Human gut Bacteroidetes can utilize yeast mannan through a selfish mechanism

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Yeasts, which have been a component of the human diet for at least 7,000 years, possess an elaborate cell wall α–mannan. The influence of yeast mannan on the ecology of the human microbiota is unknown. Here we show that yeast α–mannan is a viable food source for the Gram-negative bacterium Bacteroides thetaiotaomicron, a dominant member of the microbiota. Detailed biochemical analysis and targeted gene disruption studies support a model whereby limited cleavage of α–mannan on the surface generates large oligosaccharides that are subsequently depolymerized to mannosyl by the action of periplasmic enzymes. Co-culturing studies showed that metabolism of yeast mannan by B. thetaiotaomicron presents a ‘selfish’ model for the catabolism of this difficult to breakdown polysaccharide. Genomic comparison with B. thetaiotaomicron in conjunction with cell culture studies show that a cohort of highly successful members of the microbiota has evolved to consume sterically-restricted yeast glycans, an adaptation that may reflect the incorporation of eukaryotic microorganisms into the human diet.

The microbial community in the human large bowel, the microbiota1–2, is central to the health and nutrition of its host3–6. Glycan utilization is a key evolutionary driver underpinning the structure2–4 of this microcosm1–2, with the Bacteroidetes playing a dominant role in this process. The genomes of Bacteroidetes contain polysaccharide utilization loci (PULs)7 that encode the apparatus required to utilize complex carbohydrates, with each PUL orchestrating the degradation of a specific glycan.

The microbiota contains a cohort of bacteria that target α–mannosidic linkages (based on sequence similarity to glycoside hydrolase families (GHs) that are populated by α–mannosidases and α–mannanases8–9, indicating that α–mannose-containing glycans, such as those from yeast and other fungal α–mannans, are significant nutrients for these microbes (see Supplementary Information section 1.0). Furthermore, these glycans are implicated in the immunopathology of the inflammatory bowel disease, Crohn’s disease10,11 (Supplementary Information section 2.0). The genome of Bacteroides thetaiotaomicron, a dominant member of the microbiota, encodes 36 proteins predicted to display α–mannosidase or α–mannanase activity12. Here we unravel the mechanism by which B. thetaiotaomicron metabolizes the major α–mannose-containing glycans presented to the large bowel. The data also show that B. thetaiotaomicron expresses a specific yeast α–mannan degrading system that is distinct from the high mannose mammalian N-glycan (HMNG) depolymerizing apparatus.

Yeast α–mannan utilization by B. thetaiotaomicron

B. thetaiotaomicron utilizes α–mannan as a sole carbon source and transcriptional studies identified three B. thetaiotaomicron PULs (MAN-PUL1, MAN-PUL2 and MAN-PUL3; Fig. 1a) that were activated by α–mannan from Saccharomyces cerevisiae13, Schizosaccharomyces pombe and the pathogenic yeast Candida albicans (Fig. 1b and Extended Data Fig. 1a, b). B. thetaiotaomicron mutants lacking MAN-PUL2 or MAN-PUL1/2/3 were unable to grow on yeast mannan in vitro, (Extended Data Fig. 1c). In gnotobiotic mice fed a diet lacking glycans the B. thetaiotaomicron mutant ΔMAN-PUL1/2/3 outcompeted the wild-type bacterium, whereas wild-type B. thetaiotaomicron was the dominant species in rodents fed a yeast-mannan-rich diet, (Fig. 1c). These data underscore the importance of MAN-PUL1/2/3 when B. thetaiotaomicron is exposed to yeast mannan in vivo, while the intriguing dominance of the mutant strain in animals fed a polysaccharide-free diet is further considered in Supplementary Information section 3.2. To explore whether B. thetaiotaomicron degraded α–mannan and HMNG13 by distinct enzyme systems, the PULs activated by a HMNG, Man8GlcNAc2, were evaluated. A single PUL was activated by Man8GlcNAc2, which was distinct from MAN-PUL1/2/3 (Fig. 1c), demonstrating that degradation, and thus utilization, of α–mannan and HMNG are carried out by different PULs.

Analysis of the growth profiles of 29 human gut Bacteroidetes species revealed that nine species metabolized S. cerevisiae α–mannan, with 33 out of 34 strains of B. thetaiotaomicron growing on the glycan (Fig. 1d). These data show that B. thetaiotaomicron, along with some of its phylogenetically related neighbours, dominates yeast mannan metabolism in the Bacteroidetes phylum of the microbiota. Within fully sequenced genomes of 177 microbiota members, the presence of GHs integral to α–mannan degradation (GH38, GH92 and GH76) are restricted to five species of Bacteroides and three of Parabacteroides; no Firmicutes contain this grouping of glycoside hydrolases4.
but present in other fungal
organisms. For example, MAN-PUL1 contains an α-galactosidase, BT2620, which targets α-galactosyl linkages absent in S. cerevisiae mannan but present in other fungal α-mannans such as the yeast Schizosaccharomyces pombe14 (Extended Data Fig. 1d), explaining why inactivation of MAN-PUL1 affects growth of B. thetaitaomicrocin on this polysaccharide (Extended Data Fig. 1b). Functional diversity is also evident in MAN-PUL2, which, in addition to its catabolic role, encodes glycosyltransferases that mediate synthesis of the trisaccharide Man-α,1,6-Man-backbone by the surface endo-α-1,6-mannanases BT2623 and BT3792. The activity profiles of BT2199 and the surface endo-acting mannanases are consistent with the observed production of large oligosaccharides by non-growing cells of B. thetaiotaomicron incubated with yeast mannan (Extended Data Fig. 4b, c).

Mannan degradation at the cell surface and periplasm

The enzymatic degradation of α-mannan is restricted sterically to the periplasm where the side chains were removed by the synergistic action of α-mannosidases and sugar-6-monophosphatas15 (Fig. 2d, e). The MAN-PUL3-encoded endo-α,1,2-mannosidase BT3862 released a limited proportion of the terminal Man-α,1,6-Man disaccharides, thereby assisting in exposing the backbone. The structural basis for the specificity displayed by BT3862 is described in Extended Data Fig. 3c, d. In addition to BT3862, other surface enzymes are required to partially expose the α-1,6-Man backbone. Screening for such enzymes revealed an α-mannosidase, BT2199, that mediates very limited extracellular removal of side chains (Extended Data Fig. 4a) and thus facilitates limited cleavage of the mannan backbone by the surface endo-α-1,6-mannanases BT2623 and BT3792. The activity profiles of BT2199 and the surface endo-acting mannanases are consistent with the observed production of large oligosaccharides by non-growing cells of B. thetaiotaomicron incubated with yeast mannan (Extended Data Fig. 4b, c).

The cellular location of the key α-mannan hydrolysing enzymes (Fig. 2b, c) indicates that the polysaccharide was degraded primarily in the periplasm where the side chains were removed by the synergistic action of α-mannosidases and sugar-6-monophosphatas15 (Fig. 2d, e). The MAN-PUL3-encoded endo-α,1,2-mannosidase BT3862 released a limited proportion of the terminal Man-α,1,6-Man disaccharides, thereby assisting in exposing the backbone. The structural basis for the specificity displayed by BT3862 is described in Extended Data Fig. 3c, d. In addition to BT3862, other surface enzymes are required to partially expose the α-1,6-Man backbone. Screening for such enzymes revealed an α-mannosidase, BT2199, that mediates very limited extracellular removal of side chains (Extended Data Fig. 4a) and thus facilitates limited cleavage of the mannan backbone by the surface endo-α-1,6-mannanases BT2623 and BT3792. The activity profiles of BT2199 and the surface endo-acting mannanases are consistent with the observed production of large oligosaccharides by non-growing cells of B. thetaiotaomicron incubated with yeast mannan (Extended Data Fig. 4b, c).

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products (Extended Data Fig. 6a–c). Consistent with these product profiles, the periplasmic enzymes were ~100- to 1,000-fold more active than the surface mannanases against oligosaccharides with a degree of polymerization (d.p.) < 6, whereas against large oligosaccharides (d.p. ≥ 6) the periplasmic and surface enzymes displayed broadly similar activities (Fig. 2f and Supplementary Table 4). The linear and hence unadorned α-1,6-mannooligosaccharides generated in the periplasm were hydrolyzed by the exo-α-1,6-mannosidases BT2632 and BT3781 (Extended Data Fig. 6d, e and Supplementary Table 2), as previously proposed16. The inability of the backbone-cleaving enzymes to attack wild-type yeast α-mannan indicates that these glycoside hydrolysases cannot accommodate α-1,6-linked Man decorated at O2 (side chains are appended α-1,2 to the mannann backbone) in the active site or proximal subsites, consistent with the structures of the substrate binding clefts of BT3792 and BT3781 (Extended Data Fig. 3a–f).

A *B. thetaiotaomicron* PUL for HMNG catabolism

HMNG-PUL encodes four enzymes and two surface glycan binding proteins (Extended Data Fig. 7a). BT3990 and BT3991 target α-1,2-Man and α-1,3-Man linkages, respectively, in HMNGs17. The terminal undecorated α-1,6-Man exposed by BT3990 is hydrolysed by BT3994, which requires GlcNAC at the reducing end for activity, producing Man-α-1,6-Man-β1,4-GlcNAC (Extended Data Fig. 7b). BT3987 is a surface endo-acting N-acetylglucosaminidase, which cleaves the oligosaccharide from its polypeptide (Extended Data Fig. 7c, d). The released HMNG is held on the surface of *B. thetaiotaomicron* through the mannose-binding protein BT3986, while the SusD homologue BT3984, by recognizing GlcNAC at the reducing end of the glycan (Extended Data Fig. 7e, f and Supplementary Table 5), probably orientates the glycan into the outer membrane porin (SusHomologue BT3983) for transport into the periplasm, where the periplasmic α-mannosidases hydrolyse the oligosaccharide into a trisaccharide that is degraded by enzymes that are not encoded by HMNG-PUL. A model for the enzymatic degradation of HMNG is displayed in Fig. 4. The importance of HMNG-PUL in the metabolism of the N-glycan is consistent with the reduced growth of the Δhbm3993 mutant (lacking the extra-cellular σ factor regulator of HMNG-PUL) cultured on ManαGlcNAC2 (Extended Data Fig. 7g).

Yeast α-1-mannan degradation by *B. thetaiotaomicron*

Substrate promiscuity is a hallmark of the α-mannan degradation apparatus of *B. thetaiotaomicron*, as steric constraints limit enzymatic access to the glycan side chains, and consequently the polysaccharide backbone. Accordingly, *B. thetaiotaomicron* uniquely uses surface α-mannosidases(s), probably BT2199, to generate limited, but sufficient, side chain removal for the surface endo-acting enzymes to mediate infrequent cleavage of the backbone. The *B. thetaiotaomicron* mannan-degrading apparatus

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**Figure 2 | Mannan PULs enable colonization of gnotobiotic mice; key biochemical and cellular features of the encoded enzymes.** a. Colonization of gnotobiotic mice (n = 5) by wild type *B. thetaiotaomicron* (red) and a mutant (black) lacking MAN-PUL1/2/3 (AMAN-PUL1/2/3). On day 0 mice were gavaged with ~10^6 colony-forming units (c.f.u.) of 50:50 of the two *B. thetaiotaomicron* strains and then fed a control diet lacking *B. thetaiotaomicron*-digestible glycans (green). Left, after 7 days the control diet was supplemented with 1% YM in drinking water (pink shading). Middle, identical treatment as to the left panel except no mannan was included after day 7 (shaded green). At day 21 mannan in the water was switched between the groups in the left and middle panels, indicated by the colour panels (pink = mannan, green = no mannan). Right, mice fed the control diet (shaded green) were switched to a diet containing leavened bread (blue). The average abundance of the mannann utilization mutant (black) in mice fed the bread diet compared to the corresponding time points in mice fed the glycan-free diet (mean ± s.e.m., 81% ± 1.2% in animals fed the bread diet versus 90% ± 1.7% in mice fed the glycan-free diet; P = 0.00005 by unpaired Student’s t-test). Time points at which there was a significant difference between the mutant and wild type *B. thetaiotaomicron* on the bread diet compared to the glycan-free diet (P ≤ 0.05) are indicated with an asterisk. b. Fluorescent- and light-microscopy images of *B. thetaiotaomicron* incubated with polyclonal antibodies against BT3792 and BT3774. c. Western blots of *B. thetaiotaomicron* cells cultured on yeast mannan that were untreated with proteinase K (0 h) or incubated with 2 mg ml^-1 proteinase K for 16 h. RP, recombinant protein. Blots were probed with antibodies against the *B. thetaiotaomicron* enzymes. Localization blots and microscopy images (b, c) are representative data from three biological replicates. d. Phosphorylated high mannose N-glycan MNN4 and RNAase B incubated with BT2629 (GH92 α-mannosidase), BT3783 (phosphatase) or BT3774 (GH38 α-mannosidase) and the products analysed by capillary electrophoresis. Man₅ to Man₉ (subscript numbers refer to the number of Man units in the N-glycans) lack phosphate and mannose-1-phosphate groups. P* indicates the N-glycan contains a single phosphate, which is in the following structure: Man-α-1-phosphate-α-6-Man-α-1,2-Man. e. Catalytic efficiency of the endo-α-1,6-mannanases against α-1,6-mannooligosaccharides (see Supplementary Information Table 4 for full data). D.P., degree of polymerization of the mannooligosaccharides.
is optimized to minimize nutrient loss, illustrated by the observation that no oligosaccharides were released into the culture supernatant when *B. thetaiotaomicron* was cultured on \( \alpha \)-mannan (Extended Data Fig. 4c).

Enzymes able to attack \( \alpha \)-1,6-mannan in a periplasmic location are central to the hierarchical degradation of yeast mannan, reflecting their location and substrate specificities. The surface endo-acting enzymes generate large oligosaccharides minimizing extracellular metabolism and loss to other microbiota members. The periplasmic endo-\( \alpha \)-1,6-mannanases generate numerous short oligosaccharides, maximizing the substrate available to the periplasmic exo-acting \( \alpha \)-1,6-mannosidases. This ‘selfish’ model is consistent with the inability of *B. thetaiotaomicron* to support growth of mannose- and mannose-backbone-using strains of *Bacteroides* on intact *S. cerevisiae* \( \alpha \)-mannan (Fig. 5). It should be emphasized, however, that the degradation of at least some polysaccharides is mediated through synergistic interactions between different members of the microbiota\(^{18,19}\), illustrating the diverse mechanisms by which nutrients are used in this microbial community.

This work provides insights into the adaptation of the microbiota to yeast domestication in the human diet reflecting the regular consumption of yeast-leavened bread, fermented beverages and products such as soy sauce. *B. thetaiotaomicron*, and a limited number of other microbiota-derived Bacteroidetes, have evolved a complex machinery to digest and metabolize yeast cell-wall mannan. Phylogenetic analysis suggests that this trait penetrated the human gut Bacteroidetes at least twice, once each in the *Bacteroides* and *Parabacteroides*. It is also possible, however, that close relatives of *B. thetaiotaomicron* have gained parts of this complex catabolic trait via separate events. Analysis of 250 human metagenomic samples revealed \( \alpha \)-mannan-degrading PULs closely related to those from *B. thetaiotaomicron* in a majority (62%) of subjects (Extended Data Fig. 8) making it more common than culturally-restricted traits like red algal porphyran degradation\(^{20}\), but less common than plant cell-wall xylolucan degrading PULs, identified in divergent *Bacteroides* species\(^{21,22}\) (see Supplementary Information section 3.3).

The \( \alpha \)-mannan degrading capacity of *B. thetaiotaomicron* is consistent with the bacterium’s ‘glycan generalist’ strategy\(^{7,12,23}\), enabling the microorganism to thrive in the competitive environment of the microbiota, where the omnivorous diet of the host requires rapid adaptation to the nutrients presented to the distal gut. Many of the organisms in the microbiota produce enzymes that attack the major components of the human diet such as starch and pectins. An additional survival strategy for some members of the microbiota, such as *B. thetaiotaomicron*, is the targeting of low-abundance, highly complex dietary glycans that are not metabolized by most other organisms, exemplified here by yeast mannan. With respect to the human host the degradation of \( \alpha \)-mannan by *B. thetaiotaomicron* may also be relevant to the health-promoting effects of the human microbiota\(^{24}\). This work provides insights into a sophisticated \( \alpha \)-mannan degrading apparatus that exists...
Figure 5 | Bacteroides co-culture sharing experiments. a–d. B. thetaiotaomicron was co-cultured with Bacteroides cellosolysisicus WH2 (a, b) or with Bacteroides xylanisolvens NLAE-zI-p352 (c, d) with either mannann (a, c) or mannano (b, d) as the sole carbon source. Each non-mannan user was also cultured on mannann independently. The upper graph in each panel depicts the c.f.u. ml⁻¹ of each strain, relative to the c.f.u. ml⁻¹ at inoculation. Total c.f.u. ml⁻¹ was determined by colony counts, and the proportion of each bacteria was determined by qPCR of marker genes from genomic DNA (shown in the lower graph of each panel). Error bars represent s.d. of three biological replicates. Bc, B. cellosolysisicus; Bt, B. thetaiotaomicron; Bx, B. xylanisolvens.

within widespread members of the human microbiota, thereby revealing the impact made by the historical domestication of yeast and other dietary fungi on the structure of this microbial consortium.

Online Content Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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Supplementary Information is available in the online version of the paper.

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Author Contributions Enzyme characterization: F.C., M.J.T., J.L., M.M. and D.W.A. Capillary electrophoresis: D.B., K.P. and W.V. E.C.L. created gene deletion strains and determined phenotypes with F.C., E.C.L. and F.C. performed enzyme localisation. F.C. and E.C.L. carried out the co-culturing experiments. Gene expression analysis: E.A.C., N.P. and E.C.M. Growth analysis on purified manann and GH76: F.C., N.P., K.U. and E.C.M. Characterization of HMNG binding proteins: Y.Z. Characterization of the dβ3774 mutant: A.D. Phylogenetic reconstruction and metagenomic analysis: E.C.M. Gnotobiotic mouse experiments: E.A.C., N.P., N.T.P. and E.C.M. Purification of HMNG: T.J.T., B.S.H. and R.C. Isolation and genomic analysis of pig gut strains: T.A., C.I.Z., A.C. and G.S. performed NMR experiments on GH76 and M.P.J. on gut products. Z.H. and G.S. synthesized substrates. Crystallographic studies by A.J.T., G.J.D., M.D.S., A.B.B. and R.M. Experiments designed by F.C., E.C.L., G.J.D., S.J.W., D.W.A., E.C.M. and H.J.G. The writing team was written primarily by H.J.G. and E.C.M with contributions from S.J.W., G.J.D. and D.W.A., E.C.L. and E.C.M. prepared the figures.

Author Information The protein crystal structures reported in this study have been deposited under the following PDB accession codes: 4CR1 (BT3783-Mg binary complex); 4CS1 (BT3792); and 4UTF (BbGH99-Man-IpF-mannobiose ternary complex). Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to H.J.G. (harry.gilbert@ncl.ac.uk).

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METHODS

Producing recombinant proteins for biochemical assays. DNAs encoding the mature forms used in this study were amplified by PCR using appropriate primers. The amplified DNAs were cloned into Ncol/XhoI, NcoI/BamHI, NdeI/XhoI or NdeI/BamHI restricted pET21a or pET28a, as appropriate.

The recombinant genes generally contained a C-terminal His-tag, where appropriate, the His-tag was located at the N-terminus of the protein. The GH92 enzymes encoded by HMNG-PUL were cloned in a previous study27. The encoded recombinant genes encoding the mannan degrading enzymes, Escherichia coli strains BL21(DE3) or TUNER, containing appropriate recombinant plasmids, were cultured to mid-exponential phase in Luria Bertani broth at 37°C. This was followed by the addition of 1 mM (strain BL21(DE3)) or 0.2 mM (TUNER) isopropyl β-D-galactopyranoside (IPTG) to induce recombinant gene expression, and the culture was incubated for a further 5 h at 37°C or 16 h at 16°C, respectively.

The recombinant proteins were purified to >90% electrophoretic purity by immobilized metal ion affinity chromatography using Talon, a cobalt-based matrix, and eluted with 100 mM imidazole, as described previously27.

Producing recombinant proteins. BT3783, BT3792 and BxGH99 for crystallization. pET28a expression vectors encoding mature BT3783 (residues 27 to 314) and BT3792 (residues 155 to 514) were cloned into pET28a via NheI and XhoI sites. The gene encoding the homologue of GH99 (BxGH99) was constructed from synthesized oligonucleotide fragments (Genscript) and also cloned into pET28a using Ndel and Xhol restriction sites. Plasmids encoding BT3783 and BT3792 were transformed into E. coli BL21(DE3) Star chemically competent cells, and grown in LB broth at 37°C supplemented with 50 µg ml⁻¹ kanamycin. Production of recombinant BT3783 was induced by the addition of 0.2 mM IPTG at a culture OD₆₀₀ = 0.6, and incubation at 16°C for 16h. BT3793 was produced using the auto-induction method28 by shaking inoculated 0.5-l cultures supplemenated with 0.6% (w/v) Triton-X, 0.6% (w/v) deoxycholate, 20 mM Tris-HCl (pH 7.5) and then 48 h at 20°C. E. coli BL21 (DE3) cells harbouring the BxGH99-encoding plasmid were cultured in 0.5 l ZYM-5052 auto-induction media29, supplemented with 50 µg ml⁻¹ kanamycin, at 37°C for 8h, with induction occurring overnight at 16°C.

To purify BT3783 and BT3792, cells were collected by centrifugation and disrupted by chemical lysis procedure at 4°C. In brief, cells were resuspended in 25 ml of a solution consisting of 7% (w/v) sucrose, 50 mM Tris-HCl (pH 7.5), for 5 min. Lysozyme (Sigma-Aldrich, 10 mg) was then added and stirred for 10 min. A solution (50 ml) consisting of 0.6% (w/v) Triton-X, 0.6% (w/v) deoxycholate, 20 mM Tris-HCl (pH 7.5) was added and stirred continuing, after which 5 ml MgCl₂ was added followed by addition of DNase (Sigma-Aldrich) to a final concentration of 8.5 µg ml⁻¹.

The resulting solutions were then centrifuged for 45 min at 13000g. Similarly, cells harbouring BxGH99 were collected and resuspended in 50 mM NaH₂PO₄ (pH 8.0), 300 mM NaCl, and lysed by sonication.

BT3783 clarified cell lysates were purified by nickel Sepharose affinity chromatography by stepwise elution with imidazole. Positive fractions identified by SDS gel electrophoresis were dialysed into 20 mM Tris (pH 8.0) and concentrated to 11.25 mg ml⁻¹ with an Amicon stirred ultrafiltration unit model 8200. BT3792 clarified cell lysates were purified using nickel-affinity chromatography by immo-

Glucose-6-phosphate, generated from the released mannos, is oxidized by glucose-6-phosphate dehydrogenase with concomitant reduction of NADP⁺ to NADPH, which was monitored at 340 nm using an extinction coefficient of 6223 M⁻¹ cm⁻¹. The polysaccharide substrates used were S. cerevisiae α-mannans from wild-type or appropriate mutant strains that produce variants of the polysaccharide (Extended Data Fig. 5); these mannans were purified from stationary-phase cultures of the yeast grown in yeast extract peptone dextrose medium, as described previously25. The α-1,6-mannooligosaccharides, which were also used as substrates, were generated as follows: 1 g α-mannan from the S. cerevisiae mutant mnn2 (comprises the α-1,6-mannan backbone with no side chains) was digested to completion with the endo-mannannase BT3792. The products were freeze-dried and the small mannooligosaccharides (d.p. 2 to 5) were purified on two P2 Bio-gel columns set up in series, while the large oligosaccharides, with a d.p. of 6 to 8, were fractionated on two P4 Bio-gel columns also in series. The columns were run at 0.2 ml min⁻¹ in distilled water. The 5–6 ml fractions were evaluated by thin-layer chromatography (TLC) and those containing the same oligosaccharide were pooled.

The activity of the endo-

The specificity of enzymes against phosphorylated and neutral high mannose glycan binding proteins, following the method described in ref. 27 with the target polysaccharide at 1 mg ml⁻¹, was determined in 50 mM Na-HEPES buffer (pH7.5) containing an appropriate concentration of the polysaccharide (ranging from 0.1–6 mg ml⁻¹) and 1 mg ml⁻¹ BSA. Reactions were incubated for 30 min at 37°C and, at regular time intervals, 500-μl aliquots were removed and the amount of reducing sugar was quantified using the dinitrosalicylic acid reagent30 and a standard curve of mannose in the reaction conditions used. TLC was also used to provide a qualitative profile of the mannoooligosaccharides generated by the GH76 endo-mannannases from these reactions. Around 4 μl of the reaction was spotted on silica gel TLC plates and the plates were developed in butanol:acetic acid:water 2:1:1 and carbohydrate products detected by spraying with 0.5% orcinol in 10% sulfuric acid and heating to 100°C for 10 min. Substrate-depletion assays were used to determine the activity of the endo-mannannases against α-1,6-mannooligosaccharides. In brief, 50 μl of the oligosaccharides in 50 mM sodium phosphate buffer (pH7.5), containing 0.1 mg ml⁻¹ BSA (NaP buffer), was incubated with an appropriate concentration of enzyme (10 to 500 μl).

Aliquots were removed at regular intervals for up to 1 h and, after boiling for 10 min to inactivate the enzyme, the amount of the substrate mannoooligosaccharide remaining was quantified by high-performance anion-exchange chromatography (HPAEC) using standard methodology. In brief, the reaction products were bound to a Dionex CarboPac PA-200 column equilibrated with 100 mM NaOH. Mannan and mannoooligosaccharides were eluted with a 0–200 mM sodium acetate gradient in 100 mM NaOH at a flow rate of 0.25 ml min⁻¹, using pulsed amperometric detection. The data were used to determine catalytic efficiency (kcat/kM) as described previously25. To determine the activity of the GH99 endo-α-1,2-mannosidase BT3862, the enzyme was incubated with Man-α-1,3-Man-α-1,2-Man-α-1,2-Man-1-CH₂, and, in combination with the α-1,2-mannosidase BT3990, mannose release was monitored using the continuous assay described above (BT3990 is active on the product of BT3862, Man-α-1,2-Man-α-1,2-Man-1-CH₂). The activity of BT3862 against alym-

The specificity of enzymes against phosphorylated and neutral high mannose N-glycans was assessed by capillary electrophoresis. The methodology was essen-

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—2 μM of enzyme in 10 mM Na-Hepes buffer (pH 7.0) containing 2 mM CaCl₂. The reactions were analysed by capillary electrophoresis with laser-induced fluorescence detection (CE-LIF) using an ABI 3130 capillary DNA sequencer as described previously22.

**Mannosyl-transferase assays.** BT3775 and BT3776 at 30 μM were screened for activity using mannose and all possible η-mannobiose acceptors at a concentration of 10 mM. Reactions were performed in 10 mM Na-Hepes buffer (pH7.5), 10 mM MnCl₂, 50 mM GMP-Mannose at 37 °C over time course intervals of 0 min, 5 min, 15 min, 30 min, 60 min and 16 h. Reactions were stopped by boiling for 10 min. To explore the synergy between the two GT32 glycosyltransferases, reactions were performed with BT3775 and BT3776 simultaneously using mannan as an acceptor, and in a step-wise progression with BT3775 and mannose as the acceptor, followed by BT3776 on the purified products of the initial BT3775 reaction. Enzyme concentrations were maintained in both conditions at 30 μM. MALDI-TOF mass spectrometry of reaction products. To permethylate N-glycan fragments and mannooligosaccharides appropriate enzyme reactions were freeze-dried and suspended in dry dimethylsulfoxide (DMSO; 200 μl). Oligoglycans were per-O-methylated using standard methods23. Freshly prepared sodium hydroxide base in dry DMSO (300 μl) and then iodomethane (150 μl) was added to each sample. The tube was purged with nitrogen and vortexed vigorously. Permethylated oligosaccharides (PMOs) were extracted in 2 ml water. After removing excess iodomethane (CH₂I₂), 2 ml dichloromethane was added and vortexed to extract lipophilic components. The aqueous layer was removed after centrifugation. This was repeated five more times. After the final rinse, the dichloromethane layer was transferred into a fresh tube and dried down under N₂ stream. The samples were then dehydrated and dissolved in 50% aqueous methanol and loaded onto C18 denatured protein. A 200 μl aliquot of the sample was suspended in 200 μl dichloromethane and loaded onto silica plates and resolved in butanol/acetic acid/water buffer. The plates were dried and sugars visualized by orcinol/sulfuric acid heated to 70 °C.

**Cellular localization.** Cultures of B. theitaiotaomicron (100 ml) were grown in minimal media on yeast mannann (0.5% w/v) as a sole carbon source, to mid exponential growth phase (OD₆₀₀nm 0.6–0.8). Cells were harvested by centrifugation, washed and suspended in 10 ml PBS before being resuspended in 5 ml of the buffer. The cells were split into four 1 ml aliquots. To 3 of the aliquots 2 mg ml⁻¹ Proteinase K was added and incubated at 37 °C for 1 h. The fourth sample was left as an untreated control also for 16 h. Following incubation with the protease the samples were centrifuged at 5000g for 10 min and the supernatant discarded. The cell pellets were resuspended in 1 ml PBS and the proteins precipitated by the addition of 200 μl trichloroacetic acid and incubation on ice for 30 min. The precipitated proteins were pelleted by centrifugation and washed 4 times in 1 ml ice-cold aceton. The protein pellets were resuspended in 250 μl Laemmli buffer and subjected to SDS/PAGE. Gels were transferred to Whatman Protran BA 85 nitrocellulose membrane. Proteins of interest were detected using anti-sera raised against the corresponding protein. The secondary antibody used was a chicken anti-rabbit conjugated to horseradish peroxidase. Antibodies were detected by chemi-luminescence using Biorad Clarity Western ECL Substrate.

**Immunofluorescence microscopy.** B. theitaiotaomicron suspensions (OD₆₀₀nm = 0.8) in PBS (pH 7.0) were applied to clean Eppendorf tubes, fixed with an equal volume of 2% formalin (95% formaldehyde in PBS), and rocked for 90 min at 25 °C. The cells were then pelleted by centrifugation for 3 min at 7000g and washed twice with 1 ml of PBS. The bacterial cell pellet was resuspended in 1 ml of blocking solution (2% goat serum, 0.02% NaN₃ in PBS) and incubated at 4 °C for 1 h. After incubation cells were centrifuged again at 7000g and the supernatant discarded. For labelling, the bacteria were incubated with 0.5 ml of primary rat IgG (1/5000 dilution of IgG in blocking solution) for 2 h at 25 °C. The cells were then pelleted, by centrifugation, washed in 1 ml of PBS and resuspended in 0.4 ml goat-anti-rat IgG Alexa-Fluor 488 (Sigma), diluted 1/500 in blocking solution, and incubated 1 h at 25 °C in the dark. The cells were then pelleted, washed with PBS and resuspended in 50 μl of PBS containing ProLong Gold antifade reagent (Life Technologies). Labelled bacterial cells were mounted onto glass slides and secured with coverslips. Fluorescence was visualized using a Leica SP2 UV microscope (Leica Microsystems, Heidelberg, GmbH) with ×63 NA 1.32 lens. Alexa-Fluor 488-labelled bacteria were viewed under an ultraviolet microscope view and compared with the Cy3 channel.

**Constructing mutants of the yeast mannann and HMNG PULs in B. theitaiotaomicron.** The inactivation of the HMNG-PUL extra-cellular factor regulator was described previously24. The other mutants deployed in this study, PUL knockouts and single gene clean deletions, were introduced by allelic exchange using the pEXchange vector as described in ref. 35. The use of quantitative reverse transcriptase PCR to quantify appropriate transcripts followed the methods described in ref. 13.

**Gnotobiote mice experiments.** All animal experiments were approved by the University Committee on Use and Care of Animals at the University of Michigan. Gnotobiotic mice, germ-free and conventional, were conducd in a total of 24, 6–8-week-old male and female Swiss Webster mice (each was considered to be a single biological replica in its respective experimental and treatment group). Mice were randomly assigned into groups by a technician who was not familiar with the project. The investigators were not blinded to the identities of the treatment groups during the experiment and no data were excluded from the final analysis. For in vivo gene expression studies, mice were randomly grouped into three groups containing three animals each, and then subjected to three different dietary regiments. Two groups were fed a gamma-irradiated custom diet (glycan-free diet) that contained only glucose as the available carbohydrate as a non-digestible fibre supplement25. One of the two groups maintained on this diet was provided with purified η-mannann (1% w/v) in drinking water (Harlan-Teklad). The third group was fed a custom version of the glycane-free diet in which the glucose was replaced with dried/crumbled bread (50% w/w of final diet) that appearance of the anomeric α- and β-proton signals. Signals were assigned by two-dimensional NMR analysis (HSQC).

**Bacteroides culture and whole-cell assays.** B. theitaiotaomicron was cultured anaerobically at 37 °C in minimal media containing an appropriate carbon source, or in TYG (tryptone yeast extract glucose medium) as described previously26. Growth curves presented in the paper are averages of six biological replicates.

B. theitaiotaomicron was grown in 5 ml minimal media on 0.5% w/v S. cerevisiae mannann (Sigma) or glucose as the sole source to mid exponential phase (OD₆₀₀nm 0.6–0.8). Cells were harvested by centrifugation, 5000g for 10 min at room temperature and washed in 5 ml PBS (pH 7.1) before being resuspended in 500 μl PBS. Cells (50 μl) were assayed against yeast mannann (10 mg ml⁻¹) at 37 °C for 16 h. Assays were analysed by thin layer chromatography, 5 μl of each sample was spotted onto silica plates and resolved in butanol/acetic acid/water buffer. The plates were stained with amido black and visualized in UV light.
had been produced using yeast as a leavening agent (Zingerman’s Bake House, Ann Arbor, MI). Mice were pre-fed on each dietary condition for five days, colo-
nized with B. thetaiotaomicron or by oral gavage (10^10 cfu per animal) and main-
tained for an additional 5 days before euthanizing and collection of caecal contents
for gene expression analysis. For the in vivo competition experiment, wild-type and
mannan PUL triple mutant strains were each labelled with a unique 24 base pair
(bp) oligonucleotide tag, which is contained in a pNBU2-based chromosomal
integration vector and quantifiable by qPCR, as previously described. Each strain
was grown overnight in TYG medium and combined at approximately equal amounts
before being gavaged into mice as described above. For competition experiments,
five mice were used in each group (chosen based on the sample size used in
previous studies to observe significant changes in competitive index (39–40) and fed
varying regimens of the three diets described above for a total period of 38 days. All
germfree mice used in the competition experiment were pre-fed the gleycan-free
diet for 1 week before colonization.

**Bacteroides co-culturing experiments.** B. thetaiotaomicron, B. xylanisolvens
NLAE-el p532 and B. cellulosilyticus WH2 were cultured in minimal media con-
taining glucose (0.5% w/v) as a carbon source to mid exponential growth phase
(OD600 0.7–0.8). Cells were collected by centrifugation and washed twice with
5 ml of PBS to remove any residual glucose. The washed cells were resuspended in
5 ml of fresh minimal media with no carbon source. Minimal media (10 ml)
containing mannose or yeast mannann as the carbon source was inoculated with
equal volumes of B. thetaiotaomicron/B. xylanisolvens or B. thetaiotaomicron/B.
cellulosilyticus. A control culture of minimal media containing yeast mannann was
also inoculated with either B. xylanisolvens or B. cellulosilyticus. All cultures were
set up in triplicate. Samples (1 ml) were taken at the point of inoculation, early
exponential, late exponential and stationary phase of growth. Serial dilutions of
each sample were plated on to rich media and incubated for 2 days before colony
counts were recorded. Proportions of B. thetaiotaomicron, B. xylanisolvens and
B. cellulosilyticus per sample were determined by quantitative PCR from genomic
DNA using unique marker genes for each strain.

**Searches of human gut metagenomic data sets for mannan PULs.** A search of
250 different healthy and diseased (Crohn’s disease and ulcerative colitis) human
DNA using unique marker genes for each strain. B. thetaiotaomicron (OD600 0.7–0.8) . Cells were collected by centrifugation and washed twice with
5 ml of PBS to remove any residual glucose. The washed cells were resuspended in
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Extended Data Figure 1 | The role of specific *B. thetaiotaomicron* PULs and enzymes in utilization of mannan from *S. cerevisiae* and other yeast species.

a, Growth of wild-type *B. thetaiotaomicron* on *Candida albicans* mannan and glucose. b, Growth of wild-type *B. thetaiotaomicron* and the mutant lacking MAN-PUL1 and MAN-PUL3 (ΔMAN-PUL1/3) on *Schizosaccharomyces pombe* α-mannan. c, Growth of wild-type *B. thetaiotaomicron*, and the *B. thetaiotaomicron* mutants lacking MAN-PUL2 (ΔMAN-PUL2), or all three mannan PULs (ΔMAN-PUL1/2/3) on *S. cerevisiae* α-mannan. d, The growth profile of wild-type *B. thetaiotaomicron* and the *B. thetaiotaomicron* mutant lacking bt3774 (Δbt3774) on *S. cerevisiae* mannan. In panels a, b, c and d, each point on the growth curve represents the mean of three biological replicates.

e, Enzymes at 1 μM at 37 °C were incubated with either undecorated α-1,6-mannan (derived from mnn2 mutant of *S. cerevisiae*) (lanes 1–3) or mannan from *S. pombe* (lanes 4–9). Lanes 1 and 4, the mannans incubated in the absence of the enzymes; lanes 2 and 6, mannans incubated with the periplasmic mannanase BT3782; lanes 3 and 7, mannans incubated with the surface mannanase BT3792; lane 5, *S. pombe* mannan incubated with the GH97 α-galactosidase BT2620; lanes 8 and 9, *S. pombe* mannan incubated with BT2620/BT3782 and BT2620/BT3792, respectively. Lane 10 galactose standard; lane 11 α-1,6-mannooligosaccharides: mannose (M1), mannobiose (M2), mannotriose (M3) and mannotetraose (M4).
Extended Data Figure 2 | Product profiling of GT32 glycosyltransferases encoded by MAN-PUL2. a, HPAEC of biosynthetic reactions using mannose as an acceptor and GDP-α-Man as the donor. Mannobiose is formed in the presence of BT3775 (black) and mannotriose with BT3775 and BT3776 (red). The blue trace is a mannose standard. b, HPAEC of biosynthetic reactions with α-1,3-mannobiose as the acceptor and GDP-α-Man as the donor. BT3775 is not capable of extending mannobiose (black). In the presence of BT3775 and BT3776 mannotriose is produced (red). The blue trace is an α-1,3-mannobiose standard. c, d, MALDI-TOF analysis of the reaction products of BT3775 + BT3776 using mannose (c) and α-1,3-mannobiose (d) as an acceptor. e, NMR analysis of the α-1,3-mannobiose substrate. Peaks 1 and 3 correspond to the α-anomer and β-anomer of the mannose at the reducing end, respectively; peak 2 corresponds to the terminal α-mannosyl residue linked to O3 of the mannose at the reducing end. f, NMR analysis of the α-1,3(α-1,6)-mannotriose BT3776 product. The numbering of the peaks are the same as in e. Peak 4 corresponds to the terminal α-mannosyl residue linked to O6 of the 3,6-linked mannose at the reducing end. g, Alditol-acetate linkage analysis of mannobiose produced by BT3775 from mannose. h, Alditol-acetate linkage analysis of branched (α-1,3)(α-1,6)-mannotriose produced by BT3776 from α-1,3-mannobiose. The green circles indicate the mannose residues present in carbohydrates identified by HPAEC, MALDI-TOF and NMR.
BT3792 GH76 endo-α1,6-mannanase

BT3862 GH99 endo-α1,2-mannosidase

BT3781 GH125 exo-α1,6-mannosidase

BT3783 mannose-6-phosphatase
Extended Data Figure 3 | The structures of enzymes that play a key role in yeast mannan degradation. a, Overlay of the hydrophobic conserved residues in the predicted substrate-binding cleft of BT3792 (yellow), BT2949 (cyan) and the Listeria protein Lin0763 (green; PDB code 3K7X), and the predicted catalytic aspartates. b, Solvent representation of BT3792 in which the predicted catalytic residues, Asp258 and Asp259, are coloured green. c, Overlay of BT3862 (cyan) with a homologue of the enzyme from B. xylanisolvens, BxGH99 (green; PDB code 4UTF) in complex with Man-α-1,3-isofagomine and α-1,2-mannobiose (Man residues coloured yellow and isofagomine pink). d, Solvent-exposed surface of the substrate binding cleft of the BxGH99 (teal) ligand complex overlaid with BT3862 (grey). The subsites are numbered with the catalytic residues, Glu 333 and Glu 336, coloured red and the solvent exposed O2 of Man bound at the −2 subsite and O1 and O6 of the Man located at the +2 subsite are coloured bright green. e, Overlay of BT3781 (green; PDB code 2P0V) with the substrate and catalytic residues of the Clostridium perfringens GH125 α-mannosidase CpGH125 (cyan; PDB code 3QT9), in which the ligand 6-S-α-D-mannopyranosyl-6-thio-α-D-mannopyranose (Man-S-Man) is shown in yellow. f, Solvent-exposed surface of BT3781 in the vicinity of the active site in which the catalytic residues (Glu 174 and Glu 439) are depicted in green. The position of Man-S-Man is based on the overlay shown in e. g, Overlay of BT3783 with the catalytic and substrate binding residues of a tyrosyl-DNA phosphodiesterase (PDB code 4GYZ) in complex with Mg^{2+} (slate-blue sphere) and phosphate (coloured orange). h, A region of the solvent-accessible surface of BT3783 in which the catalytic residues are coloured green. The figure was prepared using PyMOL. A detailed description of the structures of these proteins is provided in Supplementary Information section 5.0.
a) GH92 and GH92/GH76

b) Gel electrophoresis

c) Gel electrophoresis

d) Absorbance at 600 nm over time

e) HPLC analysis

f) Confirmation of products

endo-α,1,6-mannosidase

Product

α-anomer (P-α)

β-anomer (P-β)

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Extended Data Figure 4 | The degradation of yeast mannan by *B. thetaiotaomicron* in culture and the selected enzymes expressed by the bacterium, and the stereochemistry of the reaction catalysed by GH76 endo-α-1,6-mannanases. a, GH92 α-mannosidases at high concentrations (50 μM) were incubated with yeast mannan for 5 h in the absence (labelled GH92) or in the presence (GH92/GH76) of the endo-α-1,6-mannanase BT3782. The GH92 α-mannosidases in this example were BT2199 (1), BT2130 (2) and BT3773 (3). The GH76 endo-α-1,6-mannanase only releases mannoooligosaccharides in the presence of BT2199; see also Supplementary Information section 4.1. b, *B. thetaiotaomicron* was grown on yeast mannan or glucose. Yeast mannan was incubated with no bacterium (1), *B. thetaiotaomicron* previously cultured on yeast mannan (2) and *B. thetaiotaomicron* grown on glucose (3). The cells were incubated for 5 h at 37 °C with the polysaccharide without a nitrogen source and thus were not growing. The products released by the *B. thetaiotaomicron* cells, analysed by TLC, were mediated by the activity of enzymes presented on the surface of *B. thetaiotaomicron*, and not through the action of periplasmic mannanases and mannosidases. The black box highlights very low levels of high molecular weight mannoooligosaccharides generated by the cells incubated in yeast mannan. c, *B. thetaiotaomicron* was cultured for up to 48 h (stationary phase) on yeast mannan. The supernatant of the culture at the time points indicated were analysed by TLC. In all panels the samples were chromatographed with the following α-1,6-mannooligosaccharides: mannose, M1; mannobiose, M2; mannotriose, M3; mannotetraose, M4. d, The absorbance of the culture used in c, e, BT3792 (GH76) endo-α-1,6-mannosidase is a retaining glycoside hydrolase. Enzymatic hydrolysis of 4-nitrophenyl α-D-mannopyranosyl-1,6-α-D-mannopyranoside (S) was monitored by 1H-NMR spectroscopy (500 MHz). The stacked spectra show the reaction progress over time. S_1H_α is the anomeric proton of the reducing end mannoypyranoside of the substrate, and S_1H_β is the anomeric proton of the non-reducing end mannoypyranoside. The reaction proceeds with the initial formation of the product, the α-anomer of α-1,6-mannobiose (P-α, peaks P_1H_α and P_2H_α), which slowly mutarotates to the β-anomer (P-β, peaks P_1H_β and P_2H_β). f, TLC analysis of *S. cerevisiae* mannan incubated without enzyme (lane 1), BT3774 (lane 2), BT3792 (lane 3) and BT3774 and BT3792 (lane 4). M1–M4 are α-mannooligosacharide standards numbered according to their d.p. GH76 mannanase BT3792 does not attack the backbone of *S. cerevisiae* mannan unless the side chains are first removed by the GH38 α-mannosidase BT3774, confirming that this enzyme cleaves the mannose α-1,2-linked to the mannan backbone. The data in a, b and c are representative of two biological replicates, while the data in f are representative of two technical replicates.
Extended Data Figure 5 | The activity of periplasmic α-mannosidases and the growth of different species of Bacteroides against yeast mannan.

Structures of the mannans derived from wild-type and mutants of S. cerevisiae. The tables adjacent to the different yeast structures depict the initial rate of mannan hydrolysis by the four enzymes. The growth curves adjacent to the different mannan structures show the growth profile of B. thetaiotaomicron (black), B. ovatus (red) and B. xylanisolvens (blue) on the glycans (each point represents the mean growth of 3 separate cultures ± s.d.). The porcine-derived B. xylanisolvens strain shown here acquired MAN-PUL1 by lateral gene transfer (Extended Data Fig. 9), explaining its capacity to degrade processed mannans. Vertical error bars represent standard deviation of three separate replicates in each condition.
Extended Data Figure 6 | The activity of GH76 α-mannanases and GH125 α-mannosidases a, b, BT3792 and BT3782, respectively, were incubated with α-1,6-mannonetraose at a concentration ≪ $K_M$. Substrate depletion was measured using HPAEC and the rate (right of a and b) enabled $k_{cat}/K_M$ to be determined. c, BT3792 and BT3782 were incubated with unbranched yeast mannan (derived from the S. cerevisiae mutant MNN2). The yeast mannan at 0.1% was incubated with the two GH76 α-1,6-mannanases for 1 h at 37 °C in 50 mM sodium phosphate buffer, pH 7.0. The limit products were analysed by TLC. α-1,6-Mannooligosaccharides are identified by their degree of polymerization (M1, mannose; M2, mannobiose; M3, mannotriose; M4, mannotetraose); IP, injection peak. d, e, BT2632 and BT3781 at 100 nM were incubated with 1 mg ml$^{-1}$ of the debranched mannan for 1 h in the buffer described above. TLC analysis (d) and HPAEC traces (e) of the reactions are shown. The data in c and d are representative of two technical replicates.
Extended Data Figure 7 | HMNG deconstruction by B. thetaiotaomicron.

a, Structure of the HMNG PUL. Genes drawn to scale with their orientation indicated. Genes encoding known or predicted functionalities are colour-coded and, where appropriate, are also annotated according to their CAZy glycoside hydrolase (GH) family number. SGBP represents a surface glycan binding protein.

b, BT3994 was incubated with α-1,6-mannotetraose (Man4) or the high mannose N-glycan Man5GlcNAc2, with both oligosaccharides labelled with 2-aminobenzamide (AB). At the indicated time points aliquots were removed and analysed by HPAEC using a fluorescence detection system. While Man5GlcNAc2-AB was hydrolysed by BT3994, the enzyme was not active against Man4-AB.

c, Chicken ovalbumin was incubated with buffer (1) or 1 mM of BT3987 (2) in 20 mM Na-HEPES buffer, pH 7.5, for 5 h at 37 °C, and the soluble material was permethylated and analysed by MALDI-TOF mass spectrometry. The high mannose N-glycans released are labelled.

d, Western blot of B. thetaiotaomicron cells cultured on yeast mannan that were untreated with proteinase K (0 h) or incubated with 2 mg ml⁻¹ proteinase K for 16 h (16 h). The lane labelled RP contained a purified recombinant form of BT3990. The blots were probed with antibodies against BT3990. The data in d are representative of two biological replicates.

e, f, Representative isothermal calorimetry titrations for BT3984 titrated with Gal-β1,4-GlcNAc (LacNAc; 25 mM) (e), and for BT3986 titrated with mannose (50 mM) (f). The top half of each panel shows the raw isothermal calorimetry titration heats; the bottom half, the integrated peak areas fitted using a single binding model by MicroCal Origin software. ITC was carried out in 50 mM Na-HEPES, pH 7.5 at 25 °C.

The affinities and thermodynamic parameters of binding are shown in Supplementary Table 5.

g, Growth profile of wild-type B. thetaiotaomicron (WT Bt; black) and the mutant Δbt3993 (red), which lacks the extra-cellular factor σ regulator gene of HMNG-PUL, cultured on Man8GlcNAc2 (each curve shows the mean ± s.d. of 3 separate cultures).
Extended Data Figure 8 | Metagenomic analysis of the occurrence of the yeast mannan PULs in humans. Abundance of *Bacteroides* mannan PULs in humans from a survey of metagenomic sequencing data from a total of 250 adult human samples (211 healthy, 27 ulcerative colitis, 12 Crohn’s disease; see Methods for references). Data sets were individually queried by BLAST using either each entire mannan PUL (PULs 2, 3) or a sub-fragment that was trimmed to eliminate cross-detection of other species genomes beyond *B. thetaiotaomicron* and porcine *B. xylanisolvens* (PUL1; see Methods for additional search details). Each horizontal line represents the presence of a hit in a single individual. The leftmost column summarizes the total mannan PUL content in each person (annotated according to the colour key in the upper right corner). The mannan PUL frequency across all 250 samples is shown at the bottom for each condition and is compared to the frequency of several other PULs implicated in xyloglucan and porphyran utilization. Graph at far right illustrates the variation in sequencing depth for each sample/study; black lines show the average depth in megabase pairs (Mbp) for each study; the light grey line shows the depth for each individual sample.
Extended Data Figure 9 | Presence of a conjugative transposon (cTn) that contains MAN-PUL1 in the genomes of porcine B. xylanisolvens strains and the mannan presented to these organisms. a, Shown across the top is a schematic of a cTn that has been integrated into the 3' end of a tRNA\(^{phe}\) gene in B. thetaiotaomicron strain VPI-5482. Integration is mediated by a 22 bp direct repeat sequence that is contained in tRNA\(^{phe}\) and repeated again at the other side of the cTn (right insertion site). The location of B. thetaiotaomicron MAN-PUL1 is denoted within the larger cTn element using a colour scheme identical to Fig. 1a. The lower panel shows an expanded view of the MAN-PUL1 locus in five sequenced strains of B. xylanisolvens from the faeces of pigs fed a diet enriched with distillers grains that were fermented with yeast. A nearly identical copy (both by amino acid homology and syntenic organization) of this genomic region is present in B. thetaiotaomicron and the porcine B. xylanisolvens strains. Although the draft genomes of the B. xylanisolvens strains contain gaps in all five assemblies at the left side of the MAN-PUL1, the right side insertion site was resolved in all genomes, suggesting that the B. xylanisolvens loci were also transferred by lateral transfer at some point in the history of these strains. b, Forty-three different strains from five Bacteroidetes isolated from animal guts (each indicated with a solid circle) were inoculated into minimal media containing S. cerevisiae mannan as the sole carbon source. The growth of the cultures was measured over 48 h by recording the optical density at 600 nm. c, TLC analysis of the products generated by incubating BT3774 and BT3792 with \(\alpha\)-mannan extracted from the distillers grain fed to the pigs from which the B. xylanisolvens were isolated. The data are representative of two technical replicates.
Extended Data Figure 10 | In vivo and in vitro expression of the mannan PULs. 

a, Level of *susC*-like transcripts derived from MAN-PUL1 (BT2626), MAN-PUL2 (BT3788) and MAN-PUL3 (BT3854) from *B. thetaiotaomicron* in monocolonized gnotobiotic mice fed a glycan-free diet deficient in *B. thetaiotaomicron*-digestible glycans (red), the same diet with added yeast mannan (1% w/v in drinking water) as the only usable polysaccharide (green), and a diet containing 50% bread (blue). The levels of the *susC* transcripts were quantified relative to the same mRNA species in *B. thetaiotaomicron* cultured in vitro on glucose minimal medium (MM-G). Note that in all cases, expression of MAN-PUL genes is equally high in vivo.

b, Transcription of the same mannan *susC*-like genes in response to increasing concentrations of yeast mannan in the media after 30 min exposure. The prototypic *susC* (BT3701) involved in starch metabolism is shown for comparison. c, An identical exposure experiment to that shown in b, except that glucose-grown *B. thetaiotaomicron* cells were exposed to aqueous extracts of the cereal grain diet (natural diet) fed to mice before the experiment, or the digestible glycan-free control diet (glycan-free diet) used as a base for all feeding treatments. Exposure was conducted for 30 min to determine if any diet extract contained contaminating levels of mannan that could be detected by *B. thetaiotaomicron* cells; inclusion of purified mannan (5 mg ml$^{-1}$) in addition to the glycan-free diet served as positive controls. In all panels, the results represent the mean of 3 biological replicates and error bars represent s.d.
In this Article focusing on the selfish metabolism of yeast mannan by Bacteroidetes, we also described a polysaccharide utilization locus (PUL) responsible for the degradation of high mannose mammalian N-glycan (HMNG) but omitted to cite two relevant papers\(^1,2\), for which we apologise. Both studies describe a model for the degradation of complex biantennary N-glycans by Bacteroidetes in which the degradative enzymes are encoded by PULs. These studies\(^1,2\) provide examples of how PULs can orchestrate N-glycan metabolism in addition to the HMNG PUL we describe in this Article. In all three papers it is proposed that N-glycan depolymerization occurs primarily in the periplasm.

\(^1\) Renzi, F. \textit{et al.} The N-glycan glycoprotein deglycosylation complex (Gpd) from Capnocytophaga canimorsus deglycosylates human IgG. \textit{PLoS Pathog.} \textbf{7}, e1002118 (2011).

\(^2\) Nihira, T. \textit{et al.} Discovery of β-1,4-α-mannosyl-N-acetyl-α-glucosamine phosphorylase involved in the metabolism of N-glycans. \textit{J. Biol. Chem.} \textbf{288}, 27366–27374 (2013).