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Differential bacterial capture and transport preferences facilitate co-growth on dietary fibers in the human gut

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Abstract

Metabolism of dietary glycans is pivotal in shaping the human gut microbiota. The mechanisms that promote competition for glycans amongst gut commensals, however, remain unclear. *Roseburia intestinalis*, an abundant butyrate-producing Firmicute, is a key degrader of the major dietary fiber xylan. Despite the association of this taxon to a healthy microbiota, insight is lacking into its glycan utilization machinery. Here, we investigate the apparatus that confers *R. intestinalis* growth on different xylans. *R. intestinalis* displays a large cell-attached modular xylanase that promotes multivalent and dynamic association to xylan via three known and one novel xylan-binding module. This xylanase operates in concert with an ATP-binding cassette (ABC) transporter to mediate breakdown and selective internalization of xylan-fragments. This apparatus supports co-growth between *R. intestinalis* with a model xylan-degrading *Bacteriodes* in mixed cultures. The transport protein of *R. intestinalis* prefers xylo-oligosaccharides of 4–5 xylosyl-units, whereas the counterpart from competing *Bacteroides* targets larger ligands. This insight highlights the differentiation of capture and transport preferences as a strategy to facilitate co-growth on abundant dietary fibers by gut commensals. These findings offer a unique route to manipulate the microbiota based on glycan-transport preferences in therapeutic interventions to boost or restore distinct taxa.

26 Introduction

27 The human gut microbiota (HGM) is recognized as a determinant of human health and metabolic
28 homeostasis^{1,2}. Specific signatures of the HGM are associated with local and systemic disorders
29 including irritable-bowel disease, obesity, type 2 diabetes and colon cancer³. The composition of the
30 HGM is greatly affected by dietary glycans, which are non-digestible by the host^{4,5}. Only a few
31 species out of the hundreds present in the HGM are equipped to deconstruct distinct complex
32 polysaccharides and ferment them into short chain fatty acids (SCFAs)⁶. The impact of SCFAs on host
33 health and physiology remains an important aspect of the microbiota-host interaction. Particularly
34 the SCFA butyrate, the preferred energy source for colonocytes, is known to have anti-inflammatory
35 roles and reduce the risk of colon cancer and enteric colitis⁷⁻¹⁰. Butyrate producers belonging to the
36 Firmicutes phylum are generally abundant in healthy individuals, but are markedly reduced in
37 patients with inflammatory disorders^{11,12}. Butyrate producers including *Roseburia* spp. are increased
38 in metabolic syndrome patients after faecal transfer therapy, and correlate positively to
39 improvement of insulin resistance¹³. Investigations of the metabolic preferences of butyrate
40 producers and their interplay with major HGM commensals are instrumental to develop therapeutic
41 interventions targeting butyrate-deficiency related disorders.

42 *Roseburia* is a common genus of *Clostridium* cluster XIVa within the Firmicutes that harbours
43 prevalent butyrate producers^{14,15}. This taxon adheres to mucin, consistent with an intimate
44 association with the host¹⁶. *Roseburia intestinalis* strains encode an impressive repertoire of
45 carbohydrate active enzymes (CAZymes) compared to most other Firmicutes¹⁷. *R. intestinalis*, the
46 taxonomically related *Eubacterium rectale* and species from the *Bacteroides* genus are the only
47 known HGM taxa that utilize the major hemicellulosic polysaccharide xylan¹⁸⁻²⁰. Xylan is particularly
48 abundant in cereal grains (arabinoxylan, AX), but is also found in fruits and vegetables
49 (glucuronoxylan, GX)²¹ (Fig. 1a). Xylan utilization by dominant gut commensals belonging to the

Bacteriodes genus has been investigated in detail^{22,23}, but similar knowledge is lacking for Firmicutes counterparts.

Here, we show that *Roseburia intestinalis* L1-82 grows on acetyl, arabinosyl and 4-*O*-methyl-glucuronosyl decorated dietary-relevant xylans, with a preference for cereal arabinoxylans. The growth is mediated by a multi-modular cell-attached xylanase and by an ABC transporter. The gene encoding this transporter was the most upregulated in response to xylan, consistent with a paramount role during growth on this glycan. We have characterized the xylanolytic enzymes and the transport protein, which enabled modelling xylan utilization by *R. intestinalis* and the identification of two novel xylan-specific CAZyme families. *R. intestinalis* efficiently competes with a model xylan degrader belonging to the genus *Bacteroides*, when grown on soluble and insoluble xylans. A striking finding was that the transport proteins that confer xylo-oligosaccharides capture in *R. intestinalis* and *Bacteroides* targeted ligands of different sizes, thus markedly reducing the competition for preferred ligands by either taxon. These results emphasize the competitiveness of butyrate producing Firmicutes in targeting key dietary fibers like xylan. The substantial differences in transport proteins highlight the differential capture and transport preference as a key feature to facilitate co-growth on abundant dietary fibres such as xylan.

Results

Inducible cell-attached xylanase activity mediates growth of *R. intestinalis* on substituted xylans

Anaerobic growth of *R. intestinalis* L1-82 was measured as an increase in OD_{600 nm} for growth on soluble xylans and as a decrease in pH for growth on insoluble xylans (Fig. 1b-d). *R. intestinalis* L1-82 grows rapidly on soluble xylans with a preference for wheat arabinoxylan (WAX, $\mu_{\max}=0.26 \text{ h}^{-1}$) compared to birch glucuronoxylan (BGX, $\mu_{\max}=0.13 \text{ h}^{-1}$) (Fig. 1c). Interestingly, this bacterium also utilizes highly acetylated xylans and insoluble cereal arabinoxylans from wheat (InWAX) and oat spelt (OSX), but not cornbran glucuronoarabinoxylan (CBX). Xylo-oligosaccharides and xylan-derived

monosaccharides (except glucuronic acid) were also utilized (Fig. 1b). Extracellular *endo*-1,4- β -xylanase (hereafter referred to as xylanase) activity was induced upon growth on BGX, WAX, and xylobiose (X2), despite poor growth on the latter disaccharide (Fig. 1e). The xylanase activity was cell-attached, but was released upon treatment of the cells with a high salt concentration (Fig. 1f), suggesting noncovalent attachment.

Genes encoding an ABC transporter and a multi-modular xylanase are amongst the top upregulated in response to growth of *R. intestinalis* on xylan

To elucidate the genetic basis for growth on xylans, we performed an RNA-seq transcriptional analysis of *R. intestinalis* grown on WAX, BGX, xylose and glucose. Of the 4777 predicted genes, 1–3.5% were highly upregulated (Log2 fold-change > 5) on xylans compared to glucose (Supplementary Table 1), the majority being involved in carbohydrate and energy metabolism. Besides a separate locus encoding a multi-modular xylanase of glycoside hydrolase family 10 (GH10 according to the CAZy classification, <http://www.cazy.org>²⁴), the top genes in the xylan transcriptomes cluster on a single locus (Fig. 2a,b). This locus contains eleven genes including four xylanolytic CAZymes of GH43, GH115, GH8, GH3. Only one (ROSINTL182_08192, LacI type, Pfam 00356) of three transcriptional regulator genes was highly upregulated. Strikingly, the most upregulated gene in the xylan transcriptomes encodes a solute binding protein (SBP) of an ABC transporter. Furthermore, the genes encoding the permease components of this ABC transporter were amongst the top six upregulated by xylans. Signal peptides were only predicted for the xylanase and the transporter SBP, consistent with extracellular breakdown of xylan followed by capture and uptake of xylo-oligosaccharides by the ABC transporter. The expression and the localization of the transport SBP and the xylanase at the cell surface were corroborated using immunofluorescence microscopy (Fig. 2c). Two additional loci, unique to *R. intestinalis* L1-82, lacking in other *R. intestinalis* strains, were also upregulated albeit markedly less (Supplementary Fig. 1a-d). One of these loci encodes a second

cell attached GH10 xylanase, which is also expressed at the cell surface (Supplementary Fig. 1e). The transcriptomic analysis also enabled us to assign the ABC-transporter mediating xylose import and to outline the genes involved in intracellular metabolism of xylose, arabinose and glucuronic acid (Supplementary Fig. 1f,g).

A new family of binding modules confers extended and dynamic xylan binding to the multi-modular xylanase in *R. intestinalis*

The highly upregulated *RiXyn10A*, which is conserved within the *R. intestinalis* species, is one of the largest known xylanases from human gut bacteria (Supplementary Fig. 2b). *RiXyn10A* comprises an N-terminal unassigned domain (residues 28–165), a xylan binding module of CBM22, a catalytic module of GH10, a tandem repeat of CBM9 xylan binding modules, a bacterial Ig-like domain group 2 (BIG2, pfam02368)²⁵ and a Listeria-Bacteroides repeat domain (LBR, pfam09479)²⁶. The two latter domains likely mediate cell attachment of the enzyme to the cell^{25–27} in accordance with their positive charge, which is compatible with binding to the negatively charged cell surface (residues 1100-1356, pI>10).

To generate insight into the unique modularity of *RiXyn10A*, we characterized the enzyme and truncated versions thereof (Fig. 3a-d). *RiXyn10A* incubated with BGX, WAX and InWAX generated linear and decorated oligosaccharides (Fig. 3b,c and Fig. 4). *RiXyn10A* was inactive on highly and heterogeneously substituted arabinoglucuronoxylan from corn bran, consistent with the lack of growth on this substrate by *R. intestinalis*. The enzyme was inactive on xylobiose (X2) and showed very low activity on xylotriose (X3) (Supplementary Fig. 3a). By contrast, xylotetraose (X4) and xypentaose (X5) were hydrolyzed stoichiometrically, revealing the requirement for at least four substrate-binding sub-sites for efficient hydrolysis.

A BLASTP search of the N-terminal unassigned domain (CBMx) against UniProt gave no hits indicating the lack of homologues with assigned function. CBMx confers affinity to xylan as implied

from a two times higher K_M when this domain was deleted (Fig. 3d). Affinity electrophoresis established CBMx to be a novel xylan-binding module and revealed a 30-fold stronger binding for WAX compared to BGX (Fig. 3e,f and Supplementary Fig. 3c). Surface plasmon resonance (SPR) analysis revealed the highest affinity towards xylohexaose (X6) consistent with the presence of a binding cleft large enough to accommodate at least six xylosyl units (Fig. 3e,g and Supplementary Fig. 4a-e). This analysis also indicated specificity to xylan as there was no measurable affinity to mannohexaose (Man6). The relatively low binding affinity to X6 ($K_D \approx 0.5$ mM) was corroborated using isothermal titration calorimetry (ITC) (Fig. 3e and Supplementary Fig. 4g,f). Deleting CBMx decreased the average K_D of *RiXyn10A* from 128 μ M to 65.4 μ M (*RiXyn10A* Δ CBMx) (Fig. 3e and Supplementary Fig. 4h-k), asserting that at least one or more of other CBMs possess higher affinity compared to the N-terminal new module. Homologues (sequence identity 55–27%) of the new CBM are present mainly in other bacteria from *Clostridium* XIVa cluster (Supplementary Fig. 4l), which merits the assignment of these modules into a new CBM family.

Preference of the binding protein of the ABC transporter that mediates uptake of xylan oligosaccharides in *R. intestinalis*

We showed above that the action of xylanases produces complex xylo-oligosaccharides likely decorated with arabinosyl and 4-*O*-methyl-glucuronosyl. The presence of these decorations is supported by the decrease in some of these peaks and the increase in arabinose and un-substituted xylo-oligosaccharides after treatment with debranching enzymes (see next section). No oligosaccharides were detectable (HPAEC-PAD analysis, data not shown) in spent supernatants from *R. intestinalis* growth on xylan, suggesting efficient uptake of oligomeric products. The transcriptional analysis (Fig. 2a) identified an ABC transporter likely to mediate the uptake of the xylo-oligosaccharides hydrolysis products of *RiXyn10A* from WAX and BGX. The preference of SBPs associated with oligosaccharide-specific ABC transporters has been shown to correlate well to the uptake preference of bacteria^{28,29}. We measured the affinity of *RiXBP*, the SBP of the upregulated ABC transporter, on a range of xylo-oligosaccharide ligands (Table 1 and Supplementary Fig. 5). The preferred un-substituted ligand was X5

followed by X4, and the affinity decreased steeply for smaller or larger oligosaccharides. Internal arabinosyl decorations (AX4) appeared to be preferred based on the 2.4-times higher affinity compared to the un-substituted X4. The tolerance and recognition of arabinosylated ligands is in agreement with the good growth on WAX. These results suggest that *RiXBP* is selective in capturing internally branched xylo-oligosaccharides with a xylose backbone of 4–5 xylose residues.

***R. intestinalis* degrades internalized decorated xylo-oligosaccharides by the concerted action of three hydrolases and a novel family of acetyl esterases**

Xylo-oligosaccharides are degraded in the cytoplasm after their uptake. To gain insight into intracellular xylan-oligosaccharide breakdown, we produced and characterized the α -glucuronidase *RiAgu115A* (GH115), the α -L-arabinofuranosidase *RiAbf43A* (GH43), two xylosidases *RiXyl8* (GH8) and *RiXyl3A* (GH3) as well as *RiAXE* (ROSITNL182_08194, GenBank accession EEU99941.1) from the core xylan utilization locus.

RiAgu115A released 4-*O*-methyl-glucuronic acid (MeGlcA) from glucuronoxylans (BGX and BeGX) and from BGX pretreated with *RiXyn10A* (Fig. 4a and Supplementary Fig. 6a-c). The k_{cat}/K_M of *RiAgu115A* was 16-fold higher on glucuronoxylan hydrolysate compared to intact glucuronoxylan (Supplementary Fig. 6c), indicating that *RiAgu115A* preferentially accommodates glucuronoxyloligosaccharides, consistent with the intracellular localization of this enzyme. This enzyme also cleaves MeGlcA decorations at the xylosyl penultimate to the reducing end (generated using a GH30 glucuronoxylanase, Supplementary Fig. 6b), but its activity was blocked by the presence of acetylations (Fig. 4d).

RiAbf43A is an α -L-arabinofuranosidase that exclusively releases arabinose from WAX (Fig. 4a). Kinetic analysis towards WAX and arabino-xylotetraose (AX4) (Supplementary Fig. 6d) revealed recognition of internal arabinosyl substitutions, with a 13-fold increase in k_{cat} for oligosaccharides consistent with the intracellular localization.

Both *RiXyl8* and *RiXyl3A* generated xylose from xylo-oligosaccharides, but lacked activity towards xylan (Supplementary Fig. 6g-k). *RiXyl3A* degraded xylo-oligosaccharides completely into monosaccharides, while *RiXyl8* was inactive towards X2. Reduction of xylo-oligosaccharides with NaBH₄ abolished the activity of *RiXyl8* assigning it as a reducing-end β -xylosidase³⁰ (Supplementary Fig. 6i), in contrast to *RiXyl3A* that recognizes non-reducing xylosyl moieties and maintains activity on reduced xylo-oligosaccharides. Thus, the concerted and overlapping activities of these enzymes (Supplementary Fig. 6) results in rapid depolymerization of arabinosyl and MeGlcA decorated xylo-oligosaccharides.

RiAXE, which was un-assigned, based on lack of hits in a BLASTP search of UniProt, was highly upregulated on xylans (Fig. 2a). This enzyme possesses the conserved residues in the SGNH lipases-esterases superfamily (Pfam cd00229), which also includes CAZy carbohydrate esterase families CE2, CE3, CE12 and CE16. We established that *RiAXE* is an acetyl esterase, but low sequence identities to these families (<12%) merit assigning *RiAXE* into a new carbohydrate esterase family. Indeed homologues of this enzyme are encoded by several *Clostridium* cluster XIVa strains from the human gut and by a range of Firmicutes (Supplementary Fig. 7i).

Assaying *RiAXE* activity towards AcBGX oligosaccharides (generated with *RiXyn10A*) using NMR revealed efficient deacetylation of both C2 and C3, but with a preference for C2 decorations (Fig. 4b and Supplementary 7). Analysis of the deacetylation by MALDI-ToF MS left a single acetyl group on the AcBGX oligosaccharides (Fig. 4e). Inclusion of *RiAgu115A* in this reaction resulted in complete deacetylation (Fig. 4f) suggesting that the presence of MeGlcA decorations protects acetylations in the proximity of the MeGlcA unit. Analysis of the deacetylation rates also unveiled the concerted action with *RiAgu115A* and the preference to hydrolysates of *RiXyn10A* rather than intact xylan (Supplementary Fig. 7c,d). *RiAXE* specifically recognizes acetylations on xylosyl units based on lack of activity on acetylated chitin and very low activity on acetylated mannan and cellulose monoacetate

(Supplementary Fig. 7h). Taken together, the results showed that *Ri*AXE is an efficient xylan specific representative of a new acetyl esterase family.

In summary of the biochemical characterization presented above, we propose a model for the uptake and degradation of diet-derived acetylated arabinoxylan and glucuronoxylan by *R. intestinalis* L1-82 (Fig. 5a).

***R. intestinalis* competes with *Bacterioides* for xylans**

The growth potential of *R. intestinalis* was compared with the efficient xylan degrader *Bacteroides ovatus*²², by observing growth of individual cultures and in co-culture. Both strains displayed similar growth on xylan as carbon source (Fig. 5b-d and Supplementary Fig. 8a,b). In competition, both strains appeared to grow equally well on xylans (Fig. 5e-g), whereas *R. intestinalis* dominated the co-culture on X4 after 7 hours of growth (Fig. 5h). The results indicate that *R. intestinalis* is an efficient primary degrader of xylan that is able to compete with *B. ovatus* and even outcompete this bacterium on preferred smaller xylo-oligosaccharides.

Discussion

The human gut is dominated by bacteria from two phyla: the Gram-positive Firmicutes and the Gram-negative Bacteroidetes. Firmicutes are generally regarded as metabolic specialists, while Bacteroidetes (mainly from the *Bacteroides* genus) are considered generalists based on narrow versus broad glycan utilization capabilities, respectively⁶. The size and diversity of encoded CAZymes frequently reflects these metabolic labels. Although this generalization applies to *R. intestinalis*, based on the relatively limited glycan growth profiles⁵, this species possesses distinctively larger CAZymes than most known clostridial Firmicutes of the HGM¹⁷. *R. intestinalis* has been proposed as a key xylan degrader in the human gut along with specific species of *Bacteroides*^{18,19}. Growth and enumeration of *R. intestinalis* on dietary xylans including wheat bran is reported both *in vitro* and *in*

vivo^{20,32}. Insight is lacking, however, on the preferences and the molecular machinery evolved by *R. intestinalis* to target xylan as compared to species of *Bacteriodes*. In this study, we present a model that explains the molecular basis for the utilization of xylan by *R. intestinalis* L1-82 as a representative for prevalent butyrate producing clostridia (Fig. 5a). Our data establish that *R. intestinalis* is truly a primary degrader that is equipped with a highly efficient machinery for utilization of complex dietary xylans, including insoluble arabinoxylan from cereals. Identified key components of the *R. intestinalis* xylan utilization strategy include a multi-modular extracellular xylanase and an ABC transporter, which confer the capture, breakdown and internalization of decorated xylan oligosaccharides. In the cytoplasm, internalized xylo-oligosaccharides are depolymerized without loss to competing species. We demonstrate the ability of *R. intestinalis* to grow on acetylated xylan, which reflects an adaptation to this abundant decoration in dietary xylans (Fig. 1b). Acetylated xylo-oligosaccharides are metabolized after internalization due to an intracellular previously unknown esterase family capable of removing C2, C3 and double acetylations (Fig. 4b and Supplementary Fig. 7).

The extracellular multi-modular xylanase *RiXyn10A*, the ABC transporter and enzymes conferring cytoplasmic breakdown of xylan oligosaccharides were assigned as the core xylan utilization apparatus of *R. intestinalis* (Fig. 2a,b). This assignment was based on i) conservation of this apparatus within the *Roseburia* species (Supplementary Fig. 2a), ii) highest transcriptional upregulation of the encoding genes on xylan (Fig. 2a), and iii) biochemical data from the present study. The two additional xylan-upregulated loci in *R. intestinalis* L1-82 (Supplementary Fig. 1) are lacking in *R. intestinalis* XB6B4 and *R. intestinalis* M50/1, both being able to grow on xylan²⁰. The activity and expression of the xylanase *RiXyn10B*, encoded by one of these auxiliary loci (Supplementary Fig. 3d), supports the participation of more than one locus in xylan breakdown in *R. intestinalis* L1-82. Multiplicity of xylan utilization loci has been suggested to support targeting a larger structural diversity of naturally occurring xylans by *Bacteroides*²², which may also apply for *R. intestinalis*.

Our data support the role of the *R. intestinalis* core xylanase RiXyn10A in mediating the capture and breakdown of arabino- and glucuronoxylan (Fig. 1 and Fig. 3). This enzyme possesses four CBMs from two known and one novel xylan-binding families, representing the most complex modular organisation of HGM xylanases (Fig. 3a and Supplementary Fig. 2b). This organization is conserved within the currently sequenced *R. intestinalis* species, while other *Clostridium* XIVa taxa possess simpler enzymes lacking one or more of the RiXyn10A CBMs. The N-terminal CBMx of RiXyn10A displays approximately 7-fold lower affinity for X6 than the average affinity measured for the enzyme variant lacking this module (Fig. 3e). These data merit assigning this module into a novel low-affinity xylan-specific CBM family. Nonetheless, CBMx is highly selective to arabinoxylan and clearly contributes to the overall affinity of the enzyme (Fig. 3e). Low-affinity CBMs may potentiate multivalent cooperative substrate binding, with minimal reduction of turn-over due to the energetic penalty of bond-breaking during substrate displacement from the active site (*i.e* maintenance of a relatively high k_{cat}/k_{off} ratio³³). The extended binding mediated by the CBMs of RiXyn10A seems to confer an advantage in the capture and prolonged contact of the enzyme with xylan. Deletion of the binding modules (RiXyn10A-cata) caused a substantial decrease in the apparent affinity towards WAX and BGX as judged by the loss of curvature and deviation from Michaelis-Menten kinetics (Fig. 3d and Supplementary Fig. 3b). These findings are consistent with the importance of CBMs in catalysis under substrate limitations. By contrast, similar turnover rates, were obtained by the catalytic module and the full-length RiXyn10A at high (9 mg mL⁻¹) substrate concentrations (Supplementary Fig. 3b). Multiplicity and variability of CBMs seem to be a signature of extracellular enzymes from butyrate producing Firmicutes^{34,35}. By contrast, *Bacteriodes* members possess simpler outer-membrane anchored GH10 xylanases with an inserted tandem CBM4 repeat within the catalytic module²³. Xylan capture by *Bacteriodes*, however, is additionally orchestrated by moderate affinity ($K_D \approx 60 \mu\text{M}$) xylan binding proteins that protrude away from the cell surface to facilitate binding²².

276 *R. intestinalis* was able to compete with *B. ovatus* for xylans during the log-phase (Fig. 5e-g). Notably,
 277 *R. intestinalis* seemed to outcompete *B. ovatus* after propagation of the co-culture (in the late log
 278 phase) in fresh medium for two additional passages, which underscores the competitiveness of the
 279 xylan utilisation machinery of this Firmicute (Supplementary Fig. 8c). *R. intestinalis* has been
 280 reported to be associated to insoluble xylans, including wheat bran, while species of *Bacteriodes*
 281 were enriched in the solubilized xylan fractions^{18,36}. The extended binding mediated by *RiXyn10A*
 282 may play an important role in the association to insoluble substrates. Indeed, the expression of this
 283 enzyme appeared similarly high in the mono- and mixed xylan cultures with *B. ovatus*
 284 (Supplementary Fig. 8e). These observations are different from the reported down-regulation of
 285 hydrolases by *Eubacterium rectale*, which is close taxonomic relative to *Roseburia*, during co-growth
 286 with *Bacteriodes thetaiotamicron* on a fiber rich diet in previously germ-free mice³⁷.

287 The gene encoding the binding protein (*RiXBP*) of the ABC transporter that confers xylo-
 288 oligosaccharide uptake in *R. intestinalis* was the most upregulated in the xylan transcriptomes,
 289 attesting the crucial role of oligosaccharide capture and transport in the densely populated gut
 290 ecological niche. The narrow preference of this protein for decorated backbone of 4–5 xylosyl units
 291 aligned with the products of *RiXyn10A* (Fig. 3b and Fig. 4c). The affinity and size preference of *RiXBP*
 292 were found to be very different from the corresponding protein from *Bifidobacterium*²⁹, which
 293 prefers shorter xylo-oligosaccharides with a different side chain decoration pattern. Importantly,
 294 striking differences in binding affinities and preference are observed when *RiXBP* is compared to the
 295 SusD-like xylan-binding counterpart from *Bacteriodes*. Indeed, both SusD-like proteins from *B.*
 296 *ovatus*, which mediate capture and internalization of xylan-oligosaccharides $\geq$ X6 by SusC TonB-
 297 dependent permeases, displayed no measurable binding to X4 and X5²², the preferred ligands of
 298 *RiXBP*. These differential transport protein preferences are likely to be instrumental in establishing
 299 competitive uptake profiles to select oligosaccharides of specific sizes and decorations for each
 300 taxon. This is supported by the dominance of *R. intestinalis* when the co-culture with *B. ovatus* was
 301 grown on X4 (Fig. 5h).

Our study highlights the molecular apparatus that *R. intestinalis*, as a model *Clostridium* group XIVa Firmicute, has evolved to compete for abundant dietary glycans with other dominant commensal bacteria. Strikingly complex enzymes with multiple ancillary modules mediate multivalent substrate capture and breakdown. Highly over-expressed ABC transporters mediate efficient capture and uptake of xylan oligosaccharides with a different preference than the corresponding transport systems of currently known competing taxa. Based on these findings we propose that the differentiation of glycan capture and uptake preferences represents an adaptation strategy to facilitate co-growth and minimize competition for break down oligomers from major dietary fibers by different human gut taxa.

This study gives insight into the mechanism that enables co-growth of prevalent human gut commensals on the same dietary fiber and sets the stage for the design of better therapeutic strategies aiming at restoring or boosting specific taxonomic groups in a safe and more controlled manner than currently possible.

Methods

Chemicals

All chemicals were of analytical grade. Birchwood glucuronoxylan (BGX), beechwood glucuronoxylan (BeGX), corncob xylo-oligosaccharides (CCXOS) and xylose were from Carl Roth (Karlsruhe, Germany). Cornbran xylan (CBX) was a kind gift from Dr. Madhav, Yadav, United States Department of Agriculture, Agricultural Research Service. Soluble wheat arabinoxylan (low viscosity 10 centiStokes (cSt)) (WAX), insoluble wheat arabinoxylan (high viscosity 48 cSt) (InWAX), xylobiose through to xylohexaose (X2–X6), arabinoxylotriose (AX3), arabinoxylotetraose (AX4) and mannohexaose (Man6) were from Megazyme (Wicklow, Ireland). D-Glucuronic acid was from Sigma Aldrich (St. Louis, MO, USA). L-arabinose was from VWR International Ltd (Lutterworth, Leicestershire, UK). Xylo-

oligosaccharides Longlive 95P (XOS) were from Shandon Longlive Bio-technology (Shandong, China). Acetylated birchwood glucuronoxylan (AcBGX), acetylated aspen glucuronoxylan (AcAGX), acetylated spruce galactoglucomannan (AcSGGM) were prepared with steam explosion as previously described³⁸. Cellulose acetate was a kind gift from Alexander Deutsche, University of Hamburg, Germany. Acetylated chitin-oligosaccharides were prepared as previously described³⁹.

Growth experiments and RNA-seq transcriptional analysis

R. intestinalis DSM 14610 was grown in a Whitley DG250 Anaerobic Workstation (Don Whitley, UK) in YCFA medium¹⁴ supplemented with autoclaved-sterilized 0.5% (w/v) carbohydrates. Cultures (5mL) were grown in triplicates and OD_{600 nm} and pH (for insoluble substrates) were measured to assess bacterial growth until the stationary phase was reached. Growth rates were calculated from the exponential growth phase.

For the RNA-seq analysis, total RNA was extracted at mid- to late-log phase (OD_{600 nm} = 0.5–0.7) from biological triplicate cultures (10 mL) grown in YCFA supplemented with 0.5% (w/v) glucose, xylose, WAX or BGX. Cells were harvested (4000 g, 5 min, room temperature) and the pellets were frozen at -80°C until RNA extraction. The RNA was extracted using the RNeasy Mini Kit (Qiagen) according to the manufacturer's protocol after enzymatic lysis followed by mechanical disruption of the cells. A DNase treatment was included to ensure removal of DNA. The purity and quantity of the extracted RNA were assessed by an Agilent 2100 Bioanalyzer (Agilent Technologies, UK). Removal of ribosomal RNA and library construction for RNAseq were performed using the ScriptSeq™ Complete Kit (Epicentre). High-throughput sequencing was performed in a single lane in paired end reads on an Illumina Hiseq 4000 platform at BGI (Copenhagen, Denmark). In total, 400 million paired-end reads were obtained and the read quality was assessed by FastQC v0.11.5 (<http://www.bioinformatics.babraham.ac.uk/projects/fastqc/>). The R1 reads were chosen for downstream analysis. Adaptor trimming and de-multiplexing was performed using custom python

scripts (based on the Biopython SeqIO module⁴⁰) and the FASTX-Toolkit v0.0.13.2 (http://hannonlab.cshl.edu/fastx_toolkit/). Reads were further trimmed with fastx_trimmer and subsequently, filtered with fastq_quality_filter with minimum quality score 30 (-q 30) where 95% of base-pairs meet the minimum quality score (-p 95). The resulting reads were kept if longer than 20 bps (-m 20). The *R. intestinalis* L1-82 reference genome and genome annotations are based on assembly *GCA_000156535.1_ASM15653v1*, obtained from NCBI (ftp://ftp.ncbi.nlm.nih.gov/genomes/genbank/bacteria/Roseburia_intestinalis/). Reads were mapped to the reference genome using Tophat2^{41,42}, and gene counts were determined with HTseq⁴³. Differential gene expression was performed using DeSeq2 in R⁴⁴.

Xylanase activity measurements on whole cells

Cell-associated xylanase activity was determined by growing *R. intestinalis* cells in 800 μ L YCFA containing 0.5% (w/v) xylo-oligosaccharides, WAX, BGX or glucose for 15 hours. Cells were harvested (4000 g, 5 min, room temperature), resuspended in phosphate-buffered saline (PBS) to OD_{600 nm} = 0.3 and xylanase activity was assayed using the DNS assay as described below. To determine the effect of high ionic strength on the localization of xylanase activity, *R. intestinalis* cells were grown in 6 mL YCFA containing 0.5% (w/v) BGX for 15 hours. Subsequently, the culture was divided into two 3 mL aliquots and harvested as described above. Cell pellets were resuspended in 300 μ L PBS with or without 1.5 M NaCl. The suspensions were spun down and both pellets and supernatants (wash fractions) were collected. Cell pellets were washed with excess PBS and resuspended in 300 μ L PBS. The xylanase activity of cells and wash fractions was assayed using the DNS assay.

Expression and purification of *R. intestinalis* proteins mediating xylan utilization

374 Open reading frames of the proteins without signal peptide, as predicted by SignalP v.3.0
375 (<http://www.cbs.dtu.dk/services/SignalP-3.0>), were amplified from *R. intestinalis* DSM 14610
376 genomic DNA using specific primers (Supplementary Fig. 9). Amplicons were cloned into the EcoRI
377 and NcoI restriction sites of a pETM-11 (kind gift from Dr. Gunter Stier, EMBL, Center for
378 Biochemistry, Heidelberg, Germany⁴⁵ or the XhoI and NcoI restriction site of a pET28a(+) (Novagen,
379 Darmstadt, Germany) using In-Fusion cloning (Takara) to express proteins as fusions with either
380 cleavable N-terminal His₆ tags or a C-terminal ones, respectively. Standard protocols were used for
381 recombinant protein expression and purification using His-affinity and size exclusion
382 chromatography.

383 **Enzymatic activity assays**

384 Enzymatic assays were carried out in a 50 mM HEPES 0.005% (v/v) Triton X-100, pH 7.0 standard
385 assay buffer unless otherwise stated. Hydrolysis kinetics of full-length or truncated xylanases
386 (10–200 nM) were assayed towards 1–9 mg mL⁻¹ of BGX, WAX or InWAX (37°C, 900 µL, 12 min).
387 Initial hydrolysis rates were determined by removing 200 µL aliquots every third minutes and
388 quenching the reaction in 300 µL 3,5-dinitrosalicylic acid (DNS) reagent⁴⁶. Next samples were
389 incubated for 15 min at 90°C followed by A_{540 nm} measurement in 96 microtitre plates. Xylose was
390 used as a standard (0–2.5 mM). Xylanase activity was assayed for *R. intestinalis* cells washed with
391 PBS ± 1.5 M NaCl, and wash-fractions, as above with the following modification: 180 µL of 1% (w/v)
392 BGX was incubated with 20 µL cell suspension or wash-fraction for 4 hours.

393 Hydrolysis kinetics of α-glucuronidase were analyzed on 1–9 mg mL⁻¹ BeGX or a hydrolysate thereof
394 (prepared by incubation with 4 mM RiXyn10A xylanase for 15 hours at 37°C followed by heat
395 inactivation). The initial rates of (O-methyl)-D-glucuronic acid release were measured using a
396 coupled enzymatic assay (Megazyme). Reactions (770 µL) were incubated for 2 min at 37°C with
397 10–180 nM enzyme with intermittent removal of 175 µL aliquots every 15 s into 125 µL 1 M Tris pH
398 10 to quench the reaction. This was followed by mixing 270 µL of the stopped reaction with 45 µL of

399 the NAD⁺ and uronate dehydrogenase reagents. Conversion of NAD⁺ to NADH was measured at A₃₄₀
 400 nm. Glucuronic acid was used as standard (0–500 μM).

401 Hydrolysis kinetics of *RiXyl8* and *RiXyl3A* were determined towards xylobiose (X2) through to
 402 xylohexaose (X6) (0.5–12 mM) in Mcllvaine buffer pH 6.8 (10 mM citric acid and 20 mM sodium
 403 phosphate) as described in^{47,48}. Reactions (350 μL) were incubated for 12 min at 37°C with 36–78 nM
 404 *RiXyl3A* or 2.4 nM *RiXyn8*. Aliquots of 50 μL were removed every 2 minutes and stopped in 250 μL *p*-
 405 bromoaniline (2% w/v) in glacial acetic acid with thiourea (4% w/v). The stopped reactions were
 406 incubated in darkness for 10 min at 70°C, followed by incubation at 37°C for 1 hour before
 407 measuring A_{520 nm}. The concentration of released pentoses was determined using a xylose standard
 408 (0–5 mM)⁴⁹.

409 α-L-Arabinofuranosidase activity for *RiAbf43A* was assayed in Mcllvaine buffer pH 6.8 (10 mM citric
 410 acid and 20 mM sodium phosphate) using a coupled enzymatic L-arabinose/D-galactose assay
 411 (Megazyme) towards WAX (1–24 mg mL⁻¹). Reactions (75 μL) were incubated for 12 min at 37°C with
 412 0.4–1.7 μM enzyme. Aliquots of 15 μL were removed every 2 min, and the enzyme was inactivated
 413 (10 min, 90°C) and thereafter 10 μL of this solution were mixed with 10 μL of the provided NAD⁺, 20
 414 μL of provided assay buffer and 2 μL galactose mutaoatase/β-galactose dehydrogenase mix. The
 415 formation of NADH was measured as above. Arabinose was used as standard (0–5 mM).

416 The acetyl esterase specific activity of *RiAXE* was determined in 250 μL reactions containing *para*-
 417 nitrophenyl-acetate (4 mM) and 0.14 μM enzyme. A_{405 nm} was measured every 60 s for 10 minutes
 418 at 37°C in a microtiter plate reader and *p*NP (0–1 mM) was used as standard. The specific activity
 419 was determined in units (U/mg), where a U is defined as the amount of enzyme that produces 1
 420 μmol of *p*NP min⁻¹.

421 Kinetic parameters were calculated by fitting the Michaelis-Menten equation to the initial rate data
 422 using Graph Pad Prism 7. The catalytic efficiency k_{cat}/K_m , determined from the slope of the

423 normalized initial rate ($V_0/[E]$) in the Michaelis-Menten plot, is reported when saturation was not
424 attained. All experiments were performed in triplicates.

425 **Action patterns of individual and mixtures of xylanolytic enzymes**

426 Hydrolysis of xylan and xylo-oligosaccharides was performed at 37°C for 15 hours in the standard
427 assay buffer used above. Oligosaccharide hydrolysates, used to assay the sequential action of the
428 debranching xylanolytic enzymes, were generated using *RiXyn10A*, which was separated by
429 ultrafiltration (3 kDa cutoff) before the addition of debranching enzymes. The hydrolysis profiles
430 were analyzed as detailed below. To verify the mode of reducing-end attack of *RiXyl8*, 30 mg XOS in
431 standard assay buffer were reduced by NaBH_4 (1M in 100 μM NaOH). A total of 200 μL of the NaBH_4
432 was added dropwise to 800 μL of the xylo-oligosaccharides solution, which was kept on ice. As
433 control 100 μM NaOH was added to an 800 μL xylo-oligosaccharides solution. The mixture was
434 incubated 1 hour at room temperature, then quenched by 400 μL 1 M acetic acid and diluted 10x in
435 assay buffer.

436 **Matrix-assisted laser desorption-ionization (MALDI)**

437 Oligosaccharides were analyzed with an Ultraflex MALDI ToF/ToF instrument (Bruker Daltonics,
438 Bremen, Germany). The samples were applied with 2,5-dihydroxybenzoic acid (DHB) as matrix to a
439 MTP 384 ground steel target plate (Bruker Daltonics). All spectra were obtained in positive reflection
440 mode and processed using Bruker flexAnalysis 3.3.

441 **Thin layer chromatography (TLC) and High performance anion-exchange chromatography with** 442 **pulsed amperometric detection (HPAEC-PAD)**

443 Aliquots of 1 μL of enzymatic reactions were spotted on a silica gel 60 F254 plate (Merck, Germany).
444 The chromatography was performed in a butanol:acetic acid:water (2:1:1 v/v) mobile phase. The
445 plates were dried at 50°C and carbohydrate hydrolysis products were visualized by spraying with a 5-
446 methylresorcinol:ethanol:sulfuric acid (2:80:10 % v/v) developer and tarred briefly at 350°C until

bands appeared. Release of xylo-oligosaccharides and monosaccharides was analyzed by HPAEC-PAD on an ICS-3000 (Dionex, CA, USA) using a 3x250mm CarboPac PA1 column, a 3x50 mm guard column and 10 μ L injections. Xylo-oligosaccharide and standards were eluted with mobile phase of constant 0.1 mM NaOH (flowrate 0.35 mL min⁻¹) and a two-step linear gradient of sodium acetate; 0–25 min of 0–75 mM and 25–30 min of 75–400 mM. Monosaccharides and standards (0.1 mg mL⁻¹) of galactose, arabinose, glucose and xylose were eluted with 1 mM KOH for 35 min at 0.25 mL min⁻¹.

NMR spectroscopy

For the time-resolved NMR recordings: 4 mg AcBGX or AcSGGM were dissolved in 500 μ L 50 mM phosphate buffer pH 7.0 (99.9% D₂O). 2.5 μ L of *Ri*AXE to a final concentration of 64 nM was added. The recorded spectrum is a pseudo-2D type experiment recording a 1D proton NMR spectrum every 5 min with in total 220 time points. The 1D proton spectrum was recorded with 24 scans using a 30° flip angle, and relaxation delay of 1 s (total recording time of 73 s). For enzyme treatment, 2.5 μ L of *Ri*Xyn10A and *Ri*Agu115A were added to the AcBGX sample to 167 nM and 13 nM, respectively, and the sample incubated at 37°C for 24 hours prior to *Ri*AXE addition. All homo and heteronuclear NMR experiments were recorded on a BRUKER AVIIIHD 800 MHz (Bruker BioSpin AG, Fälladen, Switzerland) equipped with 5mm with cryogenic CP-TCI and all acquisitions were done at 37°C. For chemical shift assignment of AcBGX, the following spectra were recorded: 1D proton, 2D double quantum filtered correlation spectroscopy (DQF-COSY), 2D total correlation spectroscopy (TOCSY), 2D ¹³C heteronuclear single quantum coherence (HSQC), 2D ¹³C Heteronuclear 2 Bond Correlation (H2BC), 2D ¹³C HSQC-[¹H,¹H]TOCSY and 2D heteronuclear multiple bond correlation (HMBC). The acetate signal to 1.903 ppm (pH 7.0 at 37 °C, in relation to 4,4-dimethyl-4-silapentane-1-sulfonic acid, DSS⁵⁰) was used as chemical shift reference for protons, while ¹³C chemical shifts were referenced indirectly to acetate, based on the absolute frequency ratios⁵¹. The spectra were recorded, processed and analyzed using TopSpin 3.5 software (Bruker BioSpin).

Surface plasmon resonance (SPR)

Xylo-oligosaccharide binding to *RiXyn10A*, *RiXyn10AΔCBMx* and *RiXyn10A-CBMx* was analyzed using surface plasmon resonance (SPR) on a Biacore T100 (GE Healthcare). Immobilization of the proteins on a CM5 chips was performed using a random amine coupling kit (GE Healthcare) according to the manufacture's protocol with 50-150 $\mu\text{g mL}^{-1}$ protein in 10 mM sodium acetate pH 3.6-4.2, to a density of 1362, 10531 and 4041 response units (RU) for *RiXyn10AΔCBMx*, *RiXyn10A* and *RiXyn10A-CBMx*, respectively. The analysis comprised 90 s of association, 240 s of dissociation at 30 $\mu\text{L min}^{-1}$. Sensograms were recorded at 25°C in 20 mM phosphate/citrate buffer, pH 6.5, 150 mM NaCl, 0.005% (v/v) P20 (GE Healthcare). All solutions were filtered prior to analysis (0.22 μm). Experiments were performed in duplicates with seven concentrations in the range 156 μM –10 mM for X3, 75 μM –4 mM for X4, X6, Man6 and 62.5 μM –4 mM X5. Data analysis was carried out using the Biacore T100 evaluation software and dissociation constants (K_D) were determined by fitting a one-binding site model to the steady state sensograms. No binding was measured for Man6.

Isothermal titration calorimetry (ITC)

Titration were performed using a Microcal ITC₂₀₀ calorimeter (GE healthcare) at 25°C with *RiXBP* (0.1 mM) or *RiXyn10AΔCBMx* (0.25 mM) in the sample cell and xylo-oligosaccharides (2.2–5 mM) in 10 mM sodium phosphate pH 6.5 in the syringe. An initial injection of 0.5 μL , was followed by 19 x 2 μL injections separated by 120 s. The data were corrected for the heat of dilution, determined from buffer titration and a nonlinear single binding model was fitted to the normalized integrated binding isotherms using the MicroCal Origin software v7.0 to determine the thermodynamic binding parameters.

Affinity electrophoresis

Binding of CBMx to WAX (0–0.1% w/v) or BGX (0–1.0% w/v) was assessed by affinity electrophoresis⁵² in 10% native polyacrylamide gels (70 V, 3 hours, 4°C) using purified recombinant

RiXyn10A-CBMx (3.0 µg) and β-lactoglobulin (1.5 µg) as a negative control. The relative mobility (*r*) was calculated as the migration of *RiXyn10A*-CBMx relative to migration of the dye front. A linear regression of the 1/*r* versus xylan concentration allowed the determination of *K_D* as the intercept of this X-axis.

Western blot and immunofluorescence microscopy

Custom antibodies against the recombinant for the two xylanases *RiXyn10A*, *RiXyn10B* and the transport protein *RiXBP* were raised in rats and rabbit, respectively (Eurogentec, Seraing, Belgium). The specificity of the antibodies was tested by western blots using a standard protocol. The membranes were blocked for 1 hour in 1% (w/v) BSA in TBST-buffer (Tris-buffered saline, 0.1% (v/v) Tween 20) and incubated for 2 hours with the antisera (500x dilution in TBST-buffer). Subsequently, the membranes were washed three times in TBST-buffer and incubated for 2 hours with 6000x diluted secondary polyclonal goat anti-rabbit IgG-AP antibodies coupled to alkaline phosphatase (AP) (Dako, Glostrup, Denmark) and rabbit anti-rat IgG-AP (Sigma). After three washes, the proteins were visualized by exposure to Sigma-Fast BCIP/NBT reagent (Sigma).

R. intestinalis cells were grown in 6 mL YCFA containing 0.5% (w/v) WAX to OD_{600 nm}≈0.8, harvested (4000 g, 5 min, room temperature) and washed twice in PBS. The cells were resuspended in 3 mL 4% (w/v) paraformaldehyde in PBS and fixed by incubation on ice for 15 min. Thereafter the cells were washed twice in PBS and resuspended in 2 mL PBS. 50 µL of cell suspension were added to glass slides coated with poly-L-lysine, cells blocked for 1 hour in blocking buffer (1% (w/v) milk powder in PBS) and washed twice in PBS. For labelling, the cells were incubated with 50 µL anti-sera diluted 50x in blocking buffer for 2 hours, washed twice in PBS and incubated for 1 hour with 50 µL goat anti-rat IgG Alexa-Fluor 555 or goat anti-rabbit IgG Alexa-Fluor 488 (Thermo Scientific, Massachusetts, USA). Secondary antibodies were diluted 500x PBS. Finally, cells were washed two times in PBS, one drop of ProLong Gold antifade (Thermo Scientific, Massachusetts, USA) was applied and the cells secured

with a cover slide. Fluorescence was visualized using Zeiss Axioplan 2 microscope equipped with a CoolSNAP cf color camera and a Zeiss Plan-Neofluar 100X/1.3NA, oil immersion objective.

Co-culture competition assay

Bacteriodes ovatus DSM 1896 and *R. intestinalis* DSM 14610 were grown anaerobically in 20 mL YCFA supplemented with 0.5% (w/v) glucose to late-log phase and an approximately equal number of cells (estimated by OD_{600 nm}) were inoculated into CFA medium (YCFA lacking the yeast extract to minimize *B. ovatus* growth on yeast extract⁵³) containing 0.5% (w/v) WAX, BGX, InWAX or X4. The co-cultures were grown in triplicates and samples (2 mL) were taken during growth. In the propagation experiment, the co-culture was passaged into fresh media after 9 hours of growth (start OD_{600 nm} = 0.01), then grown for 12 hours and passaged again into fresh media and grown for 12 hours. Genomic DNA was extracted from samples using DNAClean® Microbial DNA isolation kit (Qiagen). Relative bacterial abundance was estimated by qPCR. The extracted DNA was diluted to 0.5 ng µL⁻¹ and amplified in technical triplicates using strain specific primers (Supplementary Fig. 9). The amplification mix contained 2 µL DNA, 5.5 µL LightCycler 480 SYBR Green I Master mix (Roche), 0.22 µL of each primer (10 pmol/µL) and 3 µL sterile water. Amplification conditions were 1 cycle of 95 °C for 5 min, 45 cycles of 95 °C for 10 s, 60 °C for 15 s and 72 °C for 45 s using a LightCycler 480 II (Roche). Relative bacterial concentrations in each sample were estimated by comparing the gene copy numbers calculated using standard curves prepared with the respective reference DNA. Western blot was performed as described above but with cell cultures instead of purified proteins.

Data availability

The protein characterized in this study are available from NCBI with the following accession numbers: [EEV01588.1](#) (ROSINTL182_06494), [EEU99940.1](#) (ROSINTL182_08193), [EEU99941.1](#) (ROSINTL182_08194), [EEU99942.1](#) (ROSINTL182_08195), [EEU99943.1](#) (ROSINTL182_08196),

[EEU99943.1](#) (ROSINTL182_08196), [EEU99894.1](#) (ROSINTL182_08199) and [EEU99897.1](#) (ROSINTL182_08202). The authors declare that the data supporting the findings of this study are available within the paper and the supplementary information or from the corresponding author on request.

References

1. Nicholson, J. K. *et al.* Host-gut microbiota metabolic interactions. *Science* (80-.). **108**, 1262–1268 (2012).
2. Sonnenburg, J. L. & Bäckhed, F. Diet–microbiota interactions as moderators of human metabolism. *Nature* **535**, 56–64 (2016).
3. Marchesi, J. R. *et al.* The gut microbiota and host health: a new clinical frontier. *Gut* 1–10 (2015).
4. David, L. A. *et al.* Diet rapidly and reproducibly alters the human gut microbiome. *Nature* **505**, 559–563 (2013).
5. Desai, M. S. *et al.* A dietary fiber-deprived gut microbiota degrades the colonic mucus barrier and enhances pathogen susceptibility. *Cell* **167**, 1339–1353.e21 (2016).
6. Cockburn, D. W. & Koropatkin, N. M. Polysaccharide degradation by the intestinal microbiota and its influence on human health and disease. *J. Mol. Biol.* **428**, 3230–3252 (2016).
7. Xu, S. *et al.* Butyrate induces apoptosis by activating PDC and inhibiting complex I through SIRT3 inactivation. *Signal Transduct. Target. Ther.* **2**, e16035 (2017).
8. Donohoe, D. R. *et al.* The warburg effect dictates the mechanism of butyrate-mediated histone acetylation and cell proliferation. *Mol. Cell* **48**, 612–626 (2012).
9. Furusawa, Y. *et al.* Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. *Nature* **506**, 254–254 (2014).
10. Morrison, D. J. & Preston, T. Formation of short chain fatty acids by the gut microbiota and their impact on human metabolism. *Gut Microbes* **7**, 189–200 (2016).
11. Takahashi, K. *et al.* Reduced abundance of butyrate-producing bacteria species in the fecal microbial community in Crohn’s disease. *Digestion* **93**, 59–65 (2016).

- 572 12. Qin, J. *et al.* A metagenome-wide association study of gut microbiota in type 2 diabetes. *Nature* **490**, 55–60 (2012).
- 573 13. Vrieze, A. *et al.* Transfer of intestinal microbiota from lean donors increases insulin sensitivity in individuals with
574 metabolic syndrome. *Gastroenterology* **143**, 913–916.e7 (2012).
- 575 14. Duncan, S. H., Hold, G. L., Barcenilla, A., Stewart, C. S. & Flint, H. J. *Roseburia intestinalis* sp. nov., a novel
576 saccharolytic, butyrate-producing bacterium from human faeces. *Int. J. Syst. Evol. Microbiol.* **52**, 1615–1620
577 (2002).
- 578 15. Louis, P. & Flint, H. J. Diversity, metabolism and microbial ecology of butyrate-producing bacteria from the human
579 large intestine. *FEMS Microbiol. Lett.* **294**, 1–8 (2009).
- 580 16. Van den Abbeele, P. *et al.* Butyrate-producing *Clostridium* cluster XIVa species specifically colonize mucins in an *in*
581 *vitro* gut model. *ISME J.* **7**, 949–61 (2013).
- 582 17. El Kaoutari, A., Armougom, F., Gordon, J. I., Raoult, D. & Henrissat, B. The abundance and variety of carbohydrate-
583 active enzymes in the human gut microbiota. *Nat. Rev. Microbiol.* **11**, 497–504 (2013).
- 584 18. Mirande, C. *et al.* Dietary fibre degradation and fermentation by two xylanolytic bacteria *Bacteroides xylanisolvens*
585 XB1AT and *Roseburia intestinalis* XB6B4 from the human intestine. *J. Appl. Microbiol.* **109**, 451–460 (2010).
- 586 19. Chassard, C., Goumy, V., Leclerc, M., Del’homme, C. & Bernalier-Donadille, A. Characterization of the xylan-
587 degrading microbial community from human faeces. *FEMS Microbiol. Ecol.* **61**, 121–131 (2007).
- 588 20. Sheridan, P. O. *et al.* Polysaccharide utilisation loci and nutritional specialisation in a dominant group of butyrate-
589 producing human colonic Firmicutes. *Microb. Genomics* **2**, (2016).
- 590 21. Selvendran, R. R. Chemistry of plant cell walls and dietary fibre. *Scand. J. Gastroenterol.* **5521**, 33–41 (1987).
- 591 22. Rogowski, A. *et al.* Glycan complexity dictates microbial resource allocation in the large intestine. *Nat. Commun.* **6**,
592 7481 (2015).
- 593 23. Zhang, M. *et al.* Xylan utilization in human gut commensal bacteria is orchestrated by unique modular
594 organization of polysaccharide-degrading enzymes. *Proc. Natl. Acad. Sci. U. S. A.* **111**, E3708–E3717 | (2014).
- 595 24. Lombard, V., Golaconda Ramulu, H., Drula, E., Coutinho, P. M. & Henrissat, B. The carbohydrate-active enzymes
596 database (CAZy) in 2013. *Nucleic Acids Res* **42**, (2014).
- 597 25. Kelly, G. *et al.* Structure of the cell-adhesion fragment of intimin from enteropathogenic *Escherichia coli*. *Nat*
598 *Struct Mol Biol* **6**, 313–318 (1999).

599 26. Ebbes, M. *et al.* Fold and Function of the InlB B-repeat. *J. Biol. Chem.* **286**, 15496–15506 (2011).

600 27. Karlsson, E. N. *et al.* The modular xylanase Xyn10A from *Rhodothermus marinus* is cell-attached, and its C-terminal
601 domain has several putative homologues among cell-attached proteins within the phylum Bacteroidetes. *FEMS*
602 *Microbiol. Lett.* **241**, 233–242 (2004).

603 28. Ejby, M. *et al.* An atp binding cassette transporter mediates the uptake of α -(1,6)-linked dietary oligosaccharides in
604 bifidobacterium and correlates with competitive growth on these substrates. *J. Biol. Chem.* **291**, 20220–20231
605 (2016).

606 29. Ejby, M. *et al.* Structural basis for arabinoxylo-oligosaccharide capture by the probiotic *Bifidobacterium animalis*
607 subsp. lactis BI-04. *Mol. Microbiol.* **90**, 1100–1112 (2013).

608 30. Honda, Y. & Kitaoka, M. A family 8 glycoside hydrolase from *Bacillus halodurans* C-125 (BH2105) is a reducing end
609 xylose-releasing exo-oligoxyranase. *J. Biol. Chem.* **279**, 55097–55103 (2004).

610 31. Anand, S., Kaur, H. & Mande, S. S. Comparative *in silico* analysis of butyrate production pathways in gut
611 commensals and pathogens. *Front. Microbiol.* **7**, 1–12 (2016).

612 32. Duncan, S. H. *et al.* Wheat bran promotes enrichment within the human colonic microbiota of butyrate-producing
613 bacteria that release ferulic acid. *Environ. Microbiol.* **18**, 2214–2225 (2016).

614 33. Morrill, J. *et al.* The GH5 1,4- β -mannanase from *Bifidobacterium animalis* subsp. lactis BI-04 possesses a low-
615 affinity mannan-binding module and highlights the diversity of mannanolytic enzymes. *BMC Biochem.* **16**, 26
616 (2015).

617 34. Cockburn, D. W. *et al.* Molecular details of a starch utilization pathway in the human gut symbiont *Eubacterium*
618 *rectale*. *Mol. Microbiol.* **95**, 209–230 (2015).

619 35. Ze, X. *et al.* Unique organization of extracellular amylases into amyloosomes in the resistant starch-utilizing human
620 colonic firmicutes bacterium *Ruminococcus bromii*. *MBio* **6**, 1–11 (2015).

621 36. De Paepe, K., Kerckhof, F.-M., Verspreet, J., Courtin, C. M. & Van de Wiele, T. Inter-individual differences
622 determine the outcome of wheat bran colonization by the human gut microbiome. *Environ. Microbiol.* **0**, 1–17
623 (2017).

624 37. Mahowald, M. A. *et al.* Characterizing a model human gut microbiota composed of members of its two dominant
625 bacterial phyla. *Proc Natl Acad Sci U S A* **106**, 5859–5864 (2009).

626 38. Biely, P. *et al.* Mode of action of acetylxyylan esterases on acetyl glucuronoxylan and acetylated oligosaccharides
627 generated by a GH10 endoxylanase. *Biochim. Biophys. Acta - Gen. Subj.* **1830**, 5075–5086 (2013).

628 39. Sørbotten, A., Horn, S. J., Eijsink, V. G. H. & Vårum, K. M. Degradation of chitosans with chitinase B from *Serratia*
629 *marcescens*. *FEBS J.* **272**, 538–549 (2005).

630 40. Cock, P. J. A. *et al.* Biopython: Freely available Python tools for computational molecular biology and
631 bioinformatics. *Bioinformatics* **25**, 1422–1423 (2009).

632 41. Kim, D. *et al.* TopHat2: accurate alignment of transcriptomes in the presence of insertions, deletions and gene
633 fusions. *Genome Biol.* **14**, R36 (2013).

634 42. Langmead, B. & Salzberg, S. L. Fast gapped-read alignment with Bowtie 2. *Nat Methods* **9**, (2012).

635 43. Anders, S., Pyl, P. T. & Huber, W. HTSeq-A Python framework to work with high-throughput sequencing data.
636 *Bioinformatics* **31**, 166–169 (2015).

637 44. Love, M. I., Huber, W. & Anders, S. Moderated estimation of fold change and dispersion for RNA-seq data with
638 DESeq2. *Genome Biol.* **15**, 550 (2014).

639 45. Dümmler, A., Lawrence, A.-M. & de Marco, A. Simplified screening for the detection of soluble fusion constructs
640 expressed in *E. coli* using a modular set of vectors. *Microb. Cell Fact.* **4**, 34 (2005).

641 46. Miller, G. L. Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal. Chem.* **31**, 426–428
642 (1959).

643 47. Roe, J. H. & Rice, E. W. A photometric method for the determination of free pentoses in animal tissue. *J. Biol.*
644 *Chem.* **173**, 507–512 (1948).

645 48. Deschatelets, L. & Yu, E. K. C. A simple pentose assay for biomass conversion studies. *Appl. Microbiol. Biotechnol.*
646 **24**, 379–385 (1986).

647 49. Dilokpimol, A. *et al.* Enzymatic synthesis of β -xylosyl-oligosaccharides by transxylosylation using two β -xylosidases
648 of glycoside hydrolase family 3 from *Aspergillus nidulans* FGSC A4. *Carbohydr. Res.* **346**, 421–429 (2011).

649 50. Govind, V., Young, K. & Maudsley, A. A. Corrigendum: Proton NMR chemical shifts and coupling constants for brain
650 metabolites. Govindaraju V, Young K, Maudsley AA, *NMR Biomed.* 2000; 13: 129-153. *NMR Biomed.* **28**, 923–924
651 (2015).

652 51. Zhang, H., Neal, S. & Wishart, D. S. RefDB: A database of uniformly referenced protein chemical shifts. *J. Biomol.*

NMR **25**, 173–195 (2003).

52. Takeo, K. Affinity electrophoresis: Principles and applications. *Electrophoresis* **5**, 187–195 (1984).

53. Scott, K. P., Martin, J. C., Duncan, S. H. & Flint, H. J. Prebiotic stimulation of human colonic butyrate-producing bacteria and bifidobacteria, in vitro. *FEMS Microbiol. Ecol.* **87**, 30–40 (2014).

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Author contributions

Growth analysis was performed by M.L.L. Transcriptomic analysis was by M.L.L, C.W, and D.A.E. Enzyme characterization was by M.L.L., M.E., S.S.P, F.L.A and B.W. qPCR was by M.L.L and M.I.B. Microscopy was by M.L.L and C.S. Experiments were designed by M.L.L and M.A.H. The manuscript

written by M.L.L and M.A.H. with contributions from T.R.L, B.W. and F.L.A. Figures were prepared by M.L.L.

Competing interests

The authors declare no competing financial interests.

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Figure legends

Figure 1 Growth of *R. intestinalis* and induction of extracellular activity. (a) Schematic representation of cereal arabinoxylan and glucuronoxylan present in dicots cell wall, e.g. in fruits and vegetables. (b) Growth level for 18 hours on xylans, oligosaccharides thereof and monosaccharide components, with glucose as a control. Green: OD_{600 nm} increase >1.0 for soluble substrates and pH drop > 0.3 for insoluble xylans; yellow: $0.3 < \Delta OD_{600\text{ nm}} < 0.5$; red: $\Delta OD_{600\text{ nm}} < 0.1$. Asterisks indicate insoluble xylans (c) Growth curves on glucose, wheat arabinoxylan (WAX), birch glucuronoxylan (BGX) and a no carbon source control. (d) Growth on insoluble wheat arabinoxylan (InWAX) and oatspelt xylan (OSX). All growth measurements are means of triplicates with standard deviations. (e) Xylanase activity of *R. intestinalis* cells grown on glucose, xylo-oligosaccharides, BGX and WAX for 18 hours. (f) Cells grown on BGX were washed (PBS buffer $\pm$ 1.5 M NaCl) and xylanase activity was measured in wash and cell fractions to verify localization of the enzymes. Xylanase activity was measured using the DNS reducing sugar assay and data are triplicates with standard deviations.

Figure 2 The core xylan utilization apparatus of *R. intestinalis*. (a) The RNA-Seq heatmap depicts Log2 fold changes of the top upregulated xylan utilization genes expressed by cells grown on xylose (X1), wheat arabinoxylan (WAX) and birch glucuronoxylan (BGX) relative to glucose (Glc). Formal locus tag numbers ROSINTL182_xxxxx are abbreviated with the last numbers after the hyphen. Signal peptides (SP) were predicted using SignalP v.3.0. (b) Gene expression depicted as mean of the normalized Deseq2 gene counts for the core xylan utilization genes shown in (a). (c) Extracellular localization of

RiXBP and *RiXyn10A*, the solute binding protein of the xylo-oligosaccharide specific ABC transporter and the xylanase, respectively, were visualized by fluorescence microscopy of *R. intestinalis* cells using primary antibodies targeting these two proteins. No auto fluorescence was observed for cells without primary antibody (data not shown).

Figure 3 A novel low affinity xylan binding module mediates extended xylan binding to the xylanase *RiXyn10A*. (a)

Domain organization of *RiXyn10A* and truncated variants. Carbohydrate binding module (CBM), novel CBM (CBMx), bacterial Ig-like domain group 2 (BIG2), Listeria-Bacteroides repeat domain (LBR). (b,c) Xylanase activity of *RiXyn10A* on WAX and BGX assayed by HPAEC-PAD and thin layer chromatography, respectively. Peaks in 3b eluting after X6 are likely to be decorated xylo-oligosaccharides. (d) Hydrolysis kinetic parameters of *RiXyn10A*, *RiXyn10A*ΔCBMx and *RiXyn10A*-cata towards WAX and BGX. Kinetics of the *RiXyn10A*-cata are not modelled by the Michaelis-Menten expression and catalytic efficiencies are estimated from linear regression of initial rate data. Data are means of triplicates with standard deviations. (e) Binding parameters of *RiXyn10A* and variants towards oligosaccharides. Dissociation constants (K_D) determined by surface plasmon resonance (SPR) are means of a duplicate with the standard deviations. * K_D (mg mL⁻¹) from affinity electrophoresis (AE), and ** K_D from isothermal titration calorimetry (ITC). (f) Binding of *RiXyn10A*-CBMx to the negative control (no polysaccharide), WAX or BGX xylans analyzed using AE. Lanes 1+2; *RiXyn10A*-CBMx (3.0 μg), Lane 3; β-lactoglobulin negative control (1.5 μg), M; marker. (g) Binding isotherms of *RiXyn10A*-CBMx to xylo-oligosaccharides. Solid lines are fits of a one binding site model to the SPR sensograms.

Figure 4 Intracellular xylo-oligosaccharide depolymerization. (a) α-glucuronidase and α-L-arabinofuranosidase activity on WAX and BGX for *RiAgu115A* and *RiAbf43A*, respectively, based on HPAEC-PAD analysis. (b) Time-resolved NMR for *RiAXE* enzymatic deacetylation of acetylated birch glucuronoxylan (AcBGX) treated with *RiXyn10A* and *RiAgu115A*. Deacetylation time course for the first 30 min and after 18 h (green 0 min, purple 30 min, orange 18 h). All verified signals with 2-O-acetylation decreased faster in the initial phase of the reaction. The proton spectra of the acetylated region show nearly complete deacetylation of the sample after 18 h. The signal at 2.13 ppm is likely attributed to another acetylated sugar residue. Acetyl groups are designated as: C2, 2-O-acetylated xylose; C3, 3-O-acetylated xylose, C23, 2,3-di-O-acetylated xylose; C3-MeGlcA; 4-O-methylglucuronic acid 2-O-substituted and 3-O-acetylated xylose; C23(2); signal for the 2-O-acetylated of C23. The assignment of the acetylated sugar signals were based on homo and heteronuclear NMR correlation experiments (Supplementary Fig. 7) (c-f) Hydrolysis products from AcBGX by (c) *RiXyn10A*, (d) *RiXyn10A* and *RiAgu115A*, (e) *RiXyn10A* and *RiAXE*, (f) *RiXyn10A*, *RiAgu115A* and *RiAXE*. Enzyme action was analyzed by MALDI-ToF MS; Xylo-oligosaccharides decorated with acetyl and methylglucuronic acid are in green, acetyl in blue, methylglucuronic acid in red, no sidechains in orange. Di-sodium adducts of a methylglucuronic acid decorated oligosaccharides (diamonds) are colored as their corresponding single sodium adducts.

Figure 5 Model for xylan utilization by *R. intestinalis* and competition assay with *Bacteriodes ovatus*. (a) *RiXyn10A* on the cell surface efficiently captures diet-derived acetylated arabinoxylan and acetylated glucuronoxylan by its CBMs and hydrolyzes it into linear and decorated xylo-oligosaccharides, which are subsequently captured by *RiXBP* for uptake into the cytoplasm. Internalized xylo-oligosaccharides are debranched and hydrolyzed into monosaccharides and acetate. Xylose and arabinose are converted to xylulose 5-phosphate before entering the pentose phosphate pathway, whereas methyl-glucuronic acid is converted to 2-oxo-3-deoxygalactonate 6-phosphate. These precursors enter glycolysis, which generates pyruvate, some of which is used to synthesize butyrate³¹ that is externalized. The asterisk next to *RiAbf43A* indicates that the enzyme is able to hydrolyze both α -1,2 and α -1,3 linked L-arabinose. Black solid arrows show steps established or confirmed in this study. Grey solid arrows indicate steps described in literature. Grey dashed arrows indicate that H₂ and butyrate are externalized by unknown mechanisms. To make the model more general for the *R. intestinalis* species, the second less upregulated extracellular xylanase *RiXynB*, unique for the L1-82 strain, is not included in the model, although it is expressed at the cell surface. (b-d) Growth of monoculture and co-cultures of *R. intestinalis* and *B. ovatus* on WAX, InWAX and BGX. Data are means of a triplicate with standard deviations. (e-h) Time course relative abundance during growth of co-cultures on xylans and xylotetraose (X4) determined by qPCR. All data are means of a biological triplicate.

Tables

Table 1: Binding energetics of the transport protein *RiXBP* to xylo-oligosaccharides determined by ITC

Ligand	K_D (μ M)	N_0	ΔH (kcal/mol)	$T\Delta S$ (kcal/mol)	ΔG (kcal/mol)	
X6	112.7 $\pm$ 7.5	1.19 $\pm$ 0.14	-9.01 $\pm$ 1.3	-3.6	-5.4	★★★★★★★★
X5	10.3 $\pm$ 1.5	0.86 $\pm$ 0.01	-13.54 $\pm$ 0.3	-6.7	-6.8	★★★★★★
X4	16.5 $\pm$ 2.6	0.68 $\pm$ 0.02	-12.8 $\pm$ 0.4	-6.3	-6.5	★★★★★
X3	225.7 $\pm$ 14.5	0.58 $\pm$ 0.23	-21.1 $\pm$ 9.5	-16.1	-5.0	★★★★
X2	n.d.					★★
AX3	215.5 $\pm$ 95.2	0.26 $\pm$ 0.04	-44.3 $\pm$ 7.1	-39.4	-4.9	★★★ ★
AX4	6.8 $\pm$ 1.2	0.58 $\pm$ 0.01	-12.3 $\pm$ 0.2	-7.0	-5.3	★★★★★ ★

Data are means of a duplicate experiment with standard deviations. n.d. indicates that no binding was observed. AX3 is an arabino-xylotriose with a non-reducing end arabinosyl and AX4 is an arabino-xylotetraose with an arabinosyl decoration at the penultimate position from the non-reducing end (see Supplementary Fig. 5h,i).

b

Substrate	Growth
Glucose (Glc)	
Arabinose (A1)	
Glucuronic acid (GlcA)	
Xylose (X1)	
Xylobiose (X2)	
Xylotriose (X3)	
Xylotetraose (X4)	
Xylohexaose (X6)	
Corn cob xylooligosaccharides (CCXOS)	
Wheat arabinoxylan (WAX)	
Birchwood glucuronoxylan (BGX)	
Acetylated birchwood glucuronoxylan (AcBGX)	
Corn bran arabinoglucuronoxylan (CBX)	
Insoluble wheat arabinoxylan (InWAX)*	
Oat spelt xylan (OSX)*	

c Growth on soluble xylans

d Growth on insoluble xylans

e Induction of xylanase activity

f Cell attachment of xylanase activity

a

Locus ID	Log2-fold change			SP	Protein	Annotation
	X1/Glc	WAX/Glc	BGX/Glc			
06494	-1.04	7.00	6.17	Yes	<i>RiXyn10A</i>	Endo-1,4- β -xylanase
08192	-0.46	6.46	5.82	No	<i>RiLac1</i>	Transcriptional regulator, LacI family
08193	0.04	8.69	8.45	No	<i>RiAbf43A</i>	α -L-arabinofuranosidase
08194	0.24	8.78	8.88	No	<i>RiAXE</i>	Acetyl xylan esterase
08195	0.63	8.55	8.35	No	<i>RiAgu115A</i>	Xylan α -1,2-glucuronidase
08196	0.78	8.60	8.29	No	<i>RiXyl8</i>	Reducing-end-xylose releasing exo-oligoxylanase
08197	0.91	8.71	8.43	No	<i>RiXPP-A</i>	ABC transporter, permease protein
08198	0.03	8.89	8.33	No	<i>RiXPP-B</i>	ABC transporter, permease protein
08199	0.49	9.12	9.07	Yes	<i>RiXBP</i>	ABC transporter, xylan binding protein
08200	0.28	0.46	0.96	No		Transcriptional regulator
08201	-0.25	-0.07	0.40	No		Transcriptional regulator
08202	-0.22	0.02	0.44	No	<i>RiXyl3A</i>	Xylan 1,4- β -xylosidase

Log2-fold change

-10

0

10

b

c

a **b** **c** **d** **e** **f** **g**

a **b** **c** **d** **e** **f**

Supplementary Table 2. Modular organization of GH10 xylanases from human gut Firmicutes and Bacteroidetes.

Phylum	Family	Strain	Accession number	Length (AA)	CBMs
Firmicutes	Lachnospiraceae	<i>Roseburia intestinalis</i> L1-82	ROSINTL182_06494	1356	X, 22, 9, 9
			ROSINTL182_6338-9	601	
		<i>Roseburia intestinalis</i> XB6B4	CBL13458.1	1356	X, 22, 9, 9
		<i>Roseburia intestinalis</i> M50/1	n.a.	1356	X, 22, 9, 9
		<i>Roseburia faecis</i> M72	CRL32809.1	1380	X, 22, 9, 9
		<i>Eubacterium rectale</i> T1-815	CRL34489.1	1028	X, 9, 9
		<i>Butyrivibrio fibrisolvens</i> 16/4	CBK74925.1	1153	9
			CBK75021.1	690	13, 2
		<i>Hungatella hathewayi</i>	CUO52114.1	421	
		<i>Ruminococcus gnavus</i>	WP_064787180.1	394	
	Ruminococcaceae	<i>Ruminococcus champanellensis</i> 18P13	CBL16579.1	633	22
			CBL17682.1	1268	22, 22, 6
		<i>Ruminococcus callidus</i> ATCC 27760	ERJ94429.1	1158	22, 22, 9
			ERJ87773.1	630	22
			ERJ97032.1	382	22
Bacteroidetes	Bacteroidaceae	<i>Bacteroides ovatus</i>	EDO13863.1	372	
			EDO10007.1 ¹	376	
			EDO14247.1	573	
			EDO10010.1 ¹	740	4, 4
			EDO14052.1	584	
			EDO10798.1	750	
		<i>Bacteroides intestinalis</i> DSM 17393	EDV05054.1	782	4, 4
			EDV05072.1 ²	746	4, 4
			EDV03684.1	738	
			EDV05059.1	910	
			EDV07678.1	725	
			EDV07007.1 ²	899	
		<i>Bacteroides xylanisolvens</i> XB1A	CBK67953.1 ³	754	4, 4
			CBH32823.1	378	

AA: amino acids, n.a.: GH10 is present, but not assigned in the genome,

1. Rogowski, A. *et al.* Glycan complexity dictates microbial resource allocation in the large intestine. *Nat. Commun.* **6**, 7481 (2015).
2. Zhang, M. *et al.* Xylan utilization in human gut commensal bacteria is orchestrated by unique modular organization of polysaccharide-degrading enzymes. *Proc. Natl. Acad. Sci. U. S. A.* **111**, E3708-E3717 (2014)
3. Despres, J. *et al.* Xylan degradation by the human gut Bacteroides xylanisolvens XB1AT involves two distinct gene clusters that are linked at the transcriptional level. *BMC Genomics* **17**, 326 (2016).

Supplementary Table 3. Thermodynamic parameters and dissociation constant for R/Xyn10A-CBMx determined by ITC.

Ligand	K_D (μ M)	N_0	ΔH (kcal/mol)	$T\Delta S$ (kcal/mol)	ΔG (kcal/mol)
X6	413 $\pm$ 125	0.74 $\pm$ 0.04	-19.9 $\pm$ 1.2	-15.3	-4.6

Data are from one experiment and binding parameters are reported with the error of the fit to the binding isotherm.

Supplementary Table 4. Homologs of CBMx identified in genomes of taxonomically related taxa to *R. intestinalis*.

Strain	Accession number	Query cover	E-value	Identity
<i>Roseburia intestinalis</i> XB6B4	CBL13458.1	100%	4e-85	100%
<i>Eubacterium rectale</i> _T1815	CRL34489.1	89%	5e-36	55%
<i>Butyrivibrio</i> sp. LC3010	WP_026509692.1	92%	1e-07	36%
<i>Roseburia faecis</i> M72	CRL32809.1	93%	9e-12	36%
<i>Bacterium</i> enrichment culture clone MC3F	AFU34339.1	86%	3e-07	30%
<i>Lachnoclostridium phytofermentans</i> ISDg	ABX41884.1	84%	5e-07	26%
<i>Clostridium</i> sp. KNHs205	WP_033165005.1	88%	1e-06	28%
<i>Butyrivibrio</i> sp. IN11a14	SCX91715.1	63%	2e-06	32%
<i>Lachnospiraceae</i> bacterium YSD2013	SCX14282.1	73%	1e-05	34%
<i>Butyrivibrio</i> sp. ob235	SEK63083.1	76%	2e-04	30%
<i>Butyrivibrio</i> sp. VCD2006	WP_026526370.1	72%	3e-04	27%

Supplementary Table 5. Kinetic parameters of *RiAgu115A*.

Substrate	K_M	k_{cat}	k_{cat}/K_M
	(mg mL ⁻¹)	(s ⁻¹)	(mL mg ⁻¹ s ⁻¹)
BeGX	n.d.	n.d.	2
BeGX + <i>RiXyn10A</i>	12 ± 3	395 ± 34	33

n.d.: Low affinity and lack of curvature of the Michaelis Menten plots precluded reliable determination of kinetic parameters. Catalytic efficiencies are from the slope of the initial rates versus substrate concentration. Data are means of a triplicate with standard deviations.

Supplementary Table 6. Kinetics of *RiAbf43A*.

Substrate	K_M	k_{cat}	k_{cat}/K_M
	(mM)	(s ⁻¹)	(s ⁻¹ mM ⁻¹)
AX4	0.8 ± 0.1	20 ± 1	25
	K_M	k_{cat}	k_{cat}/K_M
	(mg mL ⁻¹)	(s ⁻¹)	(mL mg s ⁻¹)
WAX	6.3 ± 0.4	12 ± 0	1.9

Data are means of a triplicate with standard deviations.

Supplementary Table 7. Kinetics *RiXyl3A*.

Substrate	K_M	k_{cat}	k_{cat}/K_M
	(mM)	(s ⁻¹)	(s ⁻¹ mM ⁻¹)
X2	2.7 ± 0.4	57 ± 3	21
X3	3.4 ± 0.3	60 ± 2	18
X4	2.4 ± 0.4	32 ± 2	13
X5	2.6 ± 0.5	36 ± 1	14
X6	2.1 ± 0.2	30 ± 1	15

Data are means of a triplicate with standard deviations.

Supplementary Table 8. Kinetics *RiXyl8*.

Substrate	K_M	k_{cat}	k_{cat}/K_M
	(mg/mL)	(S ⁻¹)	(s ⁻¹ ·mM ⁻¹)
X3	4.8 ± 1.0	1208 ± 124	251.7
X4	5.1 ± 1.5	892 ± 131	174.9

Data are means of a triplicate with standard deviations.

Supplementary Table 9. Deacetylation activity of *RiAXE* on acetylated xylans and aryl acetate.

Substrate	Enzyme(s)	V	$V/[E]$
		(μM s ⁻¹)	(s ⁻¹)
AcBGX	<i>RiAXE</i>	2.5	39.1
	<i>RiAXE</i> + <i>RiXyn10A</i>	3.2	50
	<i>RiAXE</i> + <i>RiXyn10A</i> + <i>RiAgu115A</i>	2.8	43.8
AcSpruce mannan	<i>RiAXE</i>	0.2	3.1
pNP-acetate	<i>RiAXE</i>	4.7 ^a ± 0.1	n.d.
Autolysis		0.07	n.d.

V : rate, $V/[E]$: normalized rate by enzyme concentration estimated from NMR experiments. ^aThe activity on paranitrophenyl acetate (pNP-acetate) is expressed in U mg⁻¹.

Supplementary Table 10. Assignment of chemical shifts for xylan deacetylation by *RiAXE*.

Structural unit	Assignment						
	H-1; C-1	H-2; C-2	H-3; C-3	H-4; C-4	H-5; C-5	H-6; C-6	Ac-H; C
X	4.42; 105.4	3.19; 75.4	3.53; 76.4	3.78; 79.2	n.d	n.d	-
C2	4.68; 102.6	4.69; 76.1	3.79; 74.2	3.86; 78.9	n.d	n.d	2.10; 23.1
C3	4.47; 104.3	3.37; 75.4	4.89; 79.9	3.78; 79.1	n.d	n.d	2.17; 23.2
C23	4.81; 102.2	4.81; 74.2	5.17; 74.1	4.05; 77.9	n.d	n.d	(2) 2.10; 22.9/ (2) 2.12; 23.0
C3MeGlcA	4.57; 104.2	3.48; 73.6	4.98; 78.1	3.94; 78.1	n.d	n.d	2.15; 23.3
MeGlcA	5.17; 96.6	3.56; 74.4	3.53; 73.3	n.d	n.d	n.d	-
α	5.18; 94.8	3.56; 74.2	3.53; 73.7	n.d	n.d	n.d	-
β	4.56; 99.3	3.25; 76.7	3.52; 77.9	3.72; 79.7	n.d	n.d	-

Supplementary Table 11. Esterase activity for *Ri*AXE measured using MALDI-TOF.

	AcBGX	AcAspen xylan	AcSpruce mannan	Cellulose mono acetate	AcChitin	InWAX
<i>Ri</i> AXE	++	++	+	+	-	-
<i>Ri</i> AXE + <i>Ri</i> Agu115A	+++	n.d.	n.d.	n.d.	n.d.	n.d.

+++ : complete deacetylation, ++:almost complete acetylation (1 ≥ acetyl/oligosaccharide),

+: minor deacetylation (1-2 acetyl/oligosaccharide), -: no deacetylation). Experiments performed twice.

Supplementary Table 12. Xylan hydrolysis kinetics of *Ri*Xyn10B.

Substrate	K_M (mg mL ⁻¹)	k_{cat} (s ⁻¹)	k_{cat}/K_M (mL mg ⁻¹ s ⁻¹)
BGX	n.d.	n.d.	9.8
WAX	4.4 ± 0.8	413 ± 32	94
InWAX	n.d.	n.d.	2.3

n.d.: Low affinity and lack of curvature of the Michaelis Menten plots precluded reliable determination of the kinetic parameters and the catalytic efficiencies are determined from the slope of the initial rate data versus substrate concentration. Data are reported as means of triplicates with standard deviations

Supplementary Table 13. Cloning and mutagenesis primers^{a,b}.

Gene	Accession number	Name	Orientation	Sequence (5' -> 3')
ROSINTL182_06494 (AA27-1356)	EEV01588.1	RiXyn10A	Forward	TTTCAGGGCGCCAT GGGGTAAAAAAGTTTTACTGCAGAT
ROSINTL182_06494 (AA27-1356)	EEV01588.1	RiXyn10A	Reverse	GACGGAGCTCGAATTTT ACTACTACTGATCTTTATCTCTTTGCA
ROSINTL182_06494 (AA156-1356)	EEV01588.1	RiXyn10AΔCBMx	Forward	TTTCAGGGCGCCAT GGCAGGAGCAGGCGATGCA
ROSINTL182_06494 (AA156-1356)	EEV01588.1	RiXyn10AΔCBMx	Reverse	GACGGAGCTCGAATTTT ACTACTACTGATCTTTATCTCTTTGCA
ROSINTL182_06494 (AA349-754)	EEV01588.1	RiXyn10A-cata	Forward	TTTCAGGGCGCCAT GTCTATTGAGAAGGACATCCCGGA
ROSINTL182_06494 (AA349-754)	EEV01588.1	RiXyn10A-cata	Reverse	GACGGAGCTCGAATTTT AGGATGCATCTACATACGCCCA
ROSINTL182_06494 (AA27-165)	EEV01588.1	RiXyn10A-CBMx	Forward	TTTCAGGGCGCCAT GGGGTAAAAAAGTTTTACTGCAGAT
ROSINTL182_06494 (AA27-165)	EEV01588.1	RiXyn10A-CBMx	Reverse	GACGGAGCTCGAATTTT AATCCCCCAATTTTGCA
ROSINTL182_08193	EEU99940.1	RiAbf43A	Forward	AGGAGATATACCAT GAGTATAGCAAAGATCCGGTTC
ROSINTL182_08193	EEU99940.1	RiAbf43A	Reverse	GGTGGTGGTGCTCGAA ACCCGGTATTCCCTCAT
ROSINTL182_08194	EEU99941.1	RiAXE	Forward	AGGAGATATACCAT GAGTGGACCTGTGGCA
ROSINTL182_08194	EEU99941.1	RiAXE	Reverse	GGTGGTGGTGCTCGAA TTCCACATAGCCAAAACCAA
ROSINTL182_08195	EEU99942.1	RiAgu115A	Forward	TTTCAGGGCGCCAT GGAAGCAATTTTGGTAAAGGATC
ROSINTL182_08195	EEU99942.1	RiAgu115A	Reverse	GACGGAGCTCGAATTTT ATCATCTGTCGCTCCTTT
ROSINTL182_08196	EEU99943.1	RiXyl8	Forward	AGGAGATATACCAT GAAAGAGGAGCGCTTTGAGA
ROSINTL182_08196	EEU99943.1	RiXyl8	Reverse	GGTGGTGGTGCTCGAA TAAATCTATAATTGCCGCTCAG
ROSINTL182_08199	EEU99894.1	RiXBP	Forward	TTTCAGGGCGCCAT GGAACAAAGCAGCCG
ROSINTL182_08199	EEU99894.1	RiXBP	Reverse	GACGGAGCTCGAATTTT ATTACTGATATTTTTTCTCTCTC
ROSINTL182_08202	EEU99897.1	RiXyl3A	Forward	AGGAGATATACCAT GGAATTAATCAGAATACAGAAAACTG
ROSINTL182_08202	EEU99897.1	RiXyl3A	Reverse	GGTGGTGGTGCTCGAATAA CATCAGACTTCCACTGTTT
ROSINTL182_06338/ ROSINTL182_06339	EEV01752.1/ EEV01731.1	RiXyn10B	Forward	TTTCAGGGCGCCAT GGCTGGCGAGAAAATG
ROSINTL182_06338/ ROSINTL182_06339	EEV01752.1/ EEV01731.1	RiXyn10B	Reverse	GACGGAGCTCGAATTTT ACTATTATCAGAATGAAATAAATTTCAA

^aBold nucleotides indicate the sequences annealing to the vector.

^bUnderlined nucleotides indicate the changed codon and italics indicate the changed bases.

Supplementary Table 14. qPCR primers use.

Target bacteria	Orientation	Sequence (5' -> 3')	Reference
<i>Roseburia</i> spp.	Forward	TACTGCATTGGAACTGTCTG	1
<i>Roseburia</i> spp.	Reverse	CGGCACCGAAGAGCAAT	1
<i>Bacteroides</i> spp.	Forward	CGATGGATAGGGTTCTGAGAGGA	2
<i>Bacteroides</i> spp.	Reverse	GCTGGCACGGAGTTAGCCGA	2
Universal primer	Forward	ACTCCTACGGGAGGCAGCAGT	3
Universal primer	Reverse	GTATTACCGCGGCTGCTGGCAC	3

1. Larsen, N. *et al.* Gut microbiota in human adults with type 2 diabetes differs from non-diabetic adults. *PLoS One* **5**, e9085 (2010).

2. Bergström, A. *et al.* Introducing GUT Low-Density Array (GULDA)-a validated approach for qPCR-based intestinal microbial community analysis. *FEMS Microbiol. Lett.* **337**, 38–47 (2012).

3. Walter, J. *et al.* Detection and identification of gastrointestinal *Lactobacillus* species by using denaturing gradient gel electrophoresis and species-specific PCR primers. *Appl. Environ. Microbiol.* **66**, 297–303 (2000).

Supplementary Figures

a

Locus ID	Log2-fold change			SP	Protein	Annotation
	X1/Glc	WAX/Glc	BGX/Glc			
05034	-0.84	6.48	5.54	Yes	SBP	ABC transporter, solute-binding protein
05035	-0.79	5.99	5.44	No	PP	ABC transporter, permease protein
05036	-0.80	6.04	5.26	No	PP	ABC transporter, permease protein
05037+05106	-0.33	6.44	5.95	Yes		NHL repeat protein
05107	-1.48	5.98	5.53	No		Hypothetical protein
05108	-0.42	5.91	5.48	No		Hypothetical protein
05109	-0.61	5.87	4.92	No	PP	ABC transporter, permease protein
05110	-0.63	5.11	3.92	No	PP	ABC transporter, permease protein
05111+05112	0.34	5.73	5.07	Yes	SBP	ABC transporter, solute-binding protein
05113	-0.77	4.95	4.78	No		Hypothetical protein
05114	-0.95	4.09	4.08	No	GH115	Xylan α -1,2-glucuronidase
05115	-0.73	4.35	4.17	No	CE	Putativ esterase

b

c

Locus ID	Log2-fold change			SP	Protein	Annotation
	X1/Glc	WAX/Glc	BGX/Glc			
06331	-0.63	5.26	4.76	No	GH43	β -xylosidase/ α -L-arabinofuranosidase
06332	-1.60	5.46	4.74	No	GH43	β -xylosidase/ α -L-arabinofuranosidase
06333	-0.70	0.44	2.24	No	AraC	Transcriptional regulator
06334	-1.22	1.03	2.44	No	GH27	α -galactosidase/ β -L-arabinopyranosidase
06335	-0.69	3.58	3.44	No	GH43	β -xylosidase/ α -L-arabinofuranosidase
06336	-1.04	3.61	3.21	No	GH95	α -L-galactosidase/ α -L-fucosidase
06337	-0.57	3.45	4.08	No	GH43	β -xylosidase/ α -L-arabinofuranosidase
06338+06339	-0.99	3.47	3.62	Yes	RiXyn10B	Endo-1,4- β -xylanase
06341	-1.12	4.09	3.80	No	GH43	β -xylosidase/ α -L-arabinofuranosidase
06342	-1.32	4.28	4.07	No	CE1	Esterase
06343	-1.23	4.49	4.21	Yes	GH43	β -xylosidase/ α -L-arabinofuranosidase
06344+06345	-1.57	4.49	4.68	No	ABC-NBD	ABC transporter, nucleotide binding domain
06346	-2.45	5.12	4.74	No	ABC-PP	ABC transporter, permease protein
06347	-2.38	5.49	5.35	No		Hypothetical ABC transporter
06348	-1.62	4.52	4.04	No		Hypothetical ABC transporter
06349	-0.95	6.49	5.44	No	UxuA	Mannonate dehydratase
06350	-1.50	5.84	5.12	No	UxuB	Mannitol/D-arabinitol dehydrogenase domain protein

d

e

Supplementary Figure 1 *R. intestinalis* L1-82 unique xylan upregulated loci. **(a)** Upregulation of a putative xylan metabolism gene cluster unique for the *R. intestinalis* L1-82 strain on xylan. **(b)** Organization of genes in (a). **(c)** Second unique *R. intestinalis* L1-82 gene cluster upregulated on xylan. **(d)** Organization of putative xylan-metabolism genes upregulated in (c). **(e)** Fluorescence microscopy of *R. intestinalis* grown on xylan showing the extracellular localization of RiXyn10B. Experiments were performed three times and locus IDs ROSINTL182_xxxxx are abbreviated with the last numbers after the hyphen. Signal peptides (SP) were predicted using SignalP v.3.0. Genes residing between two contigs have two locus IDs.

Supplementary Figure 2 *R. intestinalis* L1-82 xylose metabolism. (a) Proposed model for the metabolism of the monosaccharides xylose, arabinose and glucuronic acid in *R. intestinalis* L1-82 based on the RNA-seq data in Supplementary Table 1, and literature. (b) Upregulation of xylose import and metabolism genes in the model. The RNA-Seq heatmap depicts Log2-fold changes of genes expressed by cells grown on xylose (X1), wheat arabinoxylan (WAX) and birch glucuronoxylan (BGX) relative to glucose (Glc). Locus numbers ROSINTL182_xxxxx are abbreviated with the last numbers after the hyphen.

Supplementary Figure 3 Conservation of *R. intestinalis* core xylan utilization genes within the *Roseburia* genus. Genes are denoted according to their protein products; glycoside hydrolase (GH), carbohydrate esterase (CE), transcriptional regulators (Trans.R.), ABC transporter solute binding protein (SBP), ABC transporter permease protein (PP) and hypothetical proteins (Hypothe.). Sequence identities to *R. intestinalis* L1-82 genes are shown above the genes; Locus IDs for the genes are denoted under the respective strains. The asterisk indicates that the GH10 is not assigned in the genome.

Supplementary Figure 4 Properties of the extracellular xylanases from *R. intestinalis* (a) Action patterns of *RiXyn10A* on X2–X5 analyzed by TLC; +: reaction with enzyme, -: controls without enzyme. The dotted line indicates that lanes not relevant to the figure were spliced out for clarity. (b–g) Hydrolysis kinetics of *RiXyn10A*, *RiXyn10AΔCBMx* lacking the N-terminal module and *RiXyn10A-cata*, the catalytic module on WAX, and BGX. (h) Binding of xylans to *RiXyn10*-CBMx by affinity gel electrophoresis using native polyacrylamide gels with different concentrations of WAX (0.0–0.1% w/v) or BGX (0.0–1.0% w/v). No polysaccharides were added to the control. Lane 1+2; *RiXyn10A*-CBMx (3.0 μg), Lane 3 β-lactoglobulin (1.5 μg), M; marker. (i) Domain organization of the xylanase *RiXyn10B* encoded by a locus upregulated on xylan and which is unique for the *R. intestinalis* L1-82 strain used in the present study (Supplementary Fig. 1c–d). The bottom cartoon represents the recombinant enzyme. Experiments in (a) and (h) are performed twice and in triplicates for (b–g).

Supplementary Figure 5 Binding of CBMx and *RiXyn10A* to xylo-oligosaccharides. (a-e) Reference and blank corrected sensograms depict binding of xylo-oligosaccharides (X3-X6) and mannohexaose (Man6) as negative control to CBMx (*RiXyn10A*-CBMx) using SPR analysis. (f) ITC analysis of CBMx binding to X6. (g,i) Reference and blank corrected SPR sensograms depicting the binding of X6 to *RiXyn10A* and *RiXyn10A* Δ CBMx respectively. (h,j) One binding model fitted to the binding isotherms from the sensograms in (g,i). The experiments were in triplicates, except for the ITC run once.

Supplementary Figure 6 Binding preference of *RixBP* associated to the xylo-oligomer ABC transporter of *R. intestinalis*. (a-g) ITC analysis of *RixBP* binding to linear and branched xylo-oligosaccharides. **(h,i)** Structures of the branched arabinosylated xylo-oligosaccharides AX4 and AX3, which are mixtures with arabinofuranosyl decoration either at the C2 or C3 of xylosyl units. **(j,k)** Time course HPAEC-PAD analysis of culture supernatants of *R. intestinalis* grown in YCFA with 0.5% WAX or BGX. The observed peaks between 0 and 5 minutes are likely unutilized medium components. Experiments in (a-g) are duplicates, and in (j,k) from a duplicate.

Supplementary Figure 7 Intracellular xylo-oligosaccharide degrading enzymes from *R. intestinalis* (a) TLC analysis of the release of 4-*O*-methylglucuronic acid (MeGlcA) from BGX and BeGX by RiAgu115A. Glucuronic acid (GlcA) is used as standard. (b,c) Activity of RiAgu115A on a GH30-hydrolyzed BeBGX monitored using MALDI-ToF MS; (b) is the GH30 control and (c) is the treatment with GH30 and RiAgu115A. Activity indicates RiAgu115A releases MeGlcA from the penultimate xyloxyl to the reducing end in xylo-oligosaccharides based on the GH30 strict specificity¹, whereas a GH10 generates xylo-oligosaccharides with a MeGlcA substitution at the non-reducing end². This data shows that the RiAgu115A is able to act on both internal and terminal non-reducing end substitutions on glucuronoxylan-derived xylo-oligosaccharides. Di-sodium adducts of MeGlcA decorated oligomers (diamonds) are colored as their corresponding single sodium adducts. (d,g) Monosaccharide hydrolysis products from enzymatic treatment of WAX and BGX with RiXyn10A, RiAbf43A, RiXyl3A and RiXyl8 by HPAEC-PAD. Standards were 1; arabinose, 2; galactose, 3; glucose, 4; xylose. (e,f) RiXyl3A and RiXyl8 hydrolysis of xylo-oligosaccharides analyzed with HPAEC-PAD. (h) β -xylosidase activity for RiXyl3A and RiXyl8 towards xylo-oligosaccharides (XOS) by TLC. The + and - indicate the presence and absence of the different components, respectively. Lack of activity on substrate reduced with NaBH₄ (converts reducing end unit to its alditol) provided evidence that RiXyl8 acts on the reducing end as the alditol is not accommodated in the active site. Experiments are performed in duplicates.

1. St John, F. J., Hurlbert, J. C., Rice, J. D., Preston, J. F. & Pozharski, E. Ligand bound structures of a glycosyl hydrolase family 30 glucuronoxylan xylanohydrolase. *J. Mol. Biol.* **407**, 92–109 (2011).
2. Dodd, D. & Cann, I. K. Enzymatic deconstruction of xylan for biofuel production. *Glob Chang. Biol Bioenergy* **1**, 2–17 (2009).

Supplementary Figure 8 Activity, specificity and taxonomic distribution of the novel xylan acetyl esterase *RiAXE*. (a) Phylogenetic tree of *RiAXE* and homologs identified by a BLASTP search against the non-redundant database. Sequences with coverage >86% and identity >42% were selected. All sequences were from Firmicutes members. The resulting 131 protein sequences were aligned using Muscle¹ and a phylogenetic tree constructed by the maximum likelihood algorithm in MEGA7². Bootstraps were performed with 500 replicates. The phylogenetic tree was visualized using Figtree (<http://tree.bio.ed.ac.uk/software/figtree>). Asterisk indicates position of *RiAXE*. (b) Time course deacetylation of AcBGX treated with *RiXyn10A* and *RiAgu115A* by *RiAXE* determined by NMR. (c) Rates of deacetylation by *RiAXE* on AcBGX and AcSpruce mannan (AcSGGM) in D_2O , which may influence absolute reaction rates. (d) ^{13}C HSQC spectrum of *RiXyn10A* treated AcBGX showing the acetyl region and with the 1D proton projection. (e) same as (d) but showing the spectral region for anomeric and *O*-acetylated xylose signals. *RiXyn10A* treatment enhances signal-to-noise of resonances in the NMR spectra for the assignment and increases the total number of observable individual signals. (f,g) ^{13}C HSQC spectra for *O*-acetylated regions before (f) and after (g) deacetylation by *RiAXE*. Nearly complete deacetylation of AcBGX is reached during the time resolved NMR experiment. Chemical shifts of the most dominating signal for the monosaccharide residues mark by "+", peaks encircled by dotted lines indicate cluster of chemical shifts likely to belong to the same type of monosaccharide residue as dominating signal.

1. Edgar, R. C. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res.* **32**, 1792–1797 (2004).
2. Kumar, S., Stecher, G. & Tamura, K. MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol. Biol. Evol.* **33**, msw054 (2016).

Supplementary Figure 9 Co-culture experiment with *R. intestinalis* and *B. ovatus*. (a-b) Growth curves for monoculture and co-cultures after growth of *R. intestinalis* and *B. ovatus* with water as controls instead of carbon source. (c) Relative abundance determined by qPCR in a propagation experiment with co-cultures on WAX. After 9 hours of growth, the co-culture was passaged into fresh media, passage (I) (start OD₆₀₀ nm=0.01). This culture was grown for 12 hours and passaged into fresh media again (passage II). The western blots were carried out with (d) anti-RiXBP, (e) anti-RiXyn10A, (f) anti-RiXyn10B. R: *R. intestinalis*, B: *B. ovatus*, C: co-culture of *R. intestinalis* and *B. ovatus*. Asterisk denotes the position of the band based on theoretical molecular mass. The molecular markers size is shown in kDa. Lower molecular mass signals than expected indicate proteolytic cleavage occurring particularly with the multi-modular RiXyn10A. Experiments are performed in biological triplicates in (a-c) and in duplicates in (d-f).