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How Fine Structural Differences of Xylooligosaccharides and Arabinoxylooligosaccharides Regulate Differential Growth of *Bacteroides* Species

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ABSTRACT: Xylooligosaccharides (XOS) and arabinoxylooligosaccharides (AXOS) could be used to selectively favor growth of certain gut bacterial groups. The objective of this research was to understand how the structural differences of XOS and AXOS influenced the growth of *Bacteroides* species commonly found in the intestine. We report that the specific structural details of XOS and AXOS dictate the differential growth of *Bacteroides* species in the intestine. We also investigated the expression of two polysaccharide utilization loci (PULs) in a strain of *Bacteroides ovatus* upon growth on AXOS using three different *susC* transcripts as sentinel reporter genes. 2^3-α-L-Arabinofuranosyl-xylotriose (A4) was shown to upregulate small xylan PUL gene expression, while 2^3,3^3-di-α-L-arabinofuranosyl-xylotriose (A6) decreased expression of this PUL. These results reveal new details about the potentially very specific structure−function relationship of XOS and AXOS that could be used in targeted alteration of the microbial population in the gut through dietary interventions to maintain health.

KEYWORDS: *Bacteroides species, polysaccharide utilization loci (PUL) gene expression*

1. INTRODUCTION

The intestine is an important organ that consists of an extensive surface area and permits vital interactions with the external world, including the gut microbiota.[1] The microbiota refers to "the microbial life forms within a given habitat or host".[2] The gut microbiota exerts a significant impact on host physiology, impacting the control of energy homeostasis, the immune system, digestion and vitamin synthesis,[1] and inhibition of pathogen colonization.[3] More so, the involvement of gut microbiota in conditions such as depression[4] and autism[5] has also been suggested. A potential role between the microbiome and Parkinson's disease (PD)[6] and multiple sclerosis (MS)[7] has also been indicated.

Studies of healthy adult gut microbiota have shown that it is composed primarily of members of a few bacterial phyla, with the Bacteroidetes and Firmicutes being most numerous.[8] The Bacteroidetes generally encode more carbohydrate-active enzymes (CAZymes) than other phyla, such as Firmicutes, indicating their enhanced capacity to use a wide range of polysaccharide substrates.[9] The members of the genus *Bacteroides* are adept at using plant- and host-derived polysaccharides.[10] These *Bacteroides* are rich in CAZymes involved in the acquisition and metabolism of various glycosides, including glycoside hydrolases and polysaccharide lyases, which are organized into polysaccharide utilization loci (PULs) that are distributed throughout the genome.[10]

Different bacteria have preferential growth on different substrates. It was observed that different *Bacteroides* species preferred different substrates depending upon the structural and chemical complexity of the polysaccharide substrate.[11] This indicates that just as dietary polysaccharides have the potential to alter the growth of specific bacteria, these polysaccharides can be used for selective targeting of specific bacteria. Xylan is a polysaccharide that is found in many plant materials. Xylans consists of a backbone of β-(1 → 4)-linked xylopyranose units onto which 4-*O*-methylglucuronic acid (MeGlcA) groups, *O*-acetyl groups, or other sugars, such as arabinose, can be substituted at C(O)-2 and/or C(O)-3 positions.[12] Ferulic acid can be found attached to the C(O)-5 position of these arabinose.[13] Arabinoxylans are xylans with arabinose substitution at C-2 and/or C-3 positions of the xylan backbone. Arabinoxylans are prevalent in many cereal grains: wheat (5.5−7.2%), barley (3.9−5.4%), maize (1−2%), and rice (2−3%).[14] Arabinoxylans can be hydrolyzed into xylooligosaccharides (XOS) and arabinoxylooligosaccharides (AXOS).

The genus *Bacteroides* contains the most expanded glycolytic gene repertoires that target xylan degradation.[15] Previous research has shown closely related *Bacteroides* species to use xylan in different ways.[11] Rose et al.[16] indicated differences among the fermentation profiles for AX from different cereals (maize, rice, and wheat), which consists of different structural features. Xu[17] concluded that specific molecular regions of dietary fibers differentiate gut bacteria. Dependent upon the structural complexity of XOS and AXOS, different *Bacteroides* species could respond to these substrates differently. Understanding how these structurally different oligosaccharides influence the growth of commensal bowel bacteria could lead to manipulation of the gut microbiome through introduction of XOS and AXOS to correct dysbiosis. Thus, the objective of this

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Table 1. Carbohydrate Substrates and Their Structural and Physical Properties

sample name	name	structure	DP[a]	DS[b]	molecular weight
Glu	glucose	G	1	0	180.2
Xyl	xylose	X	1	0	150.1
Ara	arabinose	A	1	0	150.1
X2	1,4-β-D-xylobiose	X−X	2	0	282.2
X3	1,4-β-D-xylotriose	X−X−X	3	0	414.4
X4	1,4-β-D-xylotetraose	X−X−X−X	4	0	546.5
X5	1,4-β-D-xylopentaose	X−X−X−X−X	5	0	678.6
X6	1,4-β-D-xylohexaose	X−X−X−X−X−X	6	0	810.7
A2	1,5-α-L-arabinobiose	A−A	2	0	282.2
A3	3^2-α-L-arabinofuranosyl-xylobiose	X^{A3}−X	2	1	414.4
A4	2^3-α-L-arabinofuranosyl-xylotriose	X^{A2}−X−X	3	1	546.2
A5	3^3-α-L-arabinofuranosyl-xylotetraose	X−X^{A3}−X−X	4	1	678.6
A6	2^3,3^3-di-α-L-arabinofuranosyl-xylotriose	$X^{A2,A3}$−X−X	3	2	678.6
A7	2^3-α-L-plus-3^3-α-L-arabinofuranosyl-xylotriose	X^{A2}−X−X and X^{A3}−X−X	3	1	546.2
A8	2^3,3^3-di-α-L-arabinofuranosyl-xylotetraose	X−$X^{A2,A3}$−X−X	4	2	810.7
A9	2^3-α-L-plus-3^3-α-L-arabinofuranosyl-xylotetraose	X−X^{A2}−X−X and X−X^{A3}−X−X	4	1	678.6
AX L	low-molecular-weight AX	−[X−X^{A3}−X−$X^{A2,A3}$−X]$_n$−			56.7 *k*DA
AX M	medium-molecular-weight AX	−[X−X^{A3}−X−$X^{A2,A3}$−X]$_n$−			323 *k*DA
AX H	high-molecular-weight AX	−[X−X^{A3}−X−$X^{A2,A3}$−X]$_n$−			370 *k*DA

[a]DP = degree of polymerization. [b]DS = degree of substitution.

research was to understand how the structural differences of XOS and AXOS impacted the growth of *Bacteroides* species commonly found in the intestine.

2. MATERIALS AND METHODS

2.1. Materials. The bacterial fermentation experiments were carried out using six publicly available *Bacteroides* strains belonging to five different species. The strains were *Bacteroides cellulosilyticus* DSM 14838, *Bacteroides ovatus* ATCC 8483, *Bacteroides ovatus* 3-1-23, *Bacteroides eggerthii* DSM 20697, *Bacteroides intestinalis* DSM 17393, *Bacteroides xylanisolvens* XB1A, and *Bacteroides thetaiotaomicron* ATCC 29148 (VPI 5482). All strains were originally isolated from human colon or fecal samples, representative of the most commonly encountered xylanolytic organisms in the gut.

All of the carbohydrate substrates were purchased from Megazyme. These included five XOS samples, 1,4-β-D-xylobiose (X2), 1,4-β-D-xylotriose (X3), 1,4-β-D-xylotetraose (X4), 1,4-β-D-xylopentaose (X5), 1,4-β-D-xylohexaose (X6), and seven AXOS samples, 1,5-α-L-arabinobiose (A2), 3^2-α-L-arabinofuranosyl-xylobiose (A3), 2^3-α-L-arabinofuranosyl-xylotriose (A4), 3^3-α-L-arabinofuranosyl-xylotetraose (A5), 2^3,3^3-di-α-L-arabinofuranosyl-xylotriose (A6), 2^3-α-L-plus-3^3-α-L-arabinofuranosyl-xylotriose (A7), 2^3,3^3-di-α-L-arabinofuranosyl-xylotetraose (A8), 2^3-α-L-plus-3^3-α-L-arabinofuranosyl-xylotetraose (A9), three arabinoxylan polysaccharides, low-molecular-weight AX (AX L), medium-molecular-weight AX (AX M), and high-molecular-weight AX (AX H), and monosaccharides, xylose (Xyl), arabinose (Ara), and glucose (Glu) (Table 1).

2.2. Pure Strain Growth Experiments. The bacterial growth experiments were carried out according to the methods described by Martens et al.[18] Each carbohydrate substrate was prepared in stock solutions (10 mg/mL) using Millipore water. Each substrate solution was autoclaved at 121 °C for 20 min. Substrate solution (30 μL) was pipetted into one of the designated wells on a 24 × 16 plate, assigning three wells for each substrate per bacterial strain. Each bacterial strain inoculum was added to each well (30 μL), diluting the final substrate concentration to 5 mg/mL.

The bacterial inocula were prepared as follows: each bacterium was inoculated into custom-chopped meat media using sterile wood sticks from glycerol stocks from the freezer. These cultures for assay inoculations were grown for 24 h at 37 °C under an anaerobic atmosphere of 10% H_2, 5% CO_2, and 85% N_2 using an anaerobic chamber (Coy Manufacturing, Grass Lake, MI, U.S.A.).

Bacteroides minimal media was prepared using minimal media[19] supplemented with additional compounds.[20] In detail, *Bacteroides* minimal media was prepared by adding together 10 mL of *Bacteroides* salt solution [KH_2PO_4 (544 g), NaCl (35 g), and $(NH_4)_2SO_4$ (45 g) dissolved in 1 L], 100 μL each of vitamin K_3 (1 mg/mL in ethanol), $FeSO_4$ (0.4 mg/mL in 10 mM HCl), $MgCl_2$ (0.1 M in water), $CaCl_2$ [0.8% (w/v) in water], and histidine/hematin solution (1.9 mM hematin in 0.2 M histidine solution), 50 μL of vitamin B_{12} stock (0.01 mg/mL in water), 100 mg of L-cysteine, and 1 mL each of Balch's vitamin mixture, trace mineral solution, purine and pyrimidine solution, and amino acid solution,[20] bringing the volume to 50 mL with distilled water (pH 7.2), and filter-sterilizing the media.

The culture (1 mL) was drawn into a 1.5 mL centrifuge tube and centrifuged at 10× 1000*g* for 1 min. The supernatant was discarded; *Bacteroides* minimal medium (1 mL) was added; and the bacterial pellet was resuspended in it. This was again centrifuged (10× 1000*g* for 1 min) to wash the bacteria. This step was repeated another time. After the second washing was discarded, the bacterial pellet was resuspended in 1 mL of *Bacteroides* minimal medium. This was the washed culture. The washed culture was pipetted into *Bacteroides* minimal medium to prepare the "diluted bacterial culture" [1:50 ratio; optical density (OD) < 0.2]. Diluted bacterial culture was inoculated into the previously prepared microplates containing substrate solution in each well inside the anaerobic chamber.

After inoculation, the plates were allowed to equilibrate with the anaerobic conditions for about 30 min. Assay plates were sealed under the atmosphere noted above with an optically clear gas-permeable polyurethane membrane (Diversified Biotech, Boston, MA, U.S.A.). The plates were then loaded into a BioStack automated plate handling device coupled to a PowerWave HT absorbance reader (both devices from BioTek Instruments, Winooski, VT, U.S.A.). Absorbance at 600 nm (A_{600}) was measured for each well at 10−15 min intervals. Data were processed using Gen5 software (BioTek) and Microsoft Excel.

Several glycans yielded complicated polyphasic growth profiles rather than a single exponential growth phase. Thus, we quantified growth in each assay by first identifying a minimum time point (A_{min}) at which A_{600} had increased by 10% over a baseline reading taken during the first 500 min of incubation. Next, we identified the time point at which A_{600} reached its maximum (A_{max}) immediately after exponential growth. The total growth parameter was generated for each well ($A_{max} - A_{min}$), resulting in average growth. Cultures that failed to increase density by at least 0.1 (A_{600}) were scored as no

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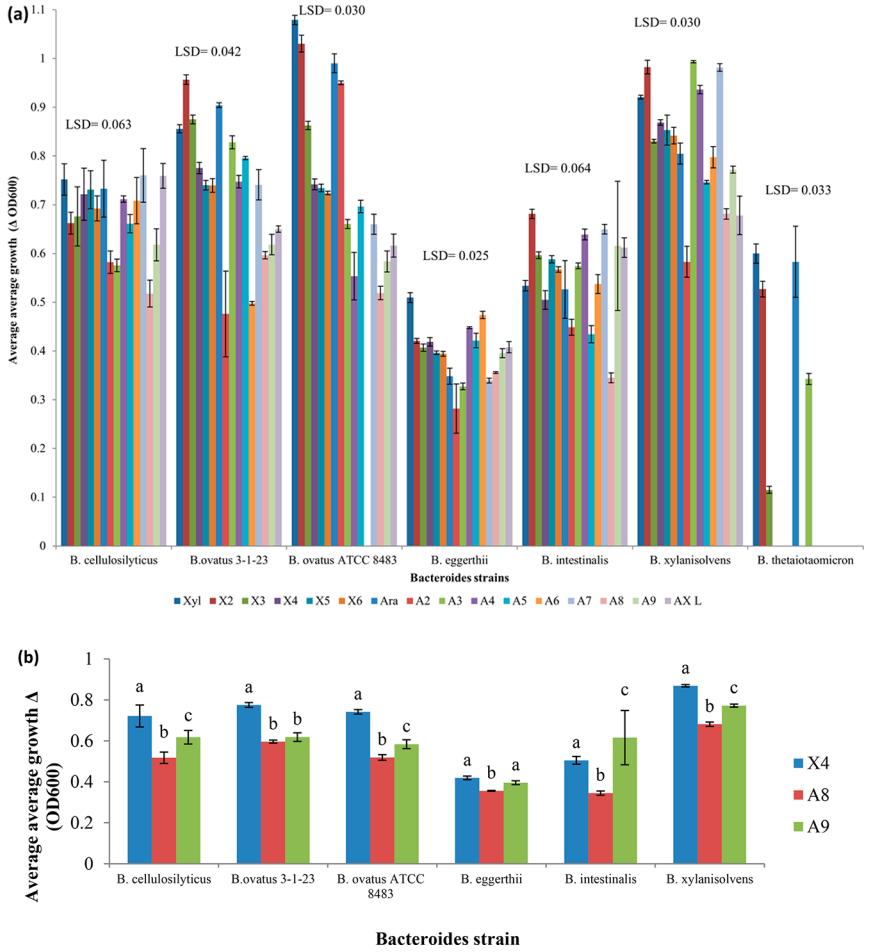

Figure 1. Growth of *Bacteroides* strains on different substrates. (a) Average average growth of each *Bacteroides* strain on 15 different carbon sources. Values are averages of three replicates. Least significant difference (LSD; $p < 0.05$) for each bacterial strain is presented above the average average growth values of the corresponding strain. (b) Average average growth of different *Bacteroides* strains on three different oligosaccharides. Letters are used to compare differences among growth within each strain. Columns with the same letters are not significantly different ($p < 0.05$) from each other within a given strain.

growth. Values were calculated as averages of three replicates to generate average average growth (ΔOD_{600}).

2.3. Quantification of Xylan PULs *susC* Transcript Expression in *B. ovatus* ATCC 8483. Quantification of transcript expression was performed by quantitative polymerase chain reaction (qPCR). Each carbohydrate substrate (Ara, Xyl, A4, A5, A6, and AX) was dissolved in sterile water to prepare stock solutions of 10 mg/mL. Arabinose and xylose (A + X) solutions were mixed in a 2:3 ratio (v/v) to mimic the monosaccharide composition of A6.

The bacterial inoculum was prepared as follows: bacteria were inoculated into custom-chopped meat media using sterile wood sticks from glycerol stocks from the freezer. This culture was grown overnight at 37 °C under an anaerobic atmosphere of 10% H_2, 5% CO_2, and 85% N_2 using an anaerobic chamber (Coy Manufacturing, Grass Lake, MI, U.S.A.). The culture (10 μL) was diluted into custom-chopped meat media (10 mL) and allowed to grow overnight under anaerobic conditions. The culture was washed, spun-down, and resuspended in *Bacteroides* minimal medium to achieve an OD of ~1.6. Each substrate was added (100 μL) to wells in the 12 × 8 well plate (100 μL), assigning two wells for each replicate and achieving three replicates for each substrate. The bacterial culture (100 μL) was added to each well and incubated under anaerobic conditions. The bacterial cultures were harvested at 1.5 h (at mid-log phase). All harvested cells were treated with RNAprotect bacterial reagent (Qiagen) and stored at −80 °C until processed. Total RNA was extracted from cells using a RNeasy kit (Qiagen), treated with Turbo DNase I (Ambion), and reverse-transcribed using Superscript III (Invitrogen). The 16S rRNAgene-normalized PUL gene abundance was assayed on an Eppendorf Realplex thermocycler and using SYBRgreen 480 I Master (Roche). Fold change in gene expression was calculated versus the A + X mixture. Values were calculated as an average of three replicates.

3. RESULTS AND DISCUSSION

3.1. Differential Growth of *Bacteroides* Species on Different Xylan Substrates. High-purity oligosaccharides with different structural complexity with respect to the degree of polymerization (DP), degree of substitution (DS), molecular weight, and substitution position were used in this study. Each of the oligosaccharides contained xylose, arabinose, or both xylose and arabinose.

Overall, *B. xylanisolvens* had the highest growth on all XOS and AXOS substrates, while *B. thetaiotaomicron* had the lowest growth (Figure 1a). In all of the species, except *B. thetaiotaomicron*, which did not grow on larger oligosaccharides, the unsubstituted oligosaccharide was the preferred substrate compared to its mono- and disubstituted counterparts with respect to average growth and specific growth rate, as evident by comparison of substrates X4, A8, and A9 (Figure 1b). When the growth among the oligosaccharides was considered, *B. eggerthii* displayed a high average growth on A6 and A4, surpassing growth on simpler oligosaccharides, such as X3, X4, X5, and X6 (Figure 1a). *B. ovatus* 3-1-23, *B. intestinalis*, and *B.*

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xylanisolvens showed preferential growth on xylobiose, which is a disaccharide, over the monosaccharide xylose (Figure 1a), while all of the other strains preferred xylose above xylobiose.

To emphasize the differential growth of each bacterium on carbohydrate substrates with different complexity, we constructed a heat map using normalized growth values (Figure 2).

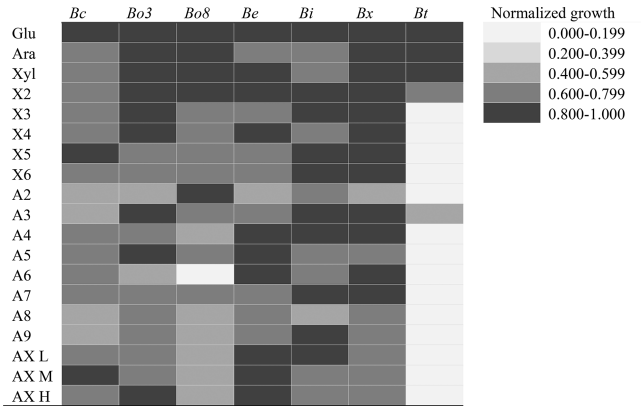

Figure 2. Heat map showing normalized growth values of seven *Bacteroides* strains: *B. cellulosilyticus* DSM 14838 (*Bc*), *B. ovatus* ATCC 8483 (*Bo8*), *B. ovatus* 3-1-23 (*Bo3*), *B. eggerthii* DSM 20697 (*Be*), *B. intestinalis* DSM 17393 (*Bi*), *B. xylanisolvens* XB1A (*Bx*), and *B. thetaiotaomicron* ATCC 29148 (VPI 5482) (*Bt*) on carbohydrate substrates: 1,4-β-D-xylobiose (X2), 1,4-β-D-xylotriose (X3), 1,4-β-D-xylotetraose (X4), 1,4-β-D-xylopentaose (X5), 1,4-β-D-xylohexaose (X6), 1,5-α-L-arabinobiose (A2), 3^2-α-L-arabinofuranosyl-xylobiose (A3), 2^3-α-L-arabinofuranosyl-xylotriose (A4), 3^3-α-L-arabinofuranosyl-xylotetraose (A5), 2^3,3^3-di-α-L-arabinofuranosyl-xylotriose (A6), 2^3-α-L-plus-3^3-α-L-arabinofuranosyl-xylotriose (A7), 2^3,3^3-di-α-L-arabinofuranosyl-xylotetraose (A8), 2^3-α-L-plus-3^3-α-L-arabinofuranosyl-xylotetraose (A9), low-molecular-weight AX (AX L), medium-molecular-weight AX (AX M), high-molecular-weight AX (AX H), xylose (Xyl), arabinose (Ara), and glucose (Glu). The normalized values were generated using average growth measurement of three replicates.

Although the bacteria in this study belonged to the same genus, they did not share similar substrate specificities. More so, the two bacterial strains that belonged to the same species, *B. ovatus*, showed different growth patterns in the heat map, indicating that the two strains used carbohydrates with specific structural details in a different manner. Of all of the strains in the study, *B. ovatus* 8483 was of specific interest as a result of its inability to grow on A6. Thus, we further investigated how A6 impacts the expression of three genes involved in carbohydrate utilization in *B. ovatus* 8483.

B. cellulosilyticus grew on all of the monosaccharide and oligosaccharide substrates (Figure 1a). When xylobiose (X2) has an arabinose substitution at the O-3 position in the terminal xylose (A3), the bacterial growth on this substituted substrate was reduced. These indicate that the bacteria preferred unsubstituted disaccharides versus substituted disaccharides. However, even if the oligosaccharide contained arabinose substitution, if the oligosaccharide also contained portions of unsubstituted xylose backbone of at least two unsubstituted xylose units at the terminal end, as in the case of A4, A5, A6, and A7, the bacteria showed a growth similar to that on unsubstituted substrates, such as X4 and X5. Thus, the xylan utilization system (Xus) protein cluster that recognizes and transports xylan through the bacterial outer membrane[21] of *B. cellulosilyticus* might have an endo acting enzyme on the Xus cluster that might be able to act on the unsubstituted regions of the substituted oligosaccharides and cleave them to shorter oligosaccharides that could be metabolized with equal efficiency to unsubstituted oligosaccharides.

However, when we compare the growth of *B. cellulosilyticus* on X4 (xylotetraose), A5 (monosubstituted xylotetraose), A8 (disubstituted xylotetraose), and A9 (mixture of monosubstituted xyloteraose), it is clear that the bacteria prefer unsubstituted XOS, followed by monosubstituted AXOS and disubstituted AXOS.

Two strains of the species *B. ovatus* were studied. Overall, the two strains shared similar average growth profiles (Figure 1a). *B. ovatus* 3-1-23 had highest growth on X2, over simpler xylose. Thus, this strain might be more adept at using the disaccharide xylobiose versus the monosaccharide xylose. Also, *B. ovatus* ATCC 8483 displayed preferential growth on arabinobiose compared to other strains. Thus, arabinobiose might be a potential substrate to be used in targeted enhancement of *B. ovatus* ATCC 8483 in the gut of individuals who have this strain in their guts. Among the AXOS substrates, *B. ovatus* 3-1-23 had relatively low growth on A6, which was a disubstituted xylotriose, while *B. ovatus* ATCC 8483, which belongs to the same species, did not grow on A6. To further explore this inability of *B. ovatus* ATCC 8483 to grow on A6, gene expression experiments were carried out. The results of these are discussed further in section 3.2.

B. eggerthii had the highest growth on xylose but did not show the same preferential growth on the monosaccharide arabinose, indicating that the bacteria use the two monosaccharides xylose and arabinose differently (Figure 1a). While no other strain used in this research had a preferential growth on A6 over other substrates within each strain, *B. eggerthii* preferred A6 as a substrate to other oligosaccharides.

B. intestinalis grew better on the xylobiose versus xylose. A7 was a mixture of 2^3-α-L-arabinofuranosyl-xylotriose and 3^3-α-L-arabinofuranosyl-xylotriose, while A4 was 2^3-α-L-arabinofuranosyl-xylotriose. The bacteria grew similarly on both A7 and A4. However, if the bacteria had a preference for O-2 substitution versus O-3 substitution, it would not have indicated similar growth on both A4 and A7. Thus, *B. intestinalis* is capable of using both O-2-substituted xylotriose and O-3-substituted xylotriose in a similar fashion.

When the growth of *B. xylanisolvens* on the three substituted xylotrioses (A4, A6, and A7) was considered, the bacteria showed a preferential growth on A4 (O-2 monosubstituted xylotriose) but the growth was hindered on A6 (O-2 and O-3 disubstituted xylotriose), indicating that the presence of disubstitution adversely affects the bacterial growth. Also, the preferential growth of the bacteria on A7 (O-2 monosubstituted xylotriose and O-3 monosubstituted xylotriose) over its growth on A4 indicates that *B. xylanisolvens* is capable of using both O-2-substituted xylotriose and O-3-substituted xylotriose effectively.

We also studied the growth of *B. thetaiotaomicron* on XOS and AXOS. Of all of the strains studied, it had the lowest overall growth on the substrates. It had a preferential growth for monosaccharides xylose and arabinose. It was able to growth on xylotriose (X3). However, beyond X3, *B. thetaiotaomicron* could not use XOS with a higher DP. When the arabinose-substituted substrates were considered, *B. thetaiotaomicron* could only use monosubstituted xylobiose (A3). Martens et al.[18] indicated that *B. thetaiotaomicron* is capable of using monosaccharides, such as A + X. Our results further suggest that *B. thetaiotaomicron* can use XOS up to DP of 3 and monosubstituted xylobiose, which

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suggests that not all arabinose substitution is a limitation to its growth. However, the overall inability of *B. thetaiotaomicron* to grow on complex oligosaccharides suggests that it only has limited enzyme systems capable of using simpler oligosaccharides.

3.2. Differential Expression of Xylan PULs in *B. ovatus* 8483 on Different Xylan Substrates. While each bacterial strain displayed differential growth preferences on different substrates, we expected the two strains, *B. ovatus* 3-1-23 and *B. ovatus* ATCC 8483, that belonged to the same species to have similar growth preferences. However, there was a drastic difference between the two strains in their ability to grow on A6. *B. ovatus* 3-1-23 was able to grow on A6, while *B. ovatus* 8483 was unable to grow on the same substrate (Figure 1a). Thus, we hypothesized that A6 is structurally unable to activate the PULs in *B. ovatus* 8483.

B. ovatus 8483 contains two PULs, spanning locus tags *bacova_03417-50* (large xylan PUL or PUL-xylL) and *bacova_04385-94* (small xylan PUL or PUL-xylS), that are activated when the organism is grown on wheat arabinoxylan.[22] A schematic representation of genes in PUL-xylL and PUL-xylS along with the locus tag (*bacova_XXXXX*) of each gene is described by Rogowski et al.[22] PUL-xylL and PUL-xylS contain genes that encode SusC-like proteins that are responsible for import of complex glycans across the outer membrane. PUL-xylL contains two genes that encode two SusC-like proteins (locus tags *bacova_3426* and *bacova_3428*). PUL-xylS also contains a gene that encodes a SusC-like protein (locus tag *bacova_4393*). We investigated the expression of these three *susC* transcripts as a representation of the expression of PUL-xylL and PUL-xylS in *B. ovatus* 8483 on four different substrates (A4, A5, A6, and AX) to evaluate if A6 is capable of activating these PULs. Fold change in gene expression in each substrate was calculated versus expression in media containing A + X (Figure 3). The four substrates were selected to represent XOS with monosubstitution of arabinose at the O-2 position (A4), monosubstitution of arabinose at the O-3 position (A5), disubstitution of arabinose at O-2 and O-3 positions (A6), and arabinoxylan polysaccharide containing O-2 and O-3 monosubstitution and O-2 and O-3 disubstitution of arabinose (AX). The gene expression pattern was similar for the two SusC-like genes in the large xylan PUL along the four substrates. When the gene that encodes the SusC-like protein (locus tag *bacova_3426*) was upregulated, the second SusC-like protein (locus tag *bacova_3428*) in the same large xylan PUL was upregulated as well. This indicates that the two SusC-like genes in PUL-xylL are not independently regulated from each other but rather have a combined relationship. A4 suppressed the expression of *bacova_3426* and *bacova_3428* by about 2-fold compared to A + X, while all of the other substrates caused an increase in SusC-like gene expression in large xylan PUL (Figure 3). When the growth profile of *B. ovatus* 8483 on A4 is considered, the bacteria has a low growth rate on A4 compared to A5. A5 also has monosubstituted arabinose attached to it. However, the substitution is via an O-3 linkage rather than an O-2 linkage. Thus, we speculate that the presence of oligosaccharides with O-2 substitution hinders the expression of genes in PUL-xylL in *B. ovatus* 8483. However, the bacteria do grow on the A4 substrate, although at a lower growth rate. When the expression of SusC-like protein-encoding genes in the small xylan PUL is considered, it has the reverse expression pattern for A4 than in large xylan PUL. In the presence of A4, the expression of SusC-like genes in PUL-xylL was down-

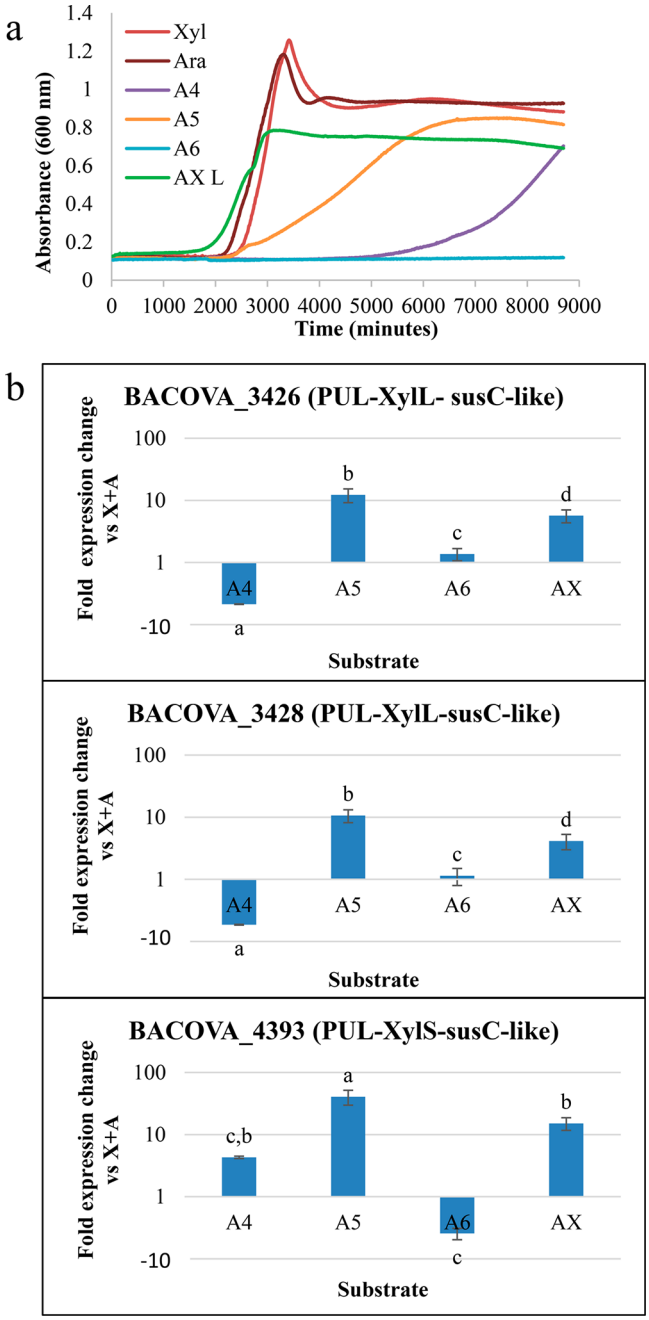

Figure 3. Growth profile and gene expression of *B. ovatus* 8483 on four different substrates. Bacteria were cultured in minimal media containing xylans as the sole carbon source. (a) Growth profile on xylose, arabinose, A4, A5, A6, and arabinoxylan (AX). (b) Differential expression of xylan PULs in *B. ovatus* 8483 on different xylan substrates. Cells were grown on different substrates, and the level of each *susC* transcript (locus tags shown) was analyzed by qPCR. The *y* axis shows the log fold change relative to a xylose/arabinose [3:2 (v/v) to mimic constitution of A6] reference. Columns with the same letters are not significantly different ($p < 0.05$) from each other.

regulated by about 2-fold, while the expression of SusC-like genes in PUL-xylS was upregulated by about 4-fold. Thus, although the presence of O-2 substitution on the xylan backbone is not identified by the large xylan PUL, the small xylan PUL could become activated by the oligosaccharides with O-2 linkage and can become used through the small xylan PUL proteins and enzymes. Furthermore, PUL-xylS could contain

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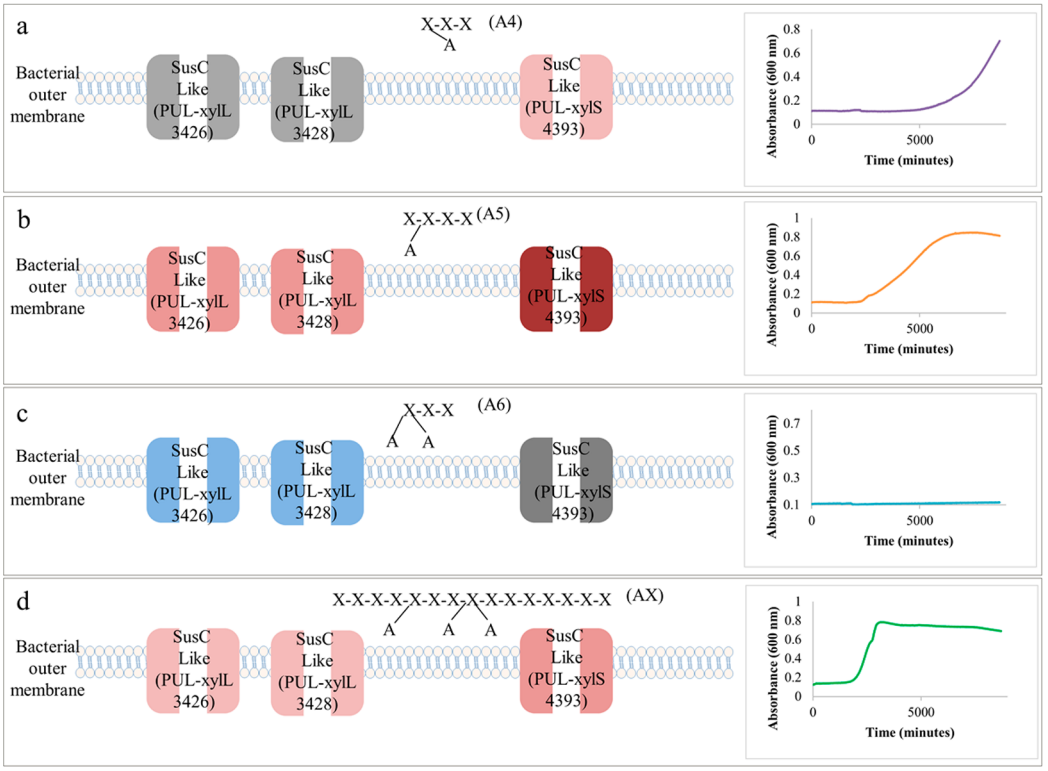

Figure 4. Schematic models for expression of SusC-like proteins in B. ovatus 8483 upon growth on AXOS with different substitution patterns and AX and corresponding growth profiles: (a) growth on A4, (b) growth on A5, (c) growth on A6, and (d) growth on AX. The expression of each SusC-like transcript is assumed to translate into SusC-like protein expression. The colors on each SusC-like protein is used to indicate expression or suppression of the product. Shades of red are used to indicate fold increase in expression, with darker shades correlating to higher expression. Shades of gray are used to indicate fold decrease in expression. Blue is used to indicate no significant change in expression.

genes that encode debranching enzymes capable of acting on α-1−2 linkages in the AXOS. Therefore, the growth of *B. ovatus* 8483 on A4 could be dependent upon PUL-xylS alone. This hypothesis could be tested by employing mutant *B. ovatus* 8482 strains that lack PUL-xylL in future experiments.

We further included A5, AXOS with O-3 susbtitution, to investigate the effect of O-3 susbtitution on the gene expression in the two xylan PULs. In the presence of A5, there was about a 10-fold increase in gene expression in the two SusC-like genes in PUL-xylL and about a 40-fold increase in gene expression in the SusC-like gene in PUL-xylS. This indicates that both large and small xylan PULs are responsive to XOS with α-1−3-linked arabinose, with PUL-xylS being triggered more robustly. Previous research by Martens et al.[18] has shown that xylotetraose is a ligand that upregulates *bacova_4393* that encodes a SusC-like protein in PUL-xylS. They also indicated that xylotetraose is not an activating ligand for the two SusC-like genes (*bacova_3426* and *bacova_3428*) in PUL-xylL. Our results further indicate that xylotetraose containing α-1−3-linked arabinose can also upregulate SusC-like genes in the small PUL. We also observed that, despite the inability of xylotetraose to activate the SusC-like genes in the large PUL, xylotetraose with α-1−3-linked arabinose (A5) could upregulate SusC-like genes in the large PUL, although to a lesser extent than the SusC-like gene in the small PUL.

Of all of the substrates studied, A6 that was a disubstituted xylotriose was the most interesting because *B. ovatus* 8483 failed to grow on it. When gene expression of SusC-like genes in PUL-xylL was been considered, there was no increase in the gene expression with respect to A6. This suggests that the regulators in association with both large and small xylan PUL in *B. ovatus* 8483 might be incapable of obtaining an activation cue from this oligosaccharide, either because they cannot bind to it directly or the enzyme and transport systems required for moving it into the periplasm or uncovering an underlying activating oligosaccharide are missing. This suggests that the inability of the bacteria to grow on A6 could be attributed to its disubstitution being at the non-reducing end, as opposed to its ability to grow on A8, which contains disubstituted xylose at an endo position.

In the presence of AX polysaccharide, genes in both large and small xylan PUL were activated and increased in expression. In addition, the fold increase in the PUL-xylS gene was larger compared to PUL-xylL genes. As previously observed, full-length wheat AX triggered robust expression of both PULs in *B. ovatus* 8483.[22] A schematic representation of the possible PUL activation and expression in *B. ovatus* 8483 on three different AXOS and AX is suggested in Figure 4.

We were able to show how the specific structural details of related oligosaccharide substrates potentially dictate the differential growth of *Bacteroides* species in the intestine. This could ensure maximum utilization of the diverse array of carbohydrates that could be present in a diet. It also implies the presence of cross-feeding mechanisms, where one species could hydrolyze a substrate to yield a product that could be used by another species, as suggested by Cockburn and Koropatkin.[23]

The preferential growth of each bacteria on specific substrates has the potential application of being used as a possible strategy to selectively manipulate the microbiota to selectively promote/hinder growth of specific bacterial strains

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in the gut. We identified A6 as a potential substrate to promote the growth of other strains while hindering the *B. ovatus* 8483 growth. Also, A7 could be a potential substrate to selectively promote the growth of *B. cellulosilyticus*, *B. intestinalis*, and *B. xylanisolvens* versus other bacterial strains used in the study.

Using three different *susC* transcripts as a proxy for expression of whole PUL, the up- and downregulation of PUL-xylL and PUL-xylS in the presence of different xylans were studied. 2^3-α-L-Arabinofuranosyl-xylotriose (A4) could be used as a substrate to upregulate PUL-xylS expression, while 2^3,3^3-di-α-L-arabinofuranosyl-xylotriose (A6) lead to downregulation of PUL-xylS expression. Thus, these specific linkages in AXOS could be used to control the gene expression and growth rates of these bacteria to achieve desired growths.

To our knowledge, there is no research conducted on the growth of these *Bacteroides* species on the array of high-purity oligosaccharides used in this study. The application of high-purity oligosaccharides with defined structures in this study provided specific information on how fine structural details, such as DP and DS, in oligosaccharides effect these bacteria.

The information derived about the potential structure–function relationship could be vital in manipulation of the bacterial population in the intestine through customized diets or prebiotic oligosaccharide mixtures to promote growth of specific bacterial strains to maintain health.

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