding model for all ligands. Man3(A) and Man3(B) comprise α 1,3-linked Man appended to the non-reducing and reducing end mannose, respectively, of Man- α 1,6-Man. The errors are to the fit of the binding curves.

Supplementary Table 6: X-ray data collection, processing and model refinement statistics.

	BT3783	BT3792	BxGH99-Man- IFG-mannobiose
Data collection			
Space group	P2 ₁	P2 ₁	I4
Cell dimensions			
<i>a</i> , <i>b</i> , <i>c</i> (Å)	65.4, 148.8, 71.5	100.1, 44.4, 103.1	108.5, 108.5, 67.8
α , β , γ (°)	90.0, 117.1, 90.0	90.0, 101.4, 90.0	90.0, 90.0, 90.0
Resolution (Å)	40-2.10 (2.21- 2.10)*	48-2.10 (2.21- 2.10)*	40.0 – 1.30 (1.32 – 1.30)*
<i>R</i> _{merge}	0.117 (0.442)	0.113 (0.476)	0.042 (0.934)
<i>I</i> / σ <i>I</i>	7.8 (3.3)	10.4 (3.3)	18.8 (1.8)
Completeness (%)	99.9 (100)	99.9 (99.9)	100.0 (100.0)
Redundancy	3.6 (3.6)	4.2 (4.2)	6.6 (6.5)
Refinement			
Resolution (Å)	2.10	2.10	1.30
No. reflections	67122	49817	91564
<i>R</i> _{work} / <i>R</i> _{free}	0.16 / 0.21	0.21 / 0.25	0.11 / 0.14
No. atoms			
Protein	8650	6085	2951
Ligand	4	n/a	44
Water	824	351	511
B-factors			
Protein	21.6	24.1	18.4
Ligand/ion	19.2	n/a	17.8
Water	30.5	26.7	38.7
R.m.s deviations			
Bond lengths (Å)	0.013	0.014	0.010
Bond angles (°)	1.529	1.597	1.52

*Highest resolution shell is shown in parentheses.