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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 *Bacteroides* 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.
Introduction

The human gut microbiota (HGM) is recognized as a determinant of human health and metabolic homeostasis\(^1,2\). Specific signatures of the HGM are associated with local and systemic disorders including irritable-bowel disease, obesity, type 2 diabetes and colon cancer\(^3\). The composition of the HGM is greatly affected by dietary glycans, which are non-digestible by the host\(^4,5\). Only a few species out of the hundreds present in the HGM are equipped to deconstruct distinct complex polysaccharides and ferment them into short chain fatty acids (SCFAs)\(^6\). The impact of SCFAs on host health and physiology remains an important aspect of the microbiota-host interaction. Particularly the SCFA butyrate, the preferred energy source for colonocytes, is known to have anti-inflammatory roles and reduce the risk of colon cancer and enteric colitis\(^7-10\). Butyrate producers belonging to the Firmicutes phylum are generally abundant in healthy individuals, but are markedly reduced in patients with inflammatory disorders\(^11,12\). Butyrate producers including *Roseburia* spp. are increased in metabolic syndrome patients after faecal transfer therapy, and correlate positively to improvement of insulin resistance\(^13\). Investigations of the metabolic preferences of butyrate producers and their interplay with major HGM commensals are instrumental to develop therapeutic interventions targeting butyrate-deficiency related disorders.

*Roseburia* is a common genus of *Clostridium* cluster XIVa within the Firmicutes that harbours prevalent butyrate producers\(^14,15\). This taxon adheres to mucin, consistent with an intimate association with the host\(^16\). *Roseburia intestinalis* strains encode an impressive repertoire of carbohydrate active enzymes (CAZymes) compared to most other Firmicutes\(^17\). *R. intestinalis*, the taxonomically related *Eubacterium rectale* and species from the *Bacteroides* genus are the only known HGM taxa that utilize the major hemicellulosic polysaccharide xylan\(^18-20\). Xylan is particularly abundant in cereal grains (arabinoxylan, AX), but is also found in fruits and vegetables (glucuronoxylan, GX)\(^21\) (Fig. 1a). Xylan utilization by dominant gut commensals belonging to the
**Bacteroides** 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 arabinoxyylans. 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 arabinoxyylan (WAX, \(\mu_{\text{max}}=0.26 \text{ h}^{-1}\)) compared to birch glucuronoxylan (BGX, \(\mu_{\text{max}}=0.13 \text{ h}^{-1}\)) (Fig. 1c). Interestingly, this bacterium also utilizes highly acetylated xylans and insoluble cereal arabinoxyylans from wheat (InWAX) and oat spelt (OSX), but not cornbran glucuronoxarabinoxyylan (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.org24), 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_0819, 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 *Ri*Xyn10A, which is conserved within the *R. intestinalis* species, is one of the largest known xylanases from human gut bacteria (Supplementary Fig. 2b). *Ri*Xyn10A 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 *Ri*Xyn10A, we characterized the enzyme and truncated versions thereof (Fig. 3a-d). *Ri*Xyn10A incubated with BGX, WAX and InWAX generated linear and decorated oligosaccharides (Fig. 3b,c and Fig. 4). *Ri*Xyn10A was inactive on highly and heterogeneously substituted arabinoglucomonoxylan 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 xylotrioce (X3) (Supplementary Fig. 3a). By contrast, xylotetraose (X4) and xylopentaose (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 $\text{RiXyn10A}$ from 128 $\mu$M to 65.4 $\mu$M ($\text{RiXyn10A}_{\Delta \text{CBMx}}$) (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 $\text{RiXyn10A}$ 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. We measured the affinity of $\text{RiXBP}$, 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 glucuronoxyl-o-oligosaccharides, 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-xylosidase (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 RXyn10A 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.
Taken together, the results showed that RiAXE 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 Bacteriodes 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, respectively.[6] 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 profiles[5], this species possesses distinctively larger CAZymes than most known clostridial Firmicutes of the HGM.[17] *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 vitro*.
Insight is lacking, however, on the preferences and the molecular machinery evolved by *R. intestinalis* to target xylan as compared to species of *Bacteroides*. 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 xylan. 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*, 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 $k_{cat}/k_{off}$ ratio$^{33}$). 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 module$^{33}$. Xylan capture by *Bacteriodes*, however, is additionally orchestrated by moderate affinity ($K_D = 60 \mu$M) xylan binding proteins that protrude away from the cell surface to facilitate binding$^{22}$. 


R. intestinalis was able to compete with B. ovatus for xylans during the log-phase (Fig. 5e-g). Notably, R. intestinalis seemed to outcompete B. ovatus after propagation of the co-culture (in the late log phase) in fresh medium for two additional passages, which underscores the competitiveness of the xylan utilisation machinery of this Firmicute (Supplementary Fig. 8c). R. intestinalis has been reported to be associated to insoluble xylans, including wheat bran, while species of Bacteriodes were enriched in the solubilized xylan fractions\textsuperscript{18,36}. The extended binding mediated by RiXyn10A may play an important role in the association to insoluble substrates. Indeed, the expression of this enzyme appeared similarly high in the mono- and mixed xylan cultures with B. ovatus (Supplementary Fig. 8e). These observations are different from the reported down-regulation of hydrolases by Eubacterium rectale, which is close taxonomic relative to Roseburia, during co-growth with Bacteroides thetaiotamicron on a fiber rich diet in previously germ-free mice\textsuperscript{37}.

The gene encoding the binding protein (RiXBP) of the ABC transporter that confers xylo-oligosaccharide uptake in R. intestinalis was the most upregulated in the xylan transcriptomes, attesting the crucial role of oligosaccharide capture and transport in the densely populated gut ecological niche. The narrow preference of this protein for decorated backbone of 4–5 xylosyl units aligned with the products of RiXyn10A (Fig. 3b and Fig. 4c). The affinity and size preference of RiXBP were found to be very different from the corresponding protein from Bifidobacterium\textsuperscript{39}, which prefers shorter xylo-oligosaccharides with a different side chain decoration pattern. Importantly, striking differences in binding affinities and preference are observed when RiXBP is compared to the SusD-like xylan-binding counterpart from Bacteriodes. Indeed, both SusD-like proteins from B. ovatus, which mediate capture and internalization of xylan-oligosaccharides ≥ X6 by SusC TonB-dependent permeases, displayed no measurable binding to X4 and X5\textsuperscript{22}, the preferred ligands of RiXBP. These differential transport protein preferences are likely to be instrumental in establishing competitive uptake profiles to select oligosaccharides of specific sizes and decorations for each taxon. This is supported by the dominance of R. intestinalis when the co-culture with B. ovatus was 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).

Corn bran 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 described. Cellulose acetate was a kind gift from Alexander Deutschle, University of Hamburg, Germany. Acetylated chitin-oligosaccharides were prepared as previously described.

**Growth experiments and RNA-seq transcriptional analysis**

*R. intestinalis* DSM 14610 was grown in a Whitley DG250 Anaerobic Workstation (Don Whitley, UK) in YCFA medium 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 module\textsuperscript{40}) 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 \textit{R. intestinalis} L1-82 reference genome and genome annotations are based on assembly \textit{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\textsuperscript{41,42}, and gene counts were determined with HTseq\textsuperscript{43}. Differential gene expression was performed using DeSeq2 in R\textsuperscript{44}.

**Xylanase activity measurements on whole cells**

Cell-associated xylanase activity was determined by growing \textit{R. intestinalis} cells in 800 µL 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\textsubscript{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, \textit{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 µL 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 µL PBS. The xylanase activity of cells and wash fractions was assayed using the DNS assay.

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

Enzymatic activity assays

Enzymatic assays were carried out in a 50 mM HEPES 0.005% (v/v) Triton X-100, pH 7.0 standard assay buffer unless otherwise stated. Hydrolysis kinetics of full-length or truncated xylanases (10–200 nM) were assayed towards 1–9 mg mL⁻¹ of BGX, WAX or InWAX (37°C, 900 µL, 12 min). Initial hydrolysis rates were determined by removing 200 µL aliquots every third minutes and quenching the reaction in 300 µL 3,5-dinitrosalicylic acid (DNS) reagent⁴⁶. Next samples were incubated for 15 min at 90°C followed by A₅₄₀ nm measurement in 96 microtitre plates. Xylose was used as a standard (0–2.5 mM). Xylanase activity was assayed for R. intestinalis cells washed with PBS ± 1.5 M NaCl, and wash-fractions, as above with the following modification: 180 µL of 1% (w/v) BGX was incubated with 20 µL cell suspension or wash-fraction for 4 hours.

Hydrolysis kinetics of α-glucuronidase were analyzed on 1–9 mg mL⁻¹ BeGX or a hydrolysate thereof (prepared by incubation with 4 mM RiXyn10A xylanase for 15 hours at 37°C followed by heat inactivation). The initial rates of (O-methyl)-D-glucuronic acid release were measured using a coupled enzymatic assay (Megazyme). Reactions (770 µL) were incubated for 2 min at 37°C with 10–180 nM enzyme with intermittent removal of 175 µL aliquots every 15 s into 125 µL 1 M Tris pH 10 to quench the reaction. This was followed by mixing 270 µL of the stopped reaction with 45 µL of
the NAD⁺ and uronate dehydrogenase reagents. Conversion of NAD⁺ to NADH was measured at A₃₄₀ nm. Glucuronic acid was used as standard (0–500 µM).

Hydrolysis kinetics of RiXyl8 and RiXyl3A were determined towards xylobiose (X₂) through to xylohexaose (X₆) (0.5–12 mM) in McIlvaine buffer pH 6.8 (10 mM citric acid and 20 mM sodium phosphate) as described in⁴⁷,⁴⁸. Reactions (350 µL) were incubated for 12 min at 37°C with 36–78 nM RiXyl3A or 2.4 nM RiXyn8. Aliquots of 50 µL were removed every 2 minutes and stopped in 250 µL p-bromoaniline (2% w/v) in glacial acetic acid with thiourea (4% w/v). The stopped reactions were incubated in darkness for 10 min at 70°C, followed by incubation at 37°C for 1 hour before measuring A₅₂₀ nm. The concentration of released pentoses was determined using a xylose standard (0–5 mM)⁴⁹.

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

The acetyl esterase specific activity of RiAXE was determined in 250 µL reactions containing para-nitrophenyl-acetate (4 mM) and 0.14 µM enzyme. A₄₀₅ nm was measured every 60 s for 10 minutes at 37°C in a microtiter plate reader and pNP (0–1 mM) was used as standard. The specific activity was determined in units (U/mg), where a U is defined as the amount of enzyme that produces 1 µmol of pNP min⁻¹.

Kinetic parameters were calculated by fitting the Michaelis-Menten equation to the initial rate data using Graph Pad Prism 7. The catalytic efficiency k₉₅/Kₘₚ determined from the slope of the
normalized initial rate \((V_0/[E])\) in the Michaelis-Menten plot, is reported when saturation was not attained. All experiments were performed in triplicates.

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

Hydrolysis of xylan and xylo-oligosachharides was performed at 37°C for 15 hours in the standard assay buffer used above. Oligosaccharide hydrolysates, used to assay the sequential action of the debranching xylanolytic enzymes, were generated using RiXyn10A, which was separated by ultrafiltration (3 kDa cutoff) before the addition of debranching enzymes. The hydrolysis profiles were analyzed as detailed below. To verify the mode of reducing-end attack of RiXyl8, 30 mg XOS in standard assay buffer were reduced by NaBH₄ (1M in 100 µM NaOH). A total of 200 µL of the NaBH₄ was added dropwise to 800 µL of the xylo-oligosaccharides solution, which was kept on ice. As control 100 µM NaOH was added to an 800 µL xylo-oligossaccharides solution. The mixture was incubated 1 hour at room temperature, then quenched by 400 µL 1 M acetic acid and diluted 10x in assay buffer.

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

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

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

Aliquots of 1 µL of enzymatic reactions were spotted on a silica gel 60 F254 plate (Merck, Germany). The chromatography was performed in a butanol:acetic acid:water (2:1:1 v/v) mobile phase. The plates were dried at 50°C and carbohydrate hydrolysis products were visualized by spraying with a 5-methylresorcinol:ethanol:sulfuric acid (2:80:10 % v/v) developer and tarred briefly at 350°C until
bands appeared. Release of xylo-oligossaccharides 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 µL 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 µL 50 mM phosphate buffer pH 7.0 (99.9% D₂O). 2.5 µL of RiAXE 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 µL of RiXyn10A and RiAgu115A 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 RiAXE 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 µg 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 µL 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)

Titrations 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 electrophoresis 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-Flour 555 or goat anti-rabbit IgG Alexa-Flour 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**

*Bacteroides 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$_{600\text{nm}}$) were inoculated into CFA medium (YCFA lacking the yeast extract to
minimize *B. ovatus* growth on yeast extract$^{53}$) containing 0.5% (w/v) WAX, BGX, InWAX or X4. The
cocultures 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$_{600\text{nm}}$= 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 $\mu$L$^{-1}$ and amplified in technical triplicates using strain specific primers (Supplementary Fig. 9)
The amplification mix contained 2 $\mu$L DNA, 5.5 $\mu$L LightCycler 480 SYBR Green I Master mix (Roche),
0.22 $\mu$L of each primer (10 pmol/$\mu$L) and 3 $\mu$L 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
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


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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: $\Delta \text{OD}_{600\, \text{nm}} > 1.0$ for soluble substrates and pH drop $> 0.3$ for insoluble xyulans; yellow: $0.3 < \Delta \text{OD}_{600\, \text{nm}} < 0.5$; red: $\Delta \text{OD}_{600\, \text{nm}} < 0.1$. Asterisks indicate insoluble xyulans (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 ± 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∆CBMx 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 (*Kd*) determined by surface plasmon resonance (SPR) are means of a duplicate with the standard deviations. * Kd (mg mL⁻¹) from affinity electrophoresis (AE), and ** Kd 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 RIXyn10-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 RIXAXE 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 homonuclear and heteronuclear NMR correlation experiments (Supplementary Fig. 7). (c-f) Hydrolysis products from AcBGX by (c) RIXyn10A, (d) RIXyn10A and *RiAgu115A*, (e) RIXyn10A and RIXAXE, (f) RIXyn10A, *RiAgu115A* and RIXAXE. 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 *Bacteroides 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 butyrate 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

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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. Sh.).
a. Arabinoxylan

- β-1,4
- β-1,4
- β-1,4
- β-1,4
- α-1,2
- α-1,3
- 3Ac
- 2Ac

- D-xylose
- L-arabinose
- D-glucuronic acid
- Methyl
- 3Ac
- 3-O-acetyl
- 2Ac
- 2-O-acetyl

Glucuronoxylan

- β-1,4
- β-1,4
- β-1,4
- β-1,4
- β-1,4
- α-1,2
- 3Ac
- 2Ac

- D-xylose
- L-arabinose
- D-glucuronic acid
- Methyl
- 3Ac
- 3-O-acetyl
- 2Ac
- 2-O-acetyl

b. Substrate

- Glucose (Glc)
- Arabinose (A1)
- Glucuronic acid (GlcA)
- Xylose (X1)
- Xylobiose (X2)
- Xylotriose (X3)
- Xylotetraose (X4)
- Xylohexaose (X6)
- Corncob xylooligossaccharides (CCXOS)
- Wheat arabinoxylan (WAX)
- Birchwood glucuronoxylan (BGX)
- Acetylated birchwood glucuronoxylan (AcBGX)
- Cornbran arabinogluconoxylan (CBX)
- Insoluble wheat arabinoxylan (InWAX)*
- Oatspelt xylan (OSX)*

OD600 nm

Time (h)

Growth on soluble xylans

Growth on insoluble xylans

Induction of xylanase activity

Cell attachment of xylanase activity
### a) Core xylan utilization gene cluster

<table>
<thead>
<tr>
<th>Locus ID</th>
<th>Log2-fold change</th>
<th>SP</th>
<th>Protein</th>
<th>Annotation</th>
</tr>
</thead>
<tbody>
<tr>
<td>06494</td>
<td>-1.04</td>
<td>Yes</td>
<td>RiXyn10A</td>
<td>Endo-1,4-β-xylanase</td>
</tr>
<tr>
<td>08192</td>
<td>-0.46</td>
<td>No</td>
<td>RiLac1</td>
<td>Transcriptional regulator, LacI family</td>
</tr>
<tr>
<td>08193</td>
<td>0.04</td>
<td>No</td>
<td>RiAbf43A</td>
<td>α-L-arabinofuranosidase</td>
</tr>
<tr>
<td>08194</td>
<td>0.24</td>
<td>No</td>
<td>RiAXE</td>
<td>Acetyl xylan esterase</td>
</tr>
<tr>
<td>08195</td>
<td>0.63</td>
<td>No</td>
<td>RiAgu115A</td>
<td>Xylan α-1,2-glucuronidase</td>
</tr>
<tr>
<td>08196</td>
<td>0.78</td>
<td>No</td>
<td>RiXyl8</td>
<td>Reducing-end-xylose releasing exo-oligoxylanase</td>
</tr>
<tr>
<td>08197</td>
<td>0.91</td>
<td>No</td>
<td>RiXPP-A</td>
<td>ABC transporter, permease protein</td>
</tr>
<tr>
<td>08198</td>
<td>0.03</td>
<td>No</td>
<td>RiXPP-B</td>
<td>ABC transporter, permease protein</td>
</tr>
<tr>
<td>08199</td>
<td>0.49</td>
<td>Yes</td>
<td>RiXBP</td>
<td>ABC transporter, xylan binding protein</td>
</tr>
<tr>
<td>08200</td>
<td>0.28</td>
<td>No</td>
<td></td>
<td>Transcriptional regulator</td>
</tr>
<tr>
<td>08201</td>
<td>-0.25</td>
<td>No</td>
<td></td>
<td>Transcriptional regulator</td>
</tr>
<tr>
<td>08202</td>
<td>-0.22</td>
<td>No</td>
<td>RiXyl3A</td>
<td>Xylan 1,4-β-xylosidase</td>
</tr>
</tbody>
</table>

### b) Mean of normalized counts

- **X1/Glc**: Orange
- **WAX/Glc**: Blue
- **BGX/Glc**: Red

### c) Images

- **RiXBP (08199)**: Fluorescent micrograph showing xylan-binding protein localization.
- **RiXyn10A (06494)**: Fluorescent micrograph showing xylanase localization.
Growth rates - InWAX

R. intestinalis L1-82

Time (h) 0.5 1 3 10 40

pH 9

Co-culture X4

H2O 100

Percentage of each strain (%)

Growth rates - WAX

0.25

OD600 nm

5

60

WAX

5

7

100 60

15 0.0

2.0 1.0 1.0 0.5 0.0

Time (h) 0 10 20 30 40

BGX

20

6.6

Time (h) 0.00 0.75 1.5 2.0

∆pH/hour 5.8

∆OD600 nm/hour 2.0 -0.003

2.0 0.75 10 80 6.0

Time (h) 10

Growth rates - BGX

OD600 nm

0.75 0.50 0.25 0.00

Passage (III)

B. ovatus

24 1.5

Percentage of each strain (%)

Co-culture propagation WAX

Passage (III)

Percentage of each strain (%)

Co-culture InWAX

0.0 0.5 1.0 1.5 2.0

WAX

Time (h) 0 7 13 24

0

20

40

60

80

100

Co-culture WAX

Time (h)

Percentage of each strain (%)

Co-culture inWax

Co-culture propagation WAX

Passage (III)

Percentage of each strain (%)

Co-culture BGX

Co-culture X4

Passage (II)

Percentage of each strain (%)

Health promoting effects

- Energy for colon epithelial cell

- Energy for hydrogen consuming microbes

- Health promoting effects

Branched and linear xylo-oligosaccharides

Arabinoxylan

Glucuronoxylan

Acetate

GH10

Xyn10A

Ri

Xyl3A

H2O

Butyrate

Ri

Agu115A

Ri

AXE

Ri

BHBP

Acetate

Ri

Abf43A

Xyn10A

Ri

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

<table>
<thead>
<tr>
<th>Phylum</th>
<th>Family</th>
<th>Strain</th>
<th>Accession number</th>
<th>Length (AA)</th>
<th>CBMs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Firmicutes</td>
<td>Lachnospiraceae</td>
<td>Roseburia</td>
<td>ROSINTL182_06494</td>
<td>1356</td>
<td>X, 22, 9, 9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>intestinalis L1-B2</td>
<td>ROSINTL182_6338-9</td>
<td>601</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Roseburia</td>
<td>CBL13458.1</td>
<td>1356</td>
<td>X, 22, 9, 9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>intestinalis X86B4</td>
<td>n.a.</td>
<td>1356</td>
<td>X, 22, 9, 9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Roseburia</td>
<td>CBL32809.1</td>
<td>1380</td>
<td>X, 22, 9, 9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>faecis M72</td>
<td>CBK74925.1</td>
<td>1153</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Eubacterium</td>
<td>CBK75021.1</td>
<td>690</td>
<td>13, 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rectale T1-815</td>
<td>421</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Butyrivibrio</td>
<td>ROSINTL182_6338-9</td>
<td>601</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>fibrisolvens 16/4</td>
<td>CRL34489.1</td>
<td>1028</td>
<td>X, 9, 9</td>
</tr>
<tr>
<td></td>
<td>Ruminococcus</td>
<td>faecis M72</td>
<td>CRL32809.1</td>
<td>1356</td>
<td>X, 22, 9, 9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>intestinalis X86B4</td>
<td>n.a.</td>
<td>1356</td>
<td>X, 22, 9, 9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>faecis M72</td>
<td>CRL32809.1</td>
<td>1356</td>
<td>X, 22, 9, 9</td>
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<tr>
<td>Bacteroidetes</td>
<td>Bacteroidaceae</td>
<td>Bacteroides oculus</td>
<td>EDO13863.1</td>
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</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>EDO10007.1^1</td>
<td>376</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>EDO124247.1</td>
<td>573</td>
<td></td>
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<td></td>
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<td>EDO10010.1^1</td>
<td>740</td>
<td>4, 4</td>
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<td>EDO14052.1</td>
<td>584</td>
<td></td>
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<tr>
<td></td>
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<td>EDO10798.1</td>
<td>750</td>
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<td></td>
<td></td>
<td>EDO10798.1</td>
<td>750</td>
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<td>EDO05054.1</td>
<td>782</td>
<td>4, 4</td>
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<td></td>
<td>EDO05072.1^2</td>
<td>746</td>
<td>4, 4</td>
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<td></td>
<td></td>
<td>EDV03684.1</td>
<td>738</td>
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</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>EDV05059.1</td>
<td>910</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
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<td>EDV07687.1</td>
<td>725</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>EDV07007.1^1</td>
<td>899</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
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<td>CBK67953.1^1</td>
<td>754</td>
<td>4, 4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CBH32823.1</td>
<td>378</td>
<td></td>
</tr>
</tbody>
</table>

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

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, 626 (2016).

Supplementary Table 3. Thermodynamic parameters and dissociation constant for RiXyn10A-CBMx determined by ITC.

<table>
<thead>
<tr>
<th>Ligand</th>
<th>$K_d$ (µM)</th>
<th>$N_H$</th>
<th>$\Delta H$ (kcal/mol)</th>
<th>$T \Delta S$ (kcal/mol)</th>
<th>$\Delta G$ (kcal/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>X6</td>
<td>413 ± 125</td>
<td>0.74 ± 0.04</td>
<td>-19.9 ± 1.2</td>
<td>-15.3</td>
<td>-4.6</td>
</tr>
</tbody>
</table>

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***

<table>
<thead>
<tr>
<th>Strain</th>
<th>Accession number</th>
<th>Query cover</th>
<th>E-value</th>
<th>Identity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roseburia intestinalis XB6B4</td>
<td>CBL13458.1</td>
<td>100%</td>
<td>4e-85</td>
<td>100%</td>
</tr>
<tr>
<td>Eubacterium rectale_T1815</td>
<td>CRL34489.1</td>
<td>89%</td>
<td>5e-36</td>
<td>55%</td>
</tr>
<tr>
<td>Butyrivibrio sp. LC3010</td>
<td>WP_026508692.1</td>
<td>92%</td>
<td>1e-07</td>
<td>36%</td>
</tr>
<tr>
<td>Roseburia faecis M72</td>
<td>CRL32809.1</td>
<td>93%</td>
<td>9e-12</td>
<td>36%</td>
</tr>
<tr>
<td>Bacterium enrichment culture clone MC3F</td>
<td>AFU34339.1</td>
<td>86%</td>
<td>3e-07</td>
<td>30%</td>
</tr>
<tr>
<td>Lachnoclostridium phytofermentans ISDg</td>
<td>ABX41884.1</td>
<td>84%</td>
<td>5e-07</td>
<td>26%</td>
</tr>
<tr>
<td>Clostridium sp. KNHs205</td>
<td>WP_023165005.1</td>
<td>88%</td>
<td>1e-06</td>
<td>28%</td>
</tr>
<tr>
<td>Butyrivibrio sp. INlla14</td>
<td>SCX91715.1</td>
<td>63%</td>
<td>2e-06</td>
<td>32%</td>
</tr>
<tr>
<td>Lachnospiraceae bacterium YSD2013</td>
<td>SCX14282.1</td>
<td>73%</td>
<td>1e-05</td>
<td>34%</td>
</tr>
<tr>
<td>Butyrivibrio sp. ob235</td>
<td>SEK63083.1</td>
<td>76%</td>
<td>2e-04</td>
<td>30%</td>
</tr>
<tr>
<td>Butyrivibrio sp. VCD2006</td>
<td>WP_026526370.1</td>
<td>72%</td>
<td>3e-04</td>
<td>27%</td>
</tr>
</tbody>
</table>

**Supplementary Table 5. Kinetic parameters of *RiAgu115A***

<table>
<thead>
<tr>
<th>Substrate</th>
<th>$K_M$ (mg mL$^{-1}$)</th>
<th>$k_{cat}$ (s$^{-1}$)</th>
<th>$k_{cat}/K_M$ (mL mg$^{-1}$ s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BeGX</td>
<td>n.d.</td>
<td>n.d.</td>
<td>2</td>
</tr>
<tr>
<td>BeGX + RiXyn10A</td>
<td>12 ± 3</td>
<td>395 ± 34</td>
<td>33</td>
</tr>
</tbody>
</table>

* 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***

<table>
<thead>
<tr>
<th>Substrate</th>
<th>$K_M$ (mM)</th>
<th>$k_{cat}$ (s$^{-1}$)</th>
<th>$k_{cat}/K_M$ (s$^{-1}$ mM$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AX4</td>
<td>0.8 ± 0.1</td>
<td>20 ± 1</td>
<td>25</td>
</tr>
<tr>
<td>WAX</td>
<td>6.3 ± 0.4</td>
<td>12 ± 0</td>
<td>1.9</td>
</tr>
</tbody>
</table>

Data are means of a triplicate with standard deviations.
### Supplementary Table 7. Kinetics of RiXyl3A.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>$K_M$ (mM)</th>
<th>$k_{cat}$ (s$^{-1}$)</th>
<th>$k_{cat}/K_M$ (s$^{-1}$ mM$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>X2</td>
<td>2.7 ± 0.4</td>
<td>57 ± 3</td>
<td>21</td>
</tr>
<tr>
<td>X3</td>
<td>3.4 ± 0.3</td>
<td>60 ± 2</td>
<td>18</td>
</tr>
<tr>
<td>X4</td>
<td>2.4 ± 0.4</td>
<td>32 ± 2</td>
<td>13</td>
</tr>
<tr>
<td>X5</td>
<td>2.6 ± 0.5</td>
<td>36 ± 1</td>
<td>14</td>
</tr>
<tr>
<td>X6</td>
<td>2.1 ± 0.2</td>
<td>30 ± 1</td>
<td>15</td>
</tr>
</tbody>
</table>

Data are means of a triplicate with standard deviations.

### Supplementary Table 8. Kinetics of RiXyl8.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>$K_M$ (mg/mL)</th>
<th>$k_{cat}$ (s$^{-1}$)</th>
<th>$k_{cat}/K_M$ (s$^{-1}$ mM$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>X3</td>
<td>4.8 ± 1.0</td>
<td>1208 ± 124</td>
<td>251.7</td>
</tr>
<tr>
<td>X4</td>
<td>5.1 ± 1.5</td>
<td>892 ± 131</td>
<td>174.9</td>
</tr>
</tbody>
</table>

Data are means of a triplicate with standard deviations.

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

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Enzyme(s)</th>
<th>$V$ (µM s$^{-1}$)</th>
<th>$V/\left[E\right]$ (s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AcBGX</td>
<td>RiAXE</td>
<td>2.5</td>
<td>39.1</td>
</tr>
<tr>
<td></td>
<td>RiAXE + RiXyn10A</td>
<td>3.2</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>RiAXE + RiXyn10A + RiAgu115A</td>
<td>2.8</td>
<td>43.8</td>
</tr>
<tr>
<td>AcSpruce mannan</td>
<td>RiAXE</td>
<td>0.2</td>
<td>3.1</td>
</tr>
<tr>
<td>pNP-acetate</td>
<td>RiAXE</td>
<td>4.7± 0.1</td>
<td>n.d</td>
</tr>
<tr>
<td>Autolysis</td>
<td></td>
<td>0.07</td>
<td>n.d</td>
</tr>
</tbody>
</table>

$V$: rate, $V/\left[E\right]$: normalized rate by enzyme concentration estimated from NMR experiments. n.d: The activity on paranitrophenyl acetate (pNP-acetate) is expressed in U mg$^{-1}$.

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

<table>
<thead>
<tr>
<th>Structural unit</th>
<th>Assignment</th>
<th>H-1, C-1</th>
<th>H-2, C-2</th>
<th>H-3, C-3</th>
<th>H-4, C-4</th>
<th>H-5, C-5</th>
<th>H-6, C-6</th>
<th>Ac-H, C-6</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>4.42, 105.4</td>
<td>3.19, 75.4</td>
<td>3.53, 76.4</td>
<td>3.78, 79.2</td>
<td>n.d</td>
<td>n.d</td>
<td>n.d</td>
<td>n.d</td>
</tr>
<tr>
<td>C2</td>
<td>4.68, 102.6</td>
<td>4.69, 76.1</td>
<td>3.79, 74.2</td>
<td>3.86, 78.9</td>
<td>n.d</td>
<td>n.d</td>
<td>n.d</td>
<td>2.10, 23.1</td>
</tr>
<tr>
<td>C3</td>
<td>4.47, 104.3</td>
<td>3.37, 75.4</td>
<td>4.88, 79.9</td>
<td>3.78, 79.1</td>
<td>n.d</td>
<td>n.d</td>
<td>n.d</td>
<td>2.17, 23.2</td>
</tr>
<tr>
<td>C23</td>
<td>4.81, 102.2</td>
<td>4.81, 74.2</td>
<td>5.17, 74.1</td>
<td>4.05, 77.9</td>
<td>n.d</td>
<td>n.d</td>
<td>n.d</td>
<td>(2) 2.10, 22.9</td>
</tr>
<tr>
<td>(2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2.15, 23.0</td>
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<tr>
<td>C2MeGlcA</td>
<td>4.57, 104.2</td>
<td>3.48, 73.6</td>
<td>4.98, 78.1</td>
<td>3.94, 78.1</td>
<td>n.d</td>
<td>n.d</td>
<td>n.d</td>
<td>2.15, 23.3</td>
</tr>
<tr>
<td>MeGlcA</td>
<td>5.17, 96.8</td>
<td>3.56, 74.4</td>
<td>3.53, 73.3</td>
<td>n.d</td>
<td>n.d</td>
<td>n.d</td>
<td>n.d</td>
<td>-</td>
</tr>
<tr>
<td>(α)</td>
<td>5.18, 94.8</td>
<td>3.56, 74.2</td>
<td>3.53, 73.7</td>
<td>n.d</td>
<td>n.d</td>
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<td>n.d</td>
<td>-</td>
</tr>
<tr>
<td>(β)</td>
<td>4.56, 99.3</td>
<td>3.25, 76.7</td>
<td>3.52, 77.9</td>
<td>3.72, 79.7</td>
<td>n.d</td>
<td>n.d</td>
<td>n.d</td>
<td>-</td>
</tr>
</tbody>
</table>
### Supplementary Table 11. Esterase activity for RiAXE measured using MALDI-TOF.

<table>
<thead>
<tr>
<th></th>
<th>AcBGX</th>
<th>AcAspen xylan</th>
<th>AcSpruce mannan</th>
<th>Cellulose mono acetate</th>
<th>AcChitin</th>
<th>InWAX</th>
</tr>
</thead>
<tbody>
<tr>
<td>RiAXE</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

+++: complete deacetylation, ++: almost complete acetylation (1 ≥ acetyl/oligosaccharide), +: minor deactylation (1-2 acetyl/oligosaccharide), -: no deacetylation. Experiments performed twice.

### Supplementary Table 12. Xylan hydrolysis kinetics of RiXyn10B.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>$K_M$ (mg mL$^{-1}$)</th>
<th>$k_{cat}$ (s$^{-1}$)</th>
<th>$k_{cat}/K_M$ (mL mg$^{-1}$ s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BGX</td>
<td>n.d.</td>
<td>n.d.</td>
<td>9.8</td>
</tr>
<tr>
<td>WAX</td>
<td>4.4 ± 0.8</td>
<td>413 ± 32</td>
<td>94</td>
</tr>
<tr>
<td>InWAX</td>
<td>n.d.</td>
<td>n.d.</td>
<td>2.3</td>
</tr>
</tbody>
</table>

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**

<table>
<thead>
<tr>
<th>Gene Accession number</th>
<th>Name Orientation</th>
<th>Sequence (5' -&gt; 3')</th>
</tr>
</thead>
<tbody>
<tr>
<td>ROSINTL182_06494 (AA27-1356) EEV01588.1</td>
<td>RiXyn10A Forward</td>
<td>TTTCAAGGCGGCGCATGGGGTGAAAAAATTATTTACTGCAGAT</td>
</tr>
<tr>
<td>ROSINTL182_06494 (AA27-1356) EEV01588.1</td>
<td>RiXyn10A Reverse</td>
<td>GACGGAGTCGCAATTTACTCTTTCTCTTCTTTCGCA</td>
</tr>
<tr>
<td>ROSINTL182_06494 (AA156-1356) EEV01588.1</td>
<td>RiXyn10A Forward</td>
<td>TTTCAAGGCGGCGCATGGGGTGAAAAAATTATTTACTGCAGAT</td>
</tr>
<tr>
<td>ROSINTL182_06494 (AA156-1356) EEV01588.1</td>
<td>RiXyn10A Reverse</td>
<td>GACGGAGTCGCAATTTACTCTTTCTCTTCTTTCGCA</td>
</tr>
<tr>
<td>ROSINTL182_06494 (AA349-754) EEV01588.1</td>
<td>RiXyn10A Forward</td>
<td>TTTCAAGGCGGCGCATGGGGTGAAAAAATTATTTACTGCAGAT</td>
</tr>
<tr>
<td>ROSINTL182_06494 (AA349-754) EEV01588.1</td>
<td>RiXyn10A Reverse</td>
<td>GACGGAGTCGCAATTTACTCTTTCTCTTCTTTCGCA</td>
</tr>
<tr>
<td>ROSINTL182_08193 EEU99940.1</td>
<td>RiAbf43A Forward</td>
<td>AGGAGATATACCATGGAGAGATATACCATG</td>
</tr>
<tr>
<td>ROSINTL182_08193 EEU99940.1</td>
<td>RiAbf43A Reverse</td>
<td>GGTGGGTGGTGCTCGAAGCCGGTGATTCCCTCATA</td>
</tr>
<tr>
<td>ROSINTL182_08194 EEU99941.1</td>
<td>RiAXE Forward</td>
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<tr>
<td>ROSINTL182_08194 EEU99941.1</td>
<td>RiAXE Reverse</td>
<td>GACGGAGTCGCAATTTACTCTTTCTCTTCTTTCGCA</td>
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<tr>
<td>ROSINTL182_08195 EEU99942.1</td>
<td>RiAgu115A Forward</td>
<td>TTTCAAGGCGGCGCATGGGGTGAAAAAATTATTTACTGCAGAT</td>
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<tr>
<td>ROSINTL182_08195 EEU99942.1</td>
<td>RiAgu115A Reverse</td>
<td>GACGGAGTCGCAATTTACTCTTTCTCTTCTTTCGCA</td>
</tr>
<tr>
<td>ROSINTL182_08196 EEU99943.1</td>
<td>RiXyl8 Forward</td>
<td>TTTCAAGGCGGCGCATGGGGTGAAAAAATTATTTACTGCAGAT</td>
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<tr>
<td>ROSINTL182_08196 EEU99943.1</td>
<td>RiXyl8 Reverse</td>
<td>GACGGAGTCGCAATTTACTCTTTCTCTTCTTTCGCA</td>
</tr>
<tr>
<td>ROSINTL182_08197 EEU99944.1</td>
<td>RiXyl3A Forward</td>
<td>TTTCAAGGCGGCGCATGGGGTGAAAAAATTATTTACTGCAGAT</td>
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<tr>
<td>ROSINTL182_08197 EEU99944.1</td>
<td>RiXyl3A Reverse</td>
<td>GACGGAGTCGCAATTTACTCTTTCTCTTCTTTCGCA</td>
</tr>
<tr>
<td>ROSINTL182_08198 EEU99945.1</td>
<td>RiXBP Forward</td>
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<tr>
<td>ROSINTL182_08198 EEU99945.1</td>
<td>RiXBP Reverse</td>
<td>GACGGAGTCGCAATTTACTCTTTCTCTTCTTTCGCA</td>
</tr>
<tr>
<td>ROSINTL182_08201 EEU99946.1</td>
<td>RiXyn10A Forward</td>
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<tr>
<td>ROSINTL182_08201 EEU99946.1</td>
<td>RiXyn10A Reverse</td>
<td>GACGGAGTCGCAATTTACTCTTTCTCTTCTTTCGCA</td>
</tr>
<tr>
<td>ROSINTL182_08202 EEU99947.1</td>
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<tr>
<td>ROSINTL182_08202 EEU99947.1</td>
<td>RiXyn10B Reverse</td>
<td>GACGGAGTCGCAATTTACTCTTTCTCTTCTTTCGCA</td>
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<tr>
<td>ROSINTL182_06338/06339 EEU99948.1</td>
<td>RiXyn10B Forward</td>
<td>TTTCAAGGCGGCGCATGGGGTGAAAAAATTATTTACTGCAGAT</td>
</tr>
<tr>
<td>ROSINTL182_06338/06339 EEU99948.1</td>
<td>RiXyn10B Reverse</td>
<td>GACGGAGTCGCAATTTACTCTTTCTCTTCTTTCGCA</td>
</tr>
</tbody>
</table>

**Bold nucleotides indicate the sequences annealing to the vector.**

**Underlined nucleotides indicate the changed codon and italics indicate the changed bases.**

**Supplementary Table 14. qPCR primers use.**

<table>
<thead>
<tr>
<th>Target bacteria</th>
<th>Orientation</th>
<th>Sequence (5' -&gt; 3')</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roseburia spp.</td>
<td>Forward</td>
<td>TACTGCATTGGAAACTGTG</td>
<td>1</td>
</tr>
<tr>
<td>Roseburia spp</td>
<td>Reverse</td>
<td>CGGAGCAGGAAAGCAAT</td>
<td>1</td>
</tr>
<tr>
<td>Bacteroides spp.</td>
<td>Forward</td>
<td>CGATGATAGGGCTTCTAGAAAACACTG</td>
<td>2</td>
</tr>
<tr>
<td>Bacteroides spp.</td>
<td>Reverse</td>
<td>GTGGCAGAATTGAGGCAAT</td>
<td>2</td>
</tr>
<tr>
<td>Universal primer</td>
<td>Forward</td>
<td>ACTTACTGCGGGAGGACGAGT</td>
<td>3</td>
</tr>
<tr>
<td>Universal primer</td>
<td>Reverse</td>
<td>GTTATAGGCGGCTGCTGGCAG</td>
<td>3</td>
</tr>
</tbody>
</table>

Supplementary Figures
### 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.

#### Table: Genes Upregulated on Xylan

<table>
<thead>
<tr>
<th>Locus ID</th>
<th>Log2-fold change</th>
<th>SP</th>
<th>Protein</th>
<th>Annotation</th>
</tr>
</thead>
<tbody>
<tr>
<td>05034</td>
<td>-0.84</td>
<td>Yes</td>
<td>SBP</td>
<td>ABC transporter, solute-binding protein</td>
</tr>
<tr>
<td>05035</td>
<td>-0.79</td>
<td>No</td>
<td>PP</td>
<td>ABC transporter, permease protein</td>
</tr>
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<td>05036</td>
<td>-0.80</td>
<td>No</td>
<td>PP</td>
<td>ABC transporter, permease protein</td>
</tr>
<tr>
<td>05037+05106</td>
<td>-0.33</td>
<td>Yes</td>
<td>NHL repeat</td>
<td></td>
</tr>
<tr>
<td>05107</td>
<td>-1.48</td>
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<td>Hypothetical protein</td>
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<td>05108</td>
<td>-0.42</td>
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<td>Hypothetical protein</td>
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<td>05110</td>
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<td>No</td>
<td>PP</td>
<td>ABC transporter, permease protein</td>
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<tr>
<td>05111+05112</td>
<td>0.34</td>
<td>Yes</td>
<td>SBP</td>
<td>ABC transporter, solute-binding protein</td>
</tr>
<tr>
<td>05113</td>
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<td>Hypothetical protein</td>
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<td>05114</td>
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<td>Xylan α-1,2-glucuronidase</td>
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<td>05115</td>
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<td>No</td>
<td>CE</td>
<td>Putative esterase</td>
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</table>

<table>
<thead>
<tr>
<th>Locus ID</th>
<th>Log2-fold change</th>
<th>SP</th>
<th>Protein</th>
<th>Annotation</th>
</tr>
</thead>
<tbody>
<tr>
<td>06331</td>
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<td>No</td>
<td>GH43</td>
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<tr>
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<td>No</td>
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<td>β-xylanosidase/α-L-arabinofuranosidase</td>
</tr>
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<td>AraC</td>
<td>Transcriptional regulator</td>
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<td>06334</td>
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<td>No</td>
<td>GH27</td>
<td>α-galactosidase/β-L-arabinopyranosidase</td>
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<td>GH95</td>
<td>α-L-galactosidase/α-L-fucosidase</td>
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<tr>
<td>06337</td>
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<td>No</td>
<td>GH43</td>
<td>β-xylanosidase/α-L-arabinofuranosidase</td>
</tr>
<tr>
<td>06338+06339</td>
<td>-0.99</td>
<td>Yes</td>
<td>RiXyn10B</td>
<td>Endo-1,4-β-xylanase</td>
</tr>
<tr>
<td>06341</td>
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<td>No</td>
<td>GH43</td>
<td>β-xylanosidase/α-L-arabinofuranosidase</td>
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<td>CE1</td>
<td>Esterase</td>
</tr>
<tr>
<td>06343</td>
<td>-1.23</td>
<td>Yes</td>
<td>GH43</td>
<td>β-xylanosidase/α-L-arabinofuranosidase</td>
</tr>
<tr>
<td>06344+06345</td>
<td>-1.57</td>
<td>No</td>
<td>ABC-NBD</td>
<td>ABC transporter, nucleotide binding domain</td>
</tr>
<tr>
<td>06346</td>
<td>-2.45</td>
<td>No</td>
<td>ABC-PP</td>
<td>ABC transporter, permease protein</td>
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<td></td>
<td>Hypothetical ABC transporter</td>
</tr>
<tr>
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<td>No</td>
<td>UxuA</td>
<td>Mannolide dehydratase</td>
</tr>
<tr>
<td>06350</td>
<td>-1.50</td>
<td>No</td>
<td>UxuB</td>
<td>Mannitol/ID-arabinitol dehydrogenase domain protein</td>
</tr>
</tbody>
</table>

#### Diagrams:

- **a**
  - **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.

- **b**
  - Locus IDs SP Protein Annotation
  - 05034: Yes SBP ABC transporter, solute-binding protein
  - 05035: No PP ABC transporter, permease protein
  - 05036: No PP ABC transporter, permease protein
  - 05037: Yes NHL repeat protein
  - 05107: No Hypothetical protein
  - 05108: No Hypothetical protein
  - 05110: No PP ABC transporter, permease protein
  - 05111: Yes SBP ABC transporter, solute-binding protein
  - 05112: No Hypothetical protein
  - 05113: No Hypothetical protein
  - 05114: No GH115 Xylan α-1,2-glucuronidase
  - 05115: No CE Putative esterase

- **c**
  - Locus IDs Log2-fold change SP Protein Annotation
  - 06331: -0.63 5.26 4.76 No GH43 β-xylanosidase/α-L-arabinofuranosidase
  - 06332: -1.60 5.46 4.74 No GH43 β-xylanosidase/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-xylanosidase/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-xylanosidase/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-xylanosidase/α-L-arabinofuranosidase
  - 06342: -1.32 4.28 4.07 No CE1 Esterase
  - 06343: -1.23 4.49 4.21 Yes GH43 β-xylanosidase/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 Mannolide dehydratase
  - 06350: -1.50 5.84 5.12 No UxuB Mannitol/ID-arabinitol dehydrogenase domain protein

- **d**
  - Diagram showing the organization of genes in (a).

- **e**
  - Fluorescence microscopy of R. intestinalis grown on xylan showing the extracellular localization of RiXyn10B.
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, RiXyn10A∆CBMx 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∆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 specificity, whereas a GH10 generates xylo-oligosaccharides with a MeGlcA substitution at the non-reducing end. 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.

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 Muscle\(^1\) and a phylogenetic tree constructed by the maximum likelihood algorithm in MEGA7. 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$_2$O, 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) deactylation 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.


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 $OD_{600}$ 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).