The human gut Firmicute *Roseburia intestinalis* is a primary degrader of dietary β-mannans

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β-Mannans are plant cell wall polysaccharides that are commonly found in human diets. However, a mechanistic understanding into the key populations that degrade this glycan is absent, especially for the dominant Firmicutes phylum. Here, we show that the prominent butyrate-producing Firmicute *Roseburia intestinalis* expresses two loci conferring metabolism of β-mannans. We combine multi-“omic” analyses and detailed biochemical studies to comprehensively characterize loci-encoded proteins that are involved in β-mannan capturing, importation, de-branching and degradation into monosaccharides. In mixed cultures, *R. intestinalis* shares the available β-mannan with *Bacteroides ovatus*, demonstrating that the apparatus allows coexistence in a competitive environment. In murine experiments, β-mannan selectively promotes beneficial gut bacteria, exemplified by increased *R. intestinalis*, and reduction of mucus-degraders. Our findings highlight that *R. intestinalis* is a primary degrader of this dietary fiber and that this metabolic capacity could be exploited to selectively promote key members of the healthy microbiota using β-mannan-based therapeutic interventions.
The human gastrointestinal tract harbors an extremely dense and diverse microbial community, known as the gut microbiota. In a mutually beneficial relationship, the gut microbiota supplies enzymes able to depolymerize dietary carbohydrates that cannot be hydrolyzed by human enzymes. The monosaccharides generated are further fermented into host-absorbable metabolites, including the short-chain fatty acids butyrate, acetate, and propionate. In particular, butyrate produced by commensal bacteria serves as the main energy source for colonocytes and it exhibits anti-carcinogenic, anti-inflammatory, and barrier protective properties in the distal gut. The relevance of this metabolic output to human health has prompted increasing interest in intentionally modulating the composition of the gut microbiota to promote wellbeing and combat disease, e.g., by the use of prebiotics. Established prebiotics have been traditionally developed based on their selective fermentation by bifidobacteria and lactobacilli generally regarded as conferring health benefits to the host. Notably, other potentially beneficial targets are the butyrate-producing Firmicutes.

Firmicutes spp., together with Faecalibacterium prausnitzii and Eubacterium rectale, constitute a group of dominant butyrate-producing Firmicutes, estimated to account for 7–24% of the total bacteria in the healthy human colon. Interest in Roseburia spp. has increased with reports that the abundance of these bacteria is reduced in individuals affected by inflammatory diseases and colorectal cancer. Complementary studies have shown that Roseburia spp. play an important role in the control of gut inflammatory processes, amelioration of atherosclerosis and in the maturation of the immune system, primarily through the production of butyrate. R. intestinalis preferentially colonizes the mucin layer and this intimate association to the host may contribute to the local level of butyrate available for the colonic epithelial cells. This species appears to be a specialist able to grow on a few glycans and has been recently shown to be a prominent xylan degrader in vitro and in the healthy human colon. β-Mannans are widespread in the human diet: they are widely used in food as thickening, stabilizing, and gelling agents (glucomannan and galactomannan, Fig. 1). They are found in the endospermic tissue of nuts (homopolymeric mannnan), coffee beans, coconut palm, tomato, and legume seeds (galactomannan) and play vital roles in the cell wall structure and as storage polysaccharides in plants. Notably, the structure of galactoglucomannan from non-edible sources (softwood) shares striking similarities with that from edible sources (Fig. 1).

Results

Two multi-gene loci mediate β-mannan utilization. R. intestinalis L1–82 grows efficiently on a variety of complex β-mannans as a sole carbon source (Fig. 2a), causing a concomitant acidification of the medium (Fig. 2b). To evaluate which fractions of β-mannan breakdown products are internalized, we analyzed the culture supernatants during R. intestinalis growth on AcGGM using high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) (Supplementary Fig. 1a, b). Neither oligosaccharides nor monosaccharides accumulated in the stationary phase culture (Supplementary Fig. 1a, b), indicating that the bacterium possesses a highly efficient apparatus to cleave and import all the sugars derived from the breakdown of this complex glucan.

To examine the molecular basis underlying β-mannan utilization by R. intestinalis, we performed an RNA sequencing (RNAseq) transcripational analysis during growth on konjac glucomannan (KGM), spruce acetylated galactoglucomannan (AcGGM) and glucose (Glc). The top 20 upregulated genes in β-mannan transcriptome encode a β-mannanase belonging to the glycoside hydrolase (GH) 26 family (GH26 according to the CAZy classification), a solute binding protein (MnBP) and two permeases (MPP) of an ABC transporter, two phosphorilases (GH130), one epimerase (Mep), two β-glucosidases (GH3) and two carbohydrate esterases (CEs) (Fig. 2c and Supplementary Data 1). These genes are located in two loci, which were designated mannan-utilization locus large (MULL: ROSINTL182_05469–83) and mannan-utilization locus small (MULS: ROSINTL182_07683–85) (Fig. 2d). Among the MULL genes expression of a Lact-type transcriptional regulator, predicted glycosyl hydrolases belonging to GH113, GH36, GH11, and a phosphomutase also increased. The response was specific to β-mannan as no differential expression of these genes was observed during growth of R. intestinalis on galactose, a building block in mannan (Supplementary Table 1).

Fig. 1 General structure of the main classes of β-mannan. Linear homopolymeric (upper structure) and linear heteropolymeric (lower three structures) β-mannans are shown. Monosaccharides (D-mannose, green circle; D-glucose, blue circle; D-galactose, yellow circle) and acetylations (2Ac, 2-O-Acetyl; 3Ac, 3-O-Acetyl; 6Ac, 6-O-Acetyl) are represented using the standard Consortium of Functional Glycomics symbols. NR end, non-reducing end; R end, reducing end; DP, degree of polymerization.
Proteomic analysis under the same growth conditions corroborated the RNAseq results; indeed, proteins encoded by the genes located in MULL and MULS were abundant in the AcGGM samples compared to the glucose samples (Fig. 2e, Supplementary Data 2).

We carried out a comparative genomic analysis to establish the distribution of β-mannans utilization loci equivalent to the identified MULL and MULS in other representative Roseburia spp. and Clostridium cluster XIVa members. The results showed that R. faecis and R. hominis shared an overall MULL and MULS organization with that of R. intestinalis (Supplementary Fig. 2, Supplementary Table 2), suggesting that the utilization of β-mannan is shared by these three Roseburia spp. However, the lack of the critical GH26 endomannanase, required to break down mannan (see later results for R. intestinalis β-mannanase RIGH26), is likely to render R. hominis only able to metabolize manno-oligosaccharides. Orthologous mannan utilization loci were identified in specific members of the Clostridium cluster XIVa, although a similar organization and complete conservation of all MULL and MULS components was not observed (Supplementary Fig. 2).

Degradation of the β-mannan backbone. RiGH26, (locus tag: ROSINTL182_07683), is a predicted extracellular modular β-mannanase comprising a carbohydrate binding module of family Mutase α-Galactosidase Mannanase/mannosidase Glucosidase Transcriptional regulator ABC transporter Esterase Phosphorylase Isomerase Epimerase

**Fig. 2** R. intestinalis upregulates several glycoside hydrolases, two carbohydrate esterases and an ABC-transporter during β-mannan consumption. **a** Growth curves of R. intestinalis in YCFA without carbon source (black) or supplemented with 0.5% of either glucose (Glc, green circles), KGM (blue circles), CGM (purple circles) or AcGGM (red circles). **b** pH measurements during R. intestinalis growth on Glc and β-mannans. In a and b, each point on the curves represent the average of three independent experiments. Error bars represent standard deviations (s.d.). **c** RNA expression profile of putative β-mannan utilization genes during R. intestinalis L1-82 growth in YCFA supplemented with 0.5% KGM (blue bars) or AcGGM (red bars). The Log2-fold change relative to cells cultured on YCFA-Glc is shown on the y-axis while the x-axis shows the putative genes involved in β-mannan catabolism. **d** Genomic organization of the large and small β-mannan utilization loci (MULL and MULS, respectively) from R. intestinalis. Genes with similar predicted functions are coded by the same color. **e** Heat map showing the proteomic detection of relevant proteins with predicted β-mannan utilization functions in triplicate samples (1–3) grown on YCFA-Glc and YCFA-AcGGM. Colors represent protein intensity expressed as Log2 of LFQ values; the color gradient ranges from 14 (green) to 34 (magenta), with black indicating 24. In c-e, locus tag numbers ROSINTL182_XXXXX are abbreviated with the last numbers after the hyphen.
27 (CBM27), a catalytic module of GH26 followed by a CBM23 (Supplementary Fig. 3a). Furthermore, two C-terminal Ig-like domains and a proline-glycine rich region likely mediate cell attachment34 and binding within the cell wall35. The extracellular localization of RiGH26 was corroborated experimentally by immunofluorescence microscopy (Fig. 3). RiGH26 exhibited activity toward decorated mannsans including KGM, carob galactomannan (CGM) and AcGGM (Fig. 4a and Supplementary Fig. 3b), generating linear and substituted manno-oligosaccharides. The enzyme was active on mannopteroase (M₄) and mannotetraose (M₅) but not mannobiase (M₂) (Supplementary Fig. 3c). Overall, the product profiles suggest capability of endo-action and indicates that RiGH26 targets large polymers and can accommodate the galactose and acetyl decorations present in these substrates. Further analysis indicated that RiGH26 is a potent enzyme as, when used at the concentration of 10 nM, it was able to hydrolyze high concentrations of spruce AcGGM (50 mg ml⁻¹) into oligosaccharides in 1 h at standard assay conditions (Supplementary Fig. 3d). No detectable activity was measured when RiGH26 was incubated with linear cello-oligosaccharides, birch xylan, curdlan, lichenan or barley β-glucans (Supplementary Fig. 3e). RiGH26 was selective for shorter oligosaccharides with its highest affinity for M₆ (Kᵅ = 165 ± 10 µM, two independent experiments) (Table 1, Supplementary Fig. 5a) and its affinity dropped for ligands smaller than a tretrasaccharide (Table 1). By contrast, RiCBM23 was selective for shorter oligosaccharides with its highest affinity for M₄ (Kᵅ = 130 ± 50 µM, two independent experiments) (Table 1, Supplementary Fig. 5b), although mannotriose (M₃) was also bound with good affinity (Table 1).

Internalization of break-down products from β-mannan. Within the MULL cluster, the three genes (ROSINTL182_05477 – ROSINTL182_05479) that encode an ATP-binding cassette (ABC) importer were shown to exhibit the highest level of increased expression during growth on β-mannan (and when compared to growth on glucose). The thermodynamic binding parameters of the ABC-transporter associated solute binding protein, RiMnBP, to linear and substituted manno-oligosaccharides were determined using isothermal titration calorimetry (ITC). RiMnBP bound a range of unsubstituted manno-oligosaccharides with a preference for M₃ (Kᵅ = 25.5 µM) followed by M₄ and M₅ (Table 2, Supplementary Fig. 6). Acetylations had a marginal effect on the binding affinity, thus providing evidence that these fragments are efficiently captured by the transport protein. Overall, these results support the predicted role of RiMnBP in the uptake of manno-oligosaccharides generated by RiGH26, showing optimal affinity for undecorated or acetyl substituted ligands with a degree of polymerization (DP) of 4–5.

Decomposition of internalized β-manno-oligosaccharides. The affinity of the solute binding protein RiMnBP to manno-oligosaccharides and the predicted intracellular location of the debranching and exo-acting enzymes is consistent with a hierarchical degradation of the internalized manno-oligosaccharides following extracellular degradation of the β-mannan polymers by RiGH26.

The ROSINTL182_05471 (RiCEX) and ROSINTL182_05473 (RICE2) gene products possess SGNH hydrolyase-type esterase domain signatures37. Comparison to previously characterized CEs revealed that RICE2 showed 25–30% identity to a CE2 from Clostridium thermocellum38 and the acetyl xylan esterase AXC2 of Cellvibrio japonicus38. In contrast, RiCEX did not display...
Fig. 4 Cleavage of the β-mannans backbone, removal of the side chains and further depolymerization of the resulting linear manno-oligosaccharides.

a  HPAEC chromatograms showing the oligosaccharide products after overnight digestion of KGM, CGM and AcGGM with RiGH26. Samples were analyzed with the following manno-oligosaccharides as external standards: M2, mannobiose; M3, mannotriose; M4, mannotetraose; M5, mannopentaose; M6, mannohexaose.

b  MALDI-TOF analysis of RiGH26-digested AcGGM incubated with either RiCE2, RiCEX or both enzymes. Peaks are labeled by DP and number of acetyl groups (Ac).

c  HPAEC chromatograms showing products generated from CGM pre-digested with RiGH26 and subsequently treated with RiGH36. Assignments for peaks not occurring in the standard samples are based on comparison with the product profiles obtained by MALDI-TOF MS of RiGH26-digested CGM (black spectrum) treated with galactose oxidase (GOX; brown spectrum). GOX converts a galactose residue in the oligosaccharides into its corresponding aldehyde, giving a mass-to-charge ratio (m/z) of -2. All assigned masses are sodium adducts. Abbreviations: Ox, oxidation; Gal1, galactose; Gal1M3, galactosylmannotriose; Gal1M4, galactosylmannotetraose; Gal2M4, digalactosylmannotetraose; Gal2M5, digalactosylmannopentaose.

d  Product profiles from RiGH26-digested CGM degradation experiments with RiGH113 analyzed by HPAEC-PAD. The release of mannose confirms the exo-activity of RiGH113.

e  HPAEC-PAD traces showing activity of RiGH3A or RiGH3B towards G5 and G4 with the corresponding controls (no enzyme). Product profiles at various time points during the reaction are shown in Supplementary Fig. 6. Taken together, the data show that RiGH3B is able to hydrolyze completely both tetramers and pentamers, producing glucose. RiGH3A shows exo-activity towards both substrates that are converted slowly to glucose and a mixture of cello-oligosaccharides. Samples were analyzed with the following cello-oligosaccharides as external standards: glucose, G1; cellobiose, G2; cellotriose, G3; cellotetraose, G4; cellopentaose, G5.

f  Chromatograms showing products generated upon incubation of RiGH130_2 with M4 and M3. The M1P released (red arrow) was identified by co-migration with the appropriate standard. In all panels, the data displayed are representative of at least three biological triplicates.
significant relatedness to other characterized CE catalogued in the CAZy database\(^1\), which excluded RiCEx from being classified in any of the 16 CE families. RiCEx and RiCE2 showed mannan acetyl esterase activity on a mixture of oligosaccharides generated via RHG26 hydrolysis of AcCGM (Fig. 4b). RiCE2 partially removed acetyl groups from the acetylated oligosaccharide substrate (Fig. 4b). RiCEx deacetylated the substrate mainly to free and monoacetylated oligosaccharides (Fig. 4b). These results indicate that RiCEx has a preference for oligosaccharides with a degree of acetyl substitution ≥2, but is less efficient on mono-substituted substrates. At the same time, it suggests that an acetyl group present at a specific position (O-2 or O-3) is resistant to enzymatic deacetylation by RiCEx. The combination of RiCEx and RiCE2 resulted in the almost complete deacetylation of the manno-oligosaccharides, indicating a cooperative interaction of the two esterases (Fig. 4b).

RHG36 released galactose from internally substituted CGM and AcCGM after the treatment with the RHG26 β-mannanase (Fig. 4c and Supplementary Fig. 7). Interestingly, RHG36 released galactose from CGM-endomannanase products with 100% efficiency (Fig. 4c, Supplementary Fig. 8a) as no oxidized product could be observed after treatment of these samples with galactose oxidase. The enzyme exhibited limited activity on large polymers (Supplementary Fig. 8b) consistent with the activity on internalized oligosaccharides in vivo. Similarly, α-galactosidase activity increased after de-acetylation of the oligosaccharides (Supplementary Fig. 8c, d). Beside cleaving single internal galactose residues from manno-oligosaccharides, this enzyme was capable of removing α-1,6-galactose from the reducing-end of a substituted manno-oligosaccharide (Supplementary Fig. 8e) and from an oligosaccharide containing two consecutive substitutions (Supplementary Fig. 8f). Corroborating these results, RHG36 cleaved galactose decorations from endomannanose products of highly substituted guar gum galactomannan (Supplementary Fig. 8b).

Sequence searches showed that the protein encoded by ROSINTL182_05483 (MULL, RHG113) exhibited 40% identity to the only characterized enzyme from this family, the endo-β-mannanase AaManA from Alicyclobacillus acidocaldarius\(^9\) (Supplementary Fig. 9a). Alignment of RHG113 and AaManA showed that the catalytic and substrate interacting residues are conserved (Supplementary Fig. 9a). When RHG113 was assayed for activity on linear manno-oligosaccharides, it revealed an ability to cleave linear manno-oligosaccharides to yield mannose and M2 (Supplementary Fig. 9b). Strikingly, time-course analysis of RHG113 activity revealed that this enzyme displays a different sub-specificity by hydrolyzing manno-oligosaccharides to mannose and a minor amount of M2 (Supplementary Fig. 9c). After overnight incubation with RHG113, M2 was partially degraded to mannose (Supplementary Fig. 9d), confirming the exo-mannosidase activity as opposed to the endo-fashion cleavage reported for the AaManA. The substituted manno-oligosaccharides galactosylmannobiose (Gal\(_2\)Man\(_2\)) and digalactosylmannopentaose (Gal\(_3\)Man\(_3\)) were hydrolyzed to a lesser extent than non-substituted substrates (Supplementary Fig. 9e); no activity could be detected on Gal\(_2\)Man\(_2\) while Gal\(_3\)Man\(_3\) was hydrolyzed to yield mannose and digalactosylmannotetraose (Gal\(_2\)Man\(_4\)), which was resistant to further hydrolysis. When the reducing end of manno-oligosaccharides was blocked (Supplementary Fig. 10a–d), no RHG113 activity could be detected demonstrating that this enzyme possesses a previously unknown reducing end mannose-releasing exo-mannosidase activity. Consistent with the view that RHG113 is an intracellular enzyme, release of mannose was detected after incubation of the enzyme with RHG26-generated CGM-oligosaccharides (Fig. 4d). The closest homologs of this enzyme are encoded by Clostridium cluster XIVA strains and a range of Firmicutes (Supplementary Fig. 10e).

Product analysis of end point assays and a time course study revealed that both RHG3A (ROSINTL182_07684) and RHG3B (ROSINTL182_07685) were β-glucosidases, with redundancy in structure (Supplementary Fig. 11a, b), active on linear cellobio-oligosaccharides (Fig. 4e). RHG3B completely hydrolyzed cellotetraose (G\(_4\)) and cellopentaose (G\(_5\)) into glucose monomers, whereas RHG3A released glucose and a range of oligosaccharides with lower efficiency compared to that of RHG3B (Supplementary Fig. 11c, d). Neither of these enzymes were active on manno-oligosaccharides (Supplementary Fig. 11e, f). While RHG3B was able to hydrolyze glucosylmannose (G\(_1\)M\(_1\)) and, partially, mannosylglucose (M\(_1\)G\(_1\)) into monomers (Supplementary Fig. 11b), RHG3A displayed activity only towards G\(_1\)M\(_1\). No activity was detected on polymeric KGM (Supplementary Fig. 12a), while glucose was released after incubation of both RHG3A and RHG3B with RHG26-generated KGM–hydrolysate (Supplementary Fig. 12b). Importantly, the latter results demonstrate that RHG26 can accept a glucose moiety at the subsite +1, generating oligosaccharides with a glucose residue at the non-reducing end.

Recombinant RHG130_2 (MULL, ROSINTL182_05474) phosphorylated M\(_4\) into M\(_3\), M\(_2\) and mannose-1-phosphate (M1P) while M\(_1\) was processed to M\(_2\) and M1P (Fig. 4f). The enzyme was inactive on cello-oligosaccharides (Supplementary Fig. 13). RHG130_2 was functional only in the presence of inorganic phosphate, confirming that RHG130_2 is a mannosyl-phosphorylase.

**Catabolism of mannobiose and mannosylglucose units.** The concerted action of the MULL and MULS encoded enzymes described above on the oligosaccharides generated by RHG26, suggest an intracellular accumulation of M2. Hydrolysis of this product into monosaccharides is accomplished through the action of two enzymes encoded by the co-transcribed genes ROSINTL182_05476 (RiMep) and ROSINTL182_05475 (RHG130_1).

RiMep was active on M\(_2\) and cellobiase (G\(_2\)), releasing M\(_1\)G\(_1\) and G\(_1\)M\(_1\), respectively (Fig. 5a). These data show that RiMep is an enzyme active on the reducing end sugar and catalyzes the conversion of disaccharide substrates to the corresponding C2 epimer. This enzyme exhibited epimerization activity not only for the substrate but also for the product as, under high enzyme amount and long reaction time, it was able to convert M\(_1\)G\(_1\) and G\(_1\)M\(_1\) to M\(_2\) and G\(_2\), respectively (Supplementary Fig. 14a). In addition, RiMep exhibited epimerization activity towards oligosaccharides with a DP > 2 but not on mono-saccharides (Supplementary Fig. 14b).

ROSINTL182_05475 encodes a specific mannosylglucose phosphorlyase belonging to the GH130 subfamily \(^1\). RHG130_1 was inactive on G\(_1\)M\(_1\) and oligosaccharides with a DP ≥ 2 (Supplementary Fig. 15). RHG130_1 displayed activity only towards M\(_1\)G\(_1\) in the presence of inorganic phosphate, releasing glucose and M1P (Fig. 5b, c).

**Catabolism of phosphorolysis products.** RiPgm catalyzes the interconversion of M1P and mannose-6-phosphate (M6P) (Fig. 5d). In addition, the enzyme displayed activity also against D-glucose 1-phosphate (GIP) yielding D-glucose 6-phosphate (G6P) (Supplementary Fig. 16a), thus indicating that RiPgm is a phosphomannomutase (PMM)/phosphoglucomutase (PGM) which can use either glucose or mannose as substrate. Consistent with the presence of a predicted magnesium-binding loop
from Thermotoga maritima β according to the standards. The reaction products were then analyzed using an HPAEC method designed for the separation of phosphorylated manno-oligosaccharides or mono-saccharides and di-saccharides. M1P, indicated with a red arrow, and G1 peaks in the PAD response (nC) time (min) 15.0 20.0 25.0 30.0 35.0 a Standards M2 G2 + RI/Mep G2 no enzyme M2 + RI/Mep M2 no enzyme PAD response (nC) b RI/GH130_1 only M1G1/M2 + RI/GH130_1 M1G1/M2 no enzyme PAD response (nC) c G1 standard M1G1/M2 no enzyme M1G1/M2 + RI/GH130_1 PAD response (nC) d M6P standard M6P no enzyme M1P + RI/Pgm PAD response (nC) e F6P + RI/GH1_D2 F6P no enzyme M6P + RI/GH1_D2 M6P no enzyme PAD response (nC)

Fig. 5 Enzymes for catabolism of mannobiase, mannosylglucose and monosaccharides deriving from complex β-mannan degradation. a) HPAEC-PAD traces showing the epimerization of M2 and G2 by RI/Mep to release M1G1 and G1M1, respectively. b, c) HPAEC-PAD of phosphorolysis reactions of RI/GH130_1 using premixed M1G1 plus M2 from Megazyme. The reaction products were then analyzed using an HPAEC method designed for the separation of phosphorylated manno-oligosaccharides or mono-saccharides and di-saccharides. M1P, indicated with a red arrow, and G1 peaks in b are marked according to the standards. d) HPAEC-PAD analysis of RPgm-catalyzed conversion of M1P to M6P. The M6P released was identified by co-migration with the M6P standard. e) Activity of RI/GH1_D2 on M6P and F6P analyzed by HPAEC-PAD.

( Supplementary Fig. 16b), the RIpgm-mediated catalytic activity was detected only when MgCl2 was present in the reaction.

ROSINTL182_05469/70 encodes a predicted bi-functional protein consisting of an N-terminal glucosidase domain (RI GH1_D1, aa 1–246) and a C-terminal family GH1 isomerase domain (RI GH1_D2, aa 247–768). RI GH1_D1 shares 44% identity to the previously characterized β-glicosidase TmGH1 from Thermotoga maritima41. The recombinant RI GH1_D1 displayed no catalytic activity against all the tested substrates, including G4, M4, M5, M6P, G6P, and fructose 6-phosphate (F6P). Thus, RI GH1_D1 functional significance is currently unclear. RI GH1_D2 is a phosphomannose isomerase catalyzing the interconversion of M6P into F6P (Fig. 5e).

R. intestinalis competes with Bacteroides for β-mannans. The ability of R. intestinalis to capture, breakdown β-mannan and efficiently internalize manno-oligosaccharides may increase its fitness to compete with other resident β-mannan degraders, including the glycan generalist Bacteroides30. To test this hypothesis, we performed in vitro co-cultivation of R. intestinalis and the efficient β-mannan degrader Bacteroides ovatus ATCC 848330. Both bacteria showed similar growth rates in individual cultures supplemented with AcGGM (Fig. 6a). Population estimates using qPCR indicated that, in the mixed cultures, both B. ovatus and R. intestinalis grew well during the exponential growth phase, suggesting that the bacteria shared the available carbon source and maintained coexistence. (Fig. 6b). During the stationary phase, when glycan availability is limited, the mean relative abundance of R. intestinalis and B. ovatus in the culture was approximately 72.5% versus 27.5%, respectively. In contrast, R. intestinalis showed slow growth on mannose (Fig. 6c) and was outcompeted when co-cultured in this carbon source with B. ovatus (Fig. 6d).

R. intestinalis responds rapidly to β-mannan supplementation. To elucidate whether dietary supplementation of β-mannan can result in expansion of key gut bacteria able to utilize this hemicellulose, germfree mice were colonized with a synthetic microbial flora composed of 14 sequenced strains of human commensal gut bacteria42. Colonized mice were fed a high-fiber diet for 14 days before being switched to a series of diet regimes with a varying amount of AcGGM (Fig. 6e). Overall, the levels of four species (R. intestinalis, Bacteroides uniformis, B. ovatus and Marvinbryantia formaxigenis) gradually increased at both AcGGM doses (Fig. 6f-i) and these strains were able to suppress the bacteria foraging on the glyco-protein rich mucus layer (Akkermansia muciniphila, Bacteroides caccae, Bacteroides thetaiotamicron, Bacteroides intestinisihominis) (Fig. 6e and j–m) and the amino acids degraders (E. coli, Clostridium symbiosum and Collinsella aerofaciens) (Fig. 6e and n). Upon feeding of a fiber-deficient diet, the fecal bacterial abundance of the mucin-eroding bacteria, the sulfate-reducer Desulfovibrio piger and the three amino acid degraders (Fig. 6e) rapidly increased with a corresponding decline of the fiber-degrading species.
Fig. 6  R. intestinalis and B. ovatus co-culture experiments and in vivo modulation of a synthetic human gut microbiota via AcGGM.  

- a, c Growth rates of mono- and mixed cultures of R. intestinalis L1–82 (Ri) and B. ovatus ATCC 8483 (Bo) on either AcGGM or mannose. Growth rate is defined as the maximum increase in absorbance at 600 nm (OD max) divided by the time (T max, in hours) to reach the maximum growth.  
- b, d In vitro competition experiment with R. intestinalis L1–82 and B. ovatus ATCC 8483 on either AcGGM or mannose as sole carbon source. The pH of the stationary phase cultures after growth on either AcGGM or mannose was 5.8 ± 0.16 and 5.6 ± 0.11 (two biological triplicates, ± indicates the s.d.), respectively, thus showing that the results are not due to differences in acid sensitivity between the two strains. The relative abundance of the bacteria for each different phases of growth was determined by quantitative PCR of species-specific vs universal primers targeting the 16 S rRNA genes.  
- e In vitro competition experiment with R. intestinalis L1–82 and B. ovatus ATCC 8483 on either AcGGM or mannose as sole carbon source. The pH of the stationary phase cultures after growth on either AcGGM or mannose was 5.8 ± 0.16 and 5.6 ± 0.11 (two biological triplicates, ± indicates the s.d.), respectively, thus showing that the results are not due to differences in acid sensitivity between the two strains. The relative abundance of the bacteria for each different phases of growth was determined by quantitative PCR of species-specific vs universal primers targeting the 16 S rRNA genes.  
- f–n Relative abundance of individual β-mannan-degrading bacteria and mucus-degraders.  

Additive relative abundances of three amino acids degraders. In f–n histogram bars show the average of seven biological replicates while error bars represent s.d. P-values were calculated by two-tailed Student’s t test. An asterisk (*) indicate a statistically significant difference (P < 0.05) in the relative abundance of each bacterium compared to that of the specific pre-FF diet. ns, not significant (P ≥ 0.05)
**Discussion**

β-Mannans are widely present in the human diet as constituents of hemicellulose in beans, some nuts and food additives, but are recalcitrant to intestinal digestion by host enzymes. These glycans instead serve as a carbon source for mannonolytic bacteria in the distal gastrointestinal tract, primarily Firmicutes and Bacteroidetes. Recent studies have characterized a few enzymes encoded by two polysaccharide utilization loci (PULs) implicated in the metabolism of galactomannan in B. ovatus and homopolymeric mannan in Bacteroides fragilis. To date, a full understanding of β-mannan utilization by Firmicutes, however, remains underexplored. The human gut butyrate-producing Firmicute R. intestinalis has previously been shown to utilize galactomannan and glucomannan as a carbon source and possesses predicted genes for the utilization of these substrates. However, no data are available relating the mannanolytic activity at a biochemical level. In this study, we show that two conserved loci, MULL and MULS, collectively provide R. intestinalis the capacity to depolymerize this plant polysaccharide. Detailed biochemical studies of the encoded enzymes allowed us to construct a model of sequential action for the mannan utilization system encoded by MULL-MULS (Fig. 7). The RiGH26 and the mannan ABC uptake system components RiMnBP/RiMPP1/RiMPP2 transcripts and proteins were the most upregulated in both the RNA sequencing and proteomic analyses, respectively (Fig. 2c, e). This highlights the crucial role of this endomannanase and the ABC transport system in the β-mannan metabolic pathway. RiGH26 is the only enzyme displayed on the cell surface (Fig. 3), allowing direct access to the intact β-mannan polymers through dynamic capture mediated by two appended carbohydrate binding modules (RiCBMs). The SPR data showed that RiCBM23 displays ~7- and 5-fold higher affinity for M3 and M4, respectively, than RiCBM27, suggesting that the two CBMs play different roles to mediate binding of RiGH26 to mannans. The RiCBMs’ $K_d$ values for the preferred manno-oligosaccharides were in the 100–200 μM range (Table 1). This moderate affinity to the bound substrate constitutes an advantage as it has lower impact on the catalytic activity compared to canonical counterparts from other organisms, and suggests an evolutionary adaptation of R. intestinalis to compete with other microbial enzymes.

![Fig. 7 Model for the degradation and utilization of complex β-mannans in R. intestinalis](https://example.com/fig7.png)

Intracellular degradation of an acetylated galactoglucomannan (AcGalactoglucomannan) occurs at the outer surface of R. intestinalis by the activity of RiGH26 (green). The extracellular recruitment of β-mannan is facilitated by interactions with CBM27 and CBM23. Import of products occurs through the ABC transporter RiMnBP/RiMPP1/RiMPP2 (orange). Within the cytoplasm, the acetyl and galactosyl decorations are removed by the two acetyl esterases RiCE2 and RiCEX (pink) and the α-galactosidase RiGH36 (yellow). The two β-glucohydrolases RiGH3A and RiGH3B (blue) release glucose from the non-reducing end of the β-manno-oligosaccharide. In addition, the reducing end manno-oligosaccharides are saccharified by the exo-acting RiGH130_2 (light green) with accumulation of M2. The M2 undergoes subsequent epimerization and phosphorylation by the concerted activity of RiMep- RiGH130_1 (light green), with release of glucose and M1P. These end products enter the glycolytic pathway either directly (for glucose) or after being converted into M6P and F6P by the phosphomannose mutase RiPgmm (red) and the isomerase RiGH1_D2 (turquoise, purple domain). Released mannose is converted to M6P by a hexokinase and processed as described above. Galactose enters glycolysis after conversion to G1P via the Leloir pathway. The pyruvate generated from glycolysis is converted to acetyl-CoA and then butyrate. Black arrows show reactions demonstrated in this study. Green arrows indicate previously demonstrated steps for the generation of butyrate from monosaccharides fermentation by R. intestinalis.
with canonical higher-affinity CBMs, but with reduced catalytic rates\(^4\). Reliance on multi-modular cell-wall anchored enzymes is a common feature in Firmicutes\(^5\); consistently, RIGH26 organization was primarily found in β-mannanase from other Roseburia species and members of the Clostridium cluster XIVa (Supplementary Fig. 4, Supplementary Table 3–5). Multiplicity of CBMs provides a contrast with the system for mannan metabolism in Bacteroides ovatus\(^30,45\), where the binding and catalytic activity are distributed between two surface located binding proteins and the single domain mannanase BoMn26B.

Collectively, our results point to a model in which the smaller manno-oligosaccharides generated by RIGH26 are imported through a dedicated β-mannan transport system consisting of RIMmBP/RIMPP1/RIMPP2 (Fig. 7). In the cytoplasm, acetylated and galactosylated manno-oligosaccharides are systematically debranched by RICE2, RICEX and RIGH36, and subsequently depolymerized. Removal of glucose units from glucomannan-oligosaccharides is carried out by RIGH3A and RIGH3B. Based on the highest transcriptional and protein regulation, the main depolymerization strategy for breakdown of unsubstituted manno-oligosaccharides is mediated by the activity of two synergistic mannoside-phosphorylases (RIGH130_2 and RIGH130_1) and an epimerase (RIMep), similar to the mannan catabolic pathway proposed in the ruminal bacterium Ruminooccus albus\(^46\). A similar system has been reported in B. fragilis\(^32\) and B. ovatus\(^30\), although only composed of an epimerase and a mannosylglucose phosphorylase (GH130_1) that, together, process GH26s-generated M\(_2\) units. The presence of the manno-oligosaccharide phosphorylases RIGH130_2 allows R. intestinalis to process undecorated manno-oligosaccharide of DP > 2, consistent with the internalization of large manno-oligosaccharides generated by RIGH26-hydrolysis of polymeric mannan. However, GH130_2s mainly catalyze the phosphorolysis of undecorated manno-oligosaccharides\(^47\). Removal of mannose units from substituted substrates is mediated by the reducing end manno-release exo-oligomannosidase RIGH113, which displays a previously undescribed specificity. The two different approaches based on the phosphorylases and the GH113 are likely to be a functional adaptation to accelerate the depolymerization process of mannan. Eventually, mannan catabolism fuels monosaccharide fermentation via glycolysis and leads to the production of butyrate, which is the primary energy source for host colonicocytes\(^5,48\).

Colonocytes oxidize butyrate to carbon dioxide\(^49\), thereby keeping the epithelial hypoxic (<1% O\(_2\)) condition. This promotes gut homeostasis by stabilizing the hypoxia-inducible transcription HIF that coordinates barrier protection in the mucosa\(^8,51\). Recently, it has been shown that antibiotic-mediated depletion of butyrate-producing Clostridia increases colonicocytes oxygenation and drives aerobic pathogen expansion in the gut lumen, resulting in Salmonella enterica-induced gastroenteritis\(^32\). Importantly, R. intestinalis has been found to affect host histone epigenetic states, direct colonic epithelial cells metabolism away from glycolysis and towards fatty acid metabolism, reduce the levels of inflammatory markers and ameliorate atherosclerosis in a diet-dependent fashion\(^18\). The athero-protective effect was in part attributed to butyrate, as this SCFA has been shown to inhibit key inflammatory pathways involved in cardiovascular disease development\(^18\).

The absence of oligosaccharides from R. intestinalis AcGGM-spent supernatant (Supplementary Fig. 1a, b) demonstrates that the β-mannan degradation apparatus is optimized for efficient uptake of all the products released by RIGH26, maximize intracellular breakdown and avoid nutrient leakage. This will limit the access to other bacteria, such as Bacteroides spp., competing for the same resource. Using AcGGM, we have shown that R. intestinalis and B. ovatus, which possesses an equally complex β-mannan degrading system, shared the available resources and maintained coexistence (Fig. 6b). Notably, R. intestinalis out-competed B. ovatus in the late exponential and stationary phase of growth; these results show that R. intestinalis is capable to bind and import the remaining β-mannan breakdown products (preferred by the RIMnBP transport protein) more efficiently than B. ovatus. Thus, it is likely that the β-mannan utilization apparatus provides R. intestinalis with a selective advantage during nutrient limitation when microbial competition for the available carbohydrates in the gut is intense. Understanding the mechanism by which β-mannan is degraded by key commensal members of the gut is crucial to designing intervention strategies through the use of targeted prebiotics which aim to program or reprogram the composition of the microbiota to maximize human health. Our in vivo study demonstrates that a diet supplemented with AcGGM can be used to manipulate the gut microbiota and to facilitate the growth of species equipped with a β-mannan degrading system, including R. intestinalis (Fig. 6e). This is supported by the increase in the relative abundance of R. intestinalis, B. uniformis, and B. ovatus, which all possess enzymes able to degrade AcGGM (BACUNI_00371 – BACUNI_00383; BACOVA_02087–02097 and BACOVA_03386–03406 respectively). R. intestinalis was highly responsive to the AcGGM within a day, with a 10 to 30 fold increase at the 2.5% and 7.5% AcGGM diet, corroborating its ability to respond dynamically to variation in this dietary fiber. Intriguingly, R. intestinalis’ response did not last over the 7 day feeding treatment and the acetogen M. formi- taxigenes seemed to replace it. A cluster of genes with predicted functions in β-mannno-oligosaccharide utilization (BRYFOR_07194- BRYFOR_07206) was identified in the genome of M. formitaxigenes (Supplementary Fig.17a). The results shown in Supplementary Fig. 17b,d suggest that R. intestinalis and M. formitaxigenes occupy different metabolic niches in the distal gut; the former consumes complex β-mannans, whereas the acetogen feasts on mono- and oligosaccharides. When in co-culture with either R. intestinalis or B. ovatus, M. formitaxigenes was out-competed in vitro (Supplementary Fig. 17e-f). A previous study with gnotobiotic mice bi-associated with the prominent saccharolytic bacterium B. thetataomicron and M. formitaxigenes showed that the presence of M. formitaxigenes caused a decrease in theecal levels of B. thetataomicron, compared with mono-associated controls. Transcriptional and metabolic analyses demonstrated that M. formitaxigenes is capable of consuming a variety of plant-derived oligosaccharides and microbial and host-derived N-glycans (such as N-acetylgalactosamine), suggesting that this ability could confer a fitness advantage when competing with the glycans-consuming Bacteroides\(^33\). Thus, it is likely that, when present as part of the synthetic microbial community described in this paper, M. formitaxigenes may be indirectly benefiting of either manno-oligosaccharides feeding/cross-feeding with other microorganisms or by its ability to grow mixotrophically, simultaneously utilizing organic carbon sources and formate or H\(_2\) for energy\(^33\). Notably, M. formitaxigenes outcompeted B. ovatus at the 7.5% AcGGM diet, underscoring the competitiveness of this acetogen in a community setting. In the context of a complex microbial community, it is likely that M. formitaxigenes makes an important contribution to host nutrition improving fermentation by acting as a formate or H\(_2\) sink and by generating acetate as main metabolic product\(^33\).

Collectively, diet-induced changes involved the promotion of mannano-lytic bacteria producing propionate, acetate and butyrate, metabolites that are known to regulate hepatic lipid, glucose homeostasis and health of the intestinal epithelium\(^11\). These SCFA-producers gained a competitive advantage over colonic mucin-degrading bacteria. Given that intermittent dietary fiber-deprivation results in a thinner mucus layer in mice, eventually
enhancing pathogen susceptibility\(^\text{42}\), our results support the concept that \(\beta\)-mannan-based interventions not only could contribute to preventing mucus barrier dysfunctions but also maintaining a gut environment that keeps pathogenic bacteria away. If confirmed in humans, these findings may help to prevent diseases affecting the integrity of the colonic mucus layer, such as ulcerative colitis\(^\text{34}\). Indeed, the fact that the \(\beta\)-mannan degradation pathway is a core trait found in the majority of the human gut microbiota\(^\text{25}\) highlights the relevance of potential therapeutic interventions through the use of \(\beta\)-mannan formulations to the general population.

Methods

**Glycans.** Carbohydrate substrates used in this study are listed in Supplementary Table 6. All glycans stocks were prepared at 10 mg ml\(^{-1}\) in dH\(_2\)O and sterilized by filtration using a 0.22 \(\mu\)m membrane filter (Sartorit AG & Co, Germany).

**Bacterial strains and growth conditions.** Unless otherwise stated, *R. intestinalis* L1-82\(^\text{23}\) was routinely grown at 37 °C without agitation in an anaerobic cabinet (Whitely A95 workstation, Don Whitely, UK) under an 85% N\(_2\)/10% H\(_2\)/5% CO\(_2\) atmosphere. Growth experiments were carried out in YCFA medium (YCFA—Yeast extract–Casein hydrolysate–Fatty Acids)\(^\text{26}\) supplemented with 0.5% (w/v) of the specific carbohydrate to be examined. Overnight cultures (300 μl) were used to inoculate 30 ml aliquots of YCFA plus the carbohydrate to be tested. These cultures were sequenced and their growth was assessed by measuring differences in pH compared to that of starting medium. Growth and final cultures for growth experiments, RNA-sequencing and proteomic analysis. Bacterial growth cultures were passaged at least three times on the same media to ensure cell growth.

**RNA-sequencing and proteomic analysis.** Bacterial growth experiments were carried out in YCFA medium (YCFA−NH\(_4\)Cl) or 100 mM sodium phosphate buffer, pH 5.8. The activity of RiPgm against AMP and GIP was tested in 10 mM sodium phosphate buffer, pH 5.8, supplemented with 1 mM MgCl\(_2\). To determine the specificity of RiGH113, the recombinant protein was sequentially incubated with 0.1 mg ml\(^{-1}\) pre-reduced or oxidized manno-oligosaccharides at 37 °C overnight. Reduction of manno-oligosaccharides was conducted by incubating 1 mg ml\(^{-1}\) manno-oligosaccharides in a volume of 75 μl with sodium dithiothreitol (NaD\(_2\)SH) in 100 mM sodium phosphate buffer. The reaction was incubated overnight at room temperature then quenched by adding 25 μl of 25 mM sodium acetate. Oxidation of manno-oligosaccharides reducing-end was obtained by incubating the substrates (1 mg ml\(^{-1}\)) with the Neurospora crassa cellulose dehydrogenase (NCdH) overnight at 37 °C. Both NaD\(_2\)SH and NCdH pretreated samples were run in standard acetonitrile solvent system before addition of the three and five independent experiments were performed to determine the enzyme activities.

**MALDI-TOF mass spectrometry of reaction products.** Reaction products generated by the enzymes used in this study were analyzed by matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS) as described previously\(^\text{65}\). Brieﬂy, 2 μl of a matrix, consisting of 9% 2,5-dihydroxybenzoic acid (DHB) in 30% acetonitrile, were applied to an MTP 384 ground steel target plate (Bruker Daltonics, Germany). Sample (1 μl) was then mixed with the matrix and dried under a stream of warm air. Samples were analyzed with an Ultraflex MALDI-TOF/TOF instrument (Bruker Daltonics, Germany), equipped with a Nitrogen 337 nm laser beam and operated in positive acquisition mode. Results were analyzed using the Bruker FlexAnalysis software (version 3.3).

**HPAE-PAF.** Mono- and oligosaccharides products were analyzed on a Dionex ICS-3000 PDA system operated by the Chromelone software version 7 (Dionex, Thermo Scientific), as described previously\(^\text{32}\). Sugars were injected onto a CarboPac PA1 2 x 250-mm analytical column (Dionex, Thermo Scientific) coupled to a CarboPac PA1 2 x 50-mm guard column kept at 30 °C. Manno-oligosaccharides and phosphorylated monosaccharides were eluted in 0.1 M NaOH at a flow rate of 0.6 ml min\(^{-1}\) by increasing the concentration of sodium acetate (NaOAc) exponentially from 0 to 0.3 M over 26 min (from 9 to 35 min after injection), before column reconditioning by 0.1 M NaOH for 10 min. Commercial manno-oligosaccharides with DP 2–6 were used as standards. For cello-oligosaccharides, the separation was done using an anion-exchange linear gradient going from 0.1 M NaOH to 0.1 M NaOH–0.1 M NaOAc over 10 min, 0.1 M NaOH–0.14 M NaOAc after 14 min, 0.1 M NaOH–0.3 M NaOAc at 16 min followed by a 2 min exponential gradient to 1 M NaOAc, before reconditioning with 0.1 M NaOH for 9 min. Cello-oligosaccharides with DP 2–6 were used as standards. For the analysis of disaccharides (G\(_1\)M\(_1\), M\(_1\)G\(_1\)) and phosphorylated monosaccharides generated from the activity of RiGH130_2, RiMep, RiGH130_1, RpPgm and RhG1113, the elution was done at 0.25 ml min\(^{-1}\) using a 40 min program. The program started with 0.01 M potassium hydroxide (KOH) for 15 min, reaching the concentration of 0.1 M KOH at 25 min after injection and was kept for additional 5 min at the same KOH concentration. Between each sample, the column was re-equilibrated by running initial conditions for 10 min.

**Protein cellular localization.** Proteins of interest were detected using anti-raisin mabs (Eurogentec) against the corresponding recombinant RiGH26 or the previously characterized RiXyn103\(^\text{28}\).

For immunofluorescence microscopy, *R. intestinalis* cells were grown in YCFA containing 8.5% AcGGM, wheat arabinoxylan (WAX) or glucose to an OD\(_{600}\) of 0.8, collected by centrifugation (4000 x g for 5 min) and washed twice in phosphate buffered saline (PBS). Cells were resuspended in 500 μl PBS and fixed by adding an equal volume of 2 x formalin (9% formaldehyde in PBS) on ice for 30 min. The bacterial pellet was washed twice with 1 ml PBS prior to resuspension in 1 ml of blocking buffer (1% bovine serum albumin, BSA, in PBS) and incubation at 4 °C for 1 h. After incubation the bacteria were harvested by budding of RH13 and the supernatant discarded. For labelling, the bacteria were incubated with 0.5 ml of anti-sera (diluted 1:500 in blocking buffer) for 2 h at 25 °C. The cells were then
pelleted, washed with 1 ml PBS and resuspended in 0.5 ml goat anti-rat IgG Alexa Fluor 488 (Sigma-Aldrich), diluted 1:500 in blocking buffer and incubated 1 h at 25 °C. After washing, cells were re-suspended in 100 μl PBS containing one drop of ProLong Gold antifade reagent (Life Technologies). Labeled bacterial cells were mounted onto glass slides and secured with coverslips. Fluorescence microscopy was performed on a Zeiss AxioObserver equipped with the ZEN Blue software. Images were acquired using an ORCA-Flash4.0 V2 Digital CMOS camera (Hamamatsu Photonics) through a 10× phase-contrast objective. A HXP 120 Illuminator (Zeiss) was used as a fluorescence light source.

Analysis of the bacterial proteome. R. intestinalis was grown in triplicate on YCFA supplemented with either 0.5% (w/v) glucose or AcGGM, respectively, as a sole carbon source. Samples (10 ml) were harvested at the mid-exponential growth phase. Cell pellet was collected by centrifugation (4500 × g, 10 min, 4 °C), resuspended in 50 mM Tris- HCl, 0.1% (v/v) Triton X-100, 200 mM NaCl, 1 mM dithiothreitol and disrupted by bead-beating using three 60 s cycles with a FastPrep24 (MP Biomedicals, CA). Proteins were precipitated with ice-cold tri-chloroacetic acid (TCA), final concentration of 10% (v/v), incubated on ice for 1 h, centrifuged (15,000 × g, 15 min, 4 °C) to pellet the precipitated proteins and washed with 300 μl ice-cold 0.1 M HCl in 90% acetone. Proteins were separated by SDS-PAGE with a 10% Mini-PROTEAN gel (Bio-Rad Laboratories, CA) and then stained with Coomassie brilliant blue R250. The gel was cut into PAGE with a 10% Mini-PROTEAN gel (Bio-Rad Laboratories, CA) and then stained with Coomassie brilliant blue R250. The gel was cut into five slices, after which proteins were reduced, alkylated, and in-gel digested according to a method published previously. The peptides were dried under vacuum, solubilized in 1% (v/v) trifluoroacetic acid (TFA) and desalted using C18 ZipTips (Merck Millipore, Germany) according to the manufacturer’s instructions.

The peptide mixture from each fraction was analyzed using a nanoHPLC-MS/MS system as described previously, using a Q-Exactive hybrid quadrupole-orbitrap mass spectrometer (Thermo Scientific) equipped with a nano-electrospray ion source. Mass spectral data were acquired using Xcalibur (v.2.2.18). MS raw files were processed with the MaxQuant software suite (version 1.4.1.2) for identification and label-free quantification (LFQ) of proteins. Proteins were identified by searching MS and MS/MS data of peptides against the UniProtKB complete proteome of R. intestinalis L1–82 (4698 sequences) supplemented with common contaminants (e.g. keratin, trypsin, and bovine serum albumin). In addition, reversed sequences of all protein entries were concatenated to the database for estimation of false-discovery rates (FDRs). Trypsin was set as proteolytic enzyme and two missed cleavages were allowed. Identiﬁcations with a FDR of 1% were considered present if the proteome analysis met the default parameters. All identiﬁcations were ﬁltered in order to achieve a protein FDR of 1%. A protein was considered “present” if it was detected in at least two of the three biological replicates in at least one glycan substrate. Missing values were imputed from a normal distribution (width of 0.3 and down shifted 1.8 standard deviations from the original distribution) in total matrix mode and differential abundance analysis was performed using an unpaired two-sided Student’s t-test with a permutation-based FDR set to 0.05. Hierarchical clustering and heat map representations were generated using Euclidean distance measure and average linkage using Perseus (version 1.5.5.3).

Substrate binding assay using SPR. The affinity of RCBM72 and RCBM23 to soluble manno-oligosaccharides and cellobio-oligosaccharides was evaluated by SPR using a Biacore T100 (GE Healthcare). The two CBMs, diluted into 10 mM sodium acetate (pH 4.1) to 2.3 μM, were immobilized on a NTA sensor chip (GE Healthcare) to a density of 3000–4000 response units (RU). Sensograms were recorded at 25 °C in phosphate/citrate buffer (20 mM phosphate/citrate buffer; 150 mM NaCl; pH 6.5; 0.05% (v/v) P2O5 surface active agent) at 30 μl per minute with association and dissociation times of 90 s and 240 s, respectively. CBMs binding was tested towards 0.2 nM – 1 mM of carbohydrate ligands dissolved in the same buffer as above. Data were analyzed using the Biacore T100 evaluation software, and equilibrium dissociation constants (Kd) were obtained by fitting a single-site binding model to either the steady-state response data or the full sensograms.

ITC. Binding of manno-oligosaccharides to RmBP was measured at 25 °C in 10 mM sodium phosphate pH 6.5 using an ITC200 microcalorimeter (MicroCal). RmBP in the sample cell was titrated by 19 injections of carbohydrate ligand separated peptide portions (120s). The low concentration of 20 nM was used: 900 μM of M1 in the syringe and 76.5 μM RmBP in the sample cell; 1365 μM of M4 or M3 in the syringe and 91 μM RmBP in the sample cell; 2270 μM of M8 in the syringe and 117 μM RmBP in the sample cell; 750 μM of diacetylated mannobiose (M2Ac2) in the syringe and 50 μM RmBP in the cell; 1500 μM of diacetylated manno- pentaoctasaccharide (M2Ac5) in the syringe and 100 μM RmBP in the cell. Thermodynamic binding parameters were determined using the MicroCal Origin software (version 7.0).

**Competition experiments.** R. intestinalis, B. ovatus and M. formatexigens cells were grown overnight under anaerobic conditions in YCFA containing 0.5% (w/v) AcGGM (VSG-AcGGM) as the sole carbon source. These subcultures were used to inoculate, in approximately equal proportions (estimated by OD562, 30 ml of the same medium. A control culture of VSG-AcGGM was also inoculated with either R. intestinalis, B. ovatus or M. formatexigens. Growth (OD562) was monitored for up to 24 h, withdrawing 1 ml samples for quantitative PCR (qPCR) analysis at selected points. Cells were pelleted, combined with 200 μl of TE buffer (pH 7.8) and bead-beated for 2 min (FastPrep96, MP Biomedicals, CA) using ≤ 10 μm acid-washed glass beads (Sigma-Aldrich). Genomic DNA was extracted using the Mag Midi kit (LGC Group, UK) according to the manufacturer’s instructions. qPCR was performed in a LightCycler 480 II system (Roche, Germany) using specific primers for each strain (Supplementary Table 9). In addition, a high-resolution melting (HRM) analysis was conducted to evaluate the specificity of the amplification and the lack of primer dimers. The raw data were imported into the LinReg PCR program and the calculated Cq values and PCR efficiency were used to deduce the ratio of R. intestinalis, B. ovatus and M. formatexigens at each time point. Statistically significant differences were determined using the unpaired two-tailed Student’s t-test.

**Human gut microbiota-associated mice and diets.** All experiments involving animals complied with all relevant ethical regulations for animal testing and research and were approved by the University of Michigan, University Committee for the Use and Care of Animals. Germfree mice were colonized with a synthetic microbiota composed of 14 fully sequenced human species according to the methodology previously adopted by Desai et al. Briefly, seven 6-week-old germfree male wild-type Swiss Webster mice that had been raised on ad libitum access to a high fiber Chow diet (LabDiet 5013) and autoclaved distilled water were gavaged for 3 consecutive days with 200 μl each of a mixture of the 14 different species. Colonized mice were maintained on this high fiber diet for 14 days before being switched to a series of diet regimes with varying fibers. This feeding sequence consisted of 7 days of feeding on a gamma-irradiated fiber-free (FF) diet (TD.140343, Harlan Teklad, USA) that does not contain AcGGM or related molecules. Mice were then switched for 7 days to a custom version of the same diet that contained AcGGM at 2.5% w/w, followed by a 7-day washout period on the FF diet, and finally 7 days of feeding on a version of this diet containing AcGGM at 7.5% w/w (in both AcGGM diets an equivalent amount of glucose was removed to accommodate the prebiotic addition). Fecal samples were taken 1 day before and 1 day after each diet transition, effectively allowing us to measure changes in response to AcGGM supplementation at 1 and 7 days of exposure to the FF diet. The relative abundance of each microbial strain at sampled time points was measured by qPCR, using previously validated primer sets, from total DNA extracted from freshly voided fecal pellets (stored at ~20 °C until extraction) exactly as described previously. Statistically significant differences were determined using the unpaired two-tailed Student’s t-test.

**Comparative genomic analysis.** Identification of similar β-mannan catabolic genes in bacteria belonging to the Clostridium XIVA cluster was performed using the Gene Ortholog Neighborhood viewer on the Integrated Microbial Genomes website (https://img.jgi.doe.gov). This was done using the genes encoding RGH26 (R0SINTL182_07683, GenBank ABY00000124.1:7167–11129) and R0MEP (R0SINTL182_05476, GenBank ABY00000025:13200–4429) as the search homolog and the default threshold e-value of 1e-5. Then, a sequence comparison was conducted where each R. intestinalis L1–82 RefSeq annotated protein sequence was subjected to BLASTp searches again others Clostridium XIVA members. Sequences with coverage >80% and amino acid similarity ≥45% were excluded.

**Reporting summary.** Further information on experimental design is available in the Nature Research Reporting Summary linked to this article.

**Data availability** All data supporting the findings of this study are available within the article and Supplementary Information, or from the corresponding author upon request. The transcriptomic data described in this article are submitted under NCBI BioProject accession number PRJNA516396. The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier PXD012448.

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Author contributions

Experiments were primarily designed by S.L.L.R., E.C.M., M.A.H., and B.W. S.L.L.R. cloned, expressed, purified and performed functional characterizations of the enzymes. Production of AcGGM was performed by L.M. and B.W. The initial growth experiments on mannans were performed by M.L.L. and these experiments were used to prepare RNA and performed the transcriptional analysis together with C.T.W. Proteomic analysis was done by S.L.L.R. and M.O.A. SPR and ITC was performed by M.E.H., who also cloned, expressed and purified the transport protein. S.L.L.R. and G.P. conducted the in vitro growth experiments, competition experiments and qPCR. S.L.L.R. performed enzyme localization studies. Mice experiment was conducted by N.A.P., R.G. and E.C.M. The manuscript was written primarily by S.L.L.R. and B.W. with contributions from P.B.P., M.O.A., M.A.H., and E.C.M. Figures were prepared by S.L.L.R., M.E.H., and N.A.P. All authors reviewed the final manuscript.

Additional information

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