Utilization of xylan-type polysaccharides in co-culture fermentations of *Bifidobacterium* and *Bacteroides* species

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ARTICLE INFO

ABSTRACT

Although most members of the genus *Bifidobacterium* are unable to utilize xylan as a carbon source, the growth of these species can be induced by this polysaccharide in the gut environment. This indicates a requirement for an association between *Bifidobacterium* species and some other members of gut microbiota. In this study, the role of cross-feeding between *Bifidobacterium* and *Bacteroides* species in the bifidogenic effect of xylan was investigated using *in-vitro* pure and co-culture fermentations. The pure culture studies showed that among the *Bifidobacterium* species tested, only *Bifidobacterium animalis* subsp. *lactis* was able to utilize xylooligosaccharides. The co-culture of this strain with *Bacteroides* species enabled it to grow in the presence of xylan. These results suggest that the ability of *Bacteroides* species to hydrolyze xylan could allow the proliferation of specific *Bifidobacterium* species in the gut through substrate cross-feeding.

1. Introduction

Dietary polysaccharides that are resistant to digestion and absorption in the human gut are totally or partially fermented in the large intestine (Knudsen, 2001). Polysaccharides are structurally complex based on their monosaccharide composition, degree of polymerization, glycosidic linkages, substitutions, and branching (Hamaker & Tuncil, 2014). The structural and functional properties of the dietary fibers and other polysaccharides vary depending on the source. Even a single fiber type (e.g. xylans, pectin, and fructans) is a heterogeneous group of several different polysaccharides. Some bacterial species are specialized by developing specific mechanisms composed of various enzymes, sensor proteins, carbohydrate-binding proteins and transporters that are specific to each non-digestible dietary polysaccharide (Martens, Kelly, Tauzin, & Brumer, 2014). *Bacteroides* species are significant members of the colon system due to their ability to digest a variety of complex polysaccharides in the colon (Hansen et al., 2013; Rogowski et al., 2015). Some species, such as *Bacteroides ovatus, Bacteroides thetaiotaomicron,* and *Bacteroides cellulosilyticus* are able to utilize several polysaccharides; however, there is no single species that can degrade all types of dietary polysaccharides (Martens et al., 2014).

Xylan is one of the most abundant natural polysaccharides. As a hydrocolloid, it can potentially be used as a food additive since it can act as a texturing and stabilizing agent (Rosa-Sibakov et al., 2016). It was shown that birch pulp xylan added to milk gels improved water holding capacity and reduced syneresis (Sedlmeyer, 2011). In bakery products, xylan influences the rheological behaviour of dough and texture (Izydorczyk & Biliaderis, 1992). Due to their film-forming capacity xylans can be used in the preparation of edible food packing materials and capsules for pharmaceutical applications (Hansen & Plackett, 2008). The xylooligosaccharides (XOS) and the arabinoxylooligosaccharides (AXOS), which are produced by partial hydrolysis of xylans using chemical, hydrothermal or enzymatic processes, have been shown to exert prebiotic functions in *in-vivo* studies (Rivière, Selak, Lantin, Leroy, & De Vuyst, 2016).

Prebiotics are defined as "a substrate that is selectively utilized by host microorganisms conferring a health benefit" (Gibson et al., 2017). The health-promoting effects of prebiotic substrates are generally ascribed to induction of the growth of several beneficial bacterial species and the production of short-chain fatty acids (SCFA) by microbial fermentation in the colon. Some *Bifidobacterium* species, which are frequently considered to be the target of the prebiotic action, are able to utilize xylan based oligosaccharides. *Bifidobacterium* species are also specialized with regard to the available oligosaccharide. Some act on the XOS and AXOS cooperatively, such that the arabinose and xylose derived from the oligosaccharides and the complete XOS and AXOS are

assimilated by different *Bifidobacterium* species (resource partitioning) (Pastell, Westermann, Meyer, Tuomainen, & Tenkanen, 2009; Rivière et al., 2014; Rivière, Selak, Geirnaert, Van den Abbeele, & De Vuyst, 2018). This allows the proliferation of several species simultaneously. Xylan in its polymeric form is also considered as an emerging prebiotic carbohydrate. Xylan and arabinoxylan (AX) proliferated bifidobacteria in *in-vitro* gut models conducted using human stool samples (Hughes et al., 2007). *In-vivo* studies with rats, mice, and pigs showed that AX as a diet constituent resulted in an increase in the number of *Bifidobacterium* counts (Neyrinck et al., 2011; Nielsen et al., 2014; Van den Abbeele et al., 2011). It is known that xylan cannot be utilized as a carbon source by bifidobacteria except for some rare species (Crittenden et al., 2002). The previous studies clearly indicated that the Bacteroidetes represent a critical xylanolytic group within the human colonic microbiota (Chassard, Goumy, Leclerc, Del'homme, & Bernalier-Donadille, 2007; Dodd, Mackie, & Cann, 2011). Thus, the bifidogenic activity of xylans may depend on the xylan degradation by *Bacteroides* species. The oligosaccharides released as a result of xylan hydrolysis by *Bacteroides* species may be available for *Bifidobacterium* species that possess the necessary metabolic systems for the utilization of those oligosaccharides (Fig. 1).

Rogowski et al. (2015) characterized the xylan degradation mechanism of *Bact. ovatus* and showed the fine-tuning of the degradation system based on the forms of xylan obtained from different plant sources. In *in-vitro* co-culture of *Bact. ovatus* and *Bifidobacterium adolescentis,* the degradation of simpler xylans (birch glucuronoxylan and wheat arabinoxylan), but not the more complex xylan (corn bran glucuronoarabinoxylan), by the former could support the growth of the latter (Rogowski et al., 2015).

Previous studies indicated clearly that the utilization of dietary fibers and other complex polysaccharides depends on the structural properties of the carbohydrates and the ability of the bacteria to act on them (Flint, Scott, Duncan, Louis, & Forano, 2012). This gives rise to the necessity of researching at the species or strain level for each type of dietary polysaccharides. In order to better understand the potential prebiotic action, specifically the bifidogenic activity of the xylans, the different forms of this polysaccharide should be tested on the target *Bifidobacterium* species and their cooperation with the xylan degrading *Bacteroides* species.

Elucidation of the degradation mechanism of each carbohydrate and the specific interactions of microorganisms can help to develop targeted prebiotic, probiotic and synbiotic preparations. In this study, five *Bifidobacterium* species were co-cultured with *Bact. ovatus* and *Bact. xylanisolvens* in an effort to show the cooperation of those two genera for the utilization of xylan.

2. Material and methods

2.1. Microorganisms

Bifidobacterium animalis subsp. *lactis* DSM-10140, *Bacteroides ovatus* DSM-1896 and *Bacteroides xylanisolvens* DSM-18836 were purchased from Leibniz Institute DSMZ- German Collection of Microorganisms and

Cell Cultures. *Bifidobacterium bifidum* NRLL B-41410, *Bifidobacterium breve* NRLL B-41408*, Bifidobacterium infantis* NRLL B-41661, *Bifidobacterium longum* NRLL B-41409 were provided by Agricultural Research Service Culture Collection (NRLL) (Peoria, IL, USA).

2.2. Substrates

Beechwood xylan (Megazyme, Bray, Ireland) was composed of (w/w) xylose (82.3 %), glucuronic acid (12.8 %), and other sugars (4.9 %). Xylooligosaccharide (XOS) was a kind gift of Shandong Longlive (Yucheng, China). According to the manufacturer, 95.5 % of XOS has a degree of polymerization between 2 and 7. Pure D-(+)-xylose was purchased from Merck (Darmstadt, Germany). The corncobs from corns grown for livestock fodder were provided by Republic of Turkey Ministry of Agriculture and Forestry, Aegean Agricultural Research Institute (Izmir, Turkey). They were obtained in a dried and ground form (particle size < 2 mm). Xylan was extracted from corncob by alkali (Egüés, Sanchez, Mondragon, & Labidi, 2012). Corncobs (180 g) were treated with 1.8 l of 10 % (w/v) NaOH at 37 °C for 3 h in a shaking incubator (Zhicheng ZHWY-200 B, PRC) at 150 rpm. The solids were removed by filtration through cheesecloth under vacuum followed by centrifugation (Centurion Scientific K3 Series, West Sussex, UK) at 4800 g and the pH was adjusted to 6.5–7.0 by addition of concentrated or 1 M HCl. Three volumes of ethanol (96 %) at 4 °C were added to precipitate xylan. The precipitate was recovered by centrifugation at 4800 g, washed with 75 % ethanol, and dried in an oven (Memmert UN30, Schwabach, Germany) at 60 °C overnight.

2.3. Batch culture fermentation

Non-pH controlled batch cultures were performed in Basal Medium (Hughes et al., 2007) containing (g/l) peptone water (2.0), yeast extract (2.0), sodium chloride (0.1), potassium phosphate dibasic (0.04), potassium phosphate monobasic (0.04), magnesium sulfate heptahydrate (0.01), calcium chloride hexahydrate (0.01), sodium bicarbonate (2.0), haemin (0.05), L-cysteine hydrochloride (0.5), resazurin (0.001), bile salt (0.5), tween 80 (2 ml/l), and vitamin K_1 (10 μl/l). The medium was boiled, put in 15 ml gas-tight Hungate tubes, and purged with N_2. The tubes were sealed with the butyl rubber stopper and scraw cap and sterilized at 121 °C for 15 min. Heat sensitive haemin and vitamin K_1 were filter-sterilized and added using sterile hypodermic syringes through the stopper. The pH value of the medium was recorded as 6.8–7.0 using a pH meter (Selecta, Barcelona, Spain) before inoculation.

The pure and the co-cultures of *Bifidobacterium* and *Bacteroides* strains were performed in the Hungate tubes containing basal medium supplemented with around 1.0 % (w/v) beechwood xylan, corncob xylan, XOS or xylose. The tubes were incubated at 37 °C for 48 h in a static incubator (Termal, Istanbul, Turkey). The organisms pre-grown in the basal medium containing 1 % (w/v) glucose at 37 °C for 18 − 24 h were used as the inocula (1 % (v/v)). The basal medium without added carbohydrate was used as a negative control.

2.4. Enumeration of bacteria

The growth was quantified by plating samples on selective solid media. The plates were incubated for 24 − 48 h in Anoxamat jar (Advanced Instruments, MA, USA) under the atmosphere composed of 10 % H_2, 10 % CO_2, and 80 % N_2. Wilkins-Chalgren anaerobe medium supplemented with 1.5 % (w/v) agar, 5 % (v/v) defibrinated horse blood (Oxoid) and Gram-negative anaerobic supplement (Oxoid) (nalidixic acid (10.0 mg/l) and vancomycin (2.5 mg/l) antibiotics; heamin (5.0 mg/l); menadione (0.5 mg/l); sodium succinate (2.5 g/l)) were used to enumerate *Bacteroides* species. The sterile horse blood and Gram-negative anaerobic supplement were added aseptically after sterilization. *Bifidobacterium* species were enumerated on Reinforced Clostridial Agar, which was prepared by adding 1.5 % (w/v) agar to
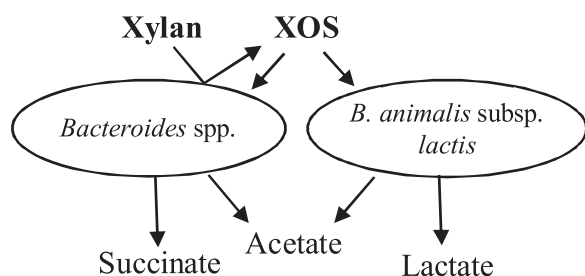

Fig. 1. The proposed mechanism for the bifidogenic effect of xylan.

Reinforced Clostridial Medium (Falony, Calmeyn, Leroy, & De Vuyst, 2009). The media were sterilized at 121 °C for 15 min.

In the initial tests, the growth was quantified by measuring optical density at 600 nm in a spectrophotometer (Shimadzu, Kyoto, Japan).

2.5. Analysis

The xylan and XOS concentrations were measured after hydrolyzing those into xylose using post-acid hydrolysis. H_2SO_4 (4 % (w/v)) was added to the samples and the mixture was kept at 121 °C for 1 h. After acid hydrolysis, $CaCO_3$ was added to increase the pH to around 5–6. The insolubles were removed by centrifugation at 4800g, for 5 min. The concentration of xylose was determined using HPLC (Perkin Elmer, Shelton, USA). Rezex RPM-monosaccharides column (Phenomenex, CA, USA) at 80 °C and a refractive index detector were used with ultra-pure water as the mobile phase at a flow rate of 0.6 ml/min. Xylan and XOS concentrations were calculated by multiplying the xylose concentrations by the anhydro correction factor (0.88). The concentrations of lactate, acetate, succinate, butyrate, and propionate were determined using HPLC with a UV detector at 210 nm. Aminex HPX-87H column (Biorad, CA, USA) at 50 °C was used with 5 mM H_2SO_4 as the mobile phase at a flow rate of 0.6 ml/min. For the quantification of the analytes, the peak areas in the chromatograms were compared with the calibration curves prepared using standard solutions at five concentrations. The standard solutions were prepared using analytical grade chemicals (Merck, Darmstadt, Germany). The carbohydrate composition of corncob extract was determined according to NREL/TP-510-42623 which is based on hydrolysing soluble carbohydrates with 4 % (v/v) H_2SO_4 for 1 h. The resulting monosaccharides were quantified in HPLC as described above (Sluiter et al., 2006). In all HPLC analysis conditions were set following column manufacturers' recommendations.

3. Results

3.1. Pure cultures

Two *Bacteroides* and five *Bifidobacterium* species were tested for their ability to utilize xylose, XOS, and beechwood and corncob xylans in pure cultures. *Bact. ovatus* and *Bact. xylanisolvens* grew on all substrates tested (Table 1). *B. bifidum, B. breve, B. infantis, B. longum* were unable to utilize these substrates. *B. animalis* subsp. *lactis* was able to utilize XOS but not xylose and xylans (Table 1). All strains showed substantial growth ($OD_{600} > 0.8$) in the basal medium containing glucose, which was used as the inoculum. The pH values of the culture broths decreased from their initial value of 7.0 as a result of the acidic end products of bacterial metabolism (Table 1). None of the strains showed growth in the negative control cultures, which did not contain any added carbohydrates.

The number of *Bact. ovatus* cells increased by 2.3 log in 24 h when growing on either XOS or beechwood xylan (Fig. 2). It utilized 3.8 g/l
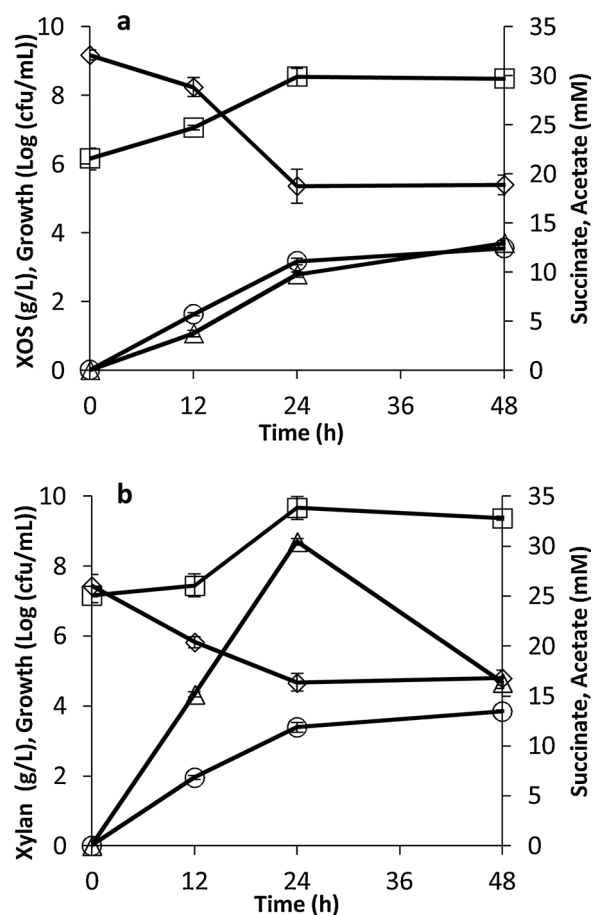

Fig. 2. Growth, carbohydrate consumption and metabolite production in the pure culture of *Bacteroides ovatus* with XOS (a) and beechwood xylan (b) as the carbon source; (square: growth; diamond: carbohydrate; triangle: acetate; circle: succinate).

XOS and 2.7 g/l xylan in 24 h, respectively (Fig. 2). The main end products were acetate and succinate, while lactate, butyrate, and propionate were not detected (Fig. 2). The succinate concentrations were similar in both cultures whereas acetate concentration was higher in the presence of xylan. Generally, the concentrations increased over 24 h and did not change notably after that except that the acetate level decreased in the culture with xylan. *Bact. xylanisolvens* cultures with XOS and beechwood xylan showed characteristics similar to *Bact. ovatus* cultures. In 24 h, the cell concentration increased by around 1.3–1.4 log, which is notably lower than the growth of *Bact. ovatus* (Fig. 3). The XOS and the xylan consumptions were also lower compared to those in *Bact. ovatus* cultures. In 24 h, 3.0 g/l XOS and 1.41 g/l beechwood xylan were consumed (Fig. 3). The metabolite profiles were comparable with both substrates except that acetate peaked earlier (12 h) in the presence

Table 1
Biomass levels (optical density at 600 nm (OD_{600})) and pH values in *Bacteroides* and *Bifidobacterium* cultures on xylose, XOS, and xylans after 48 h.

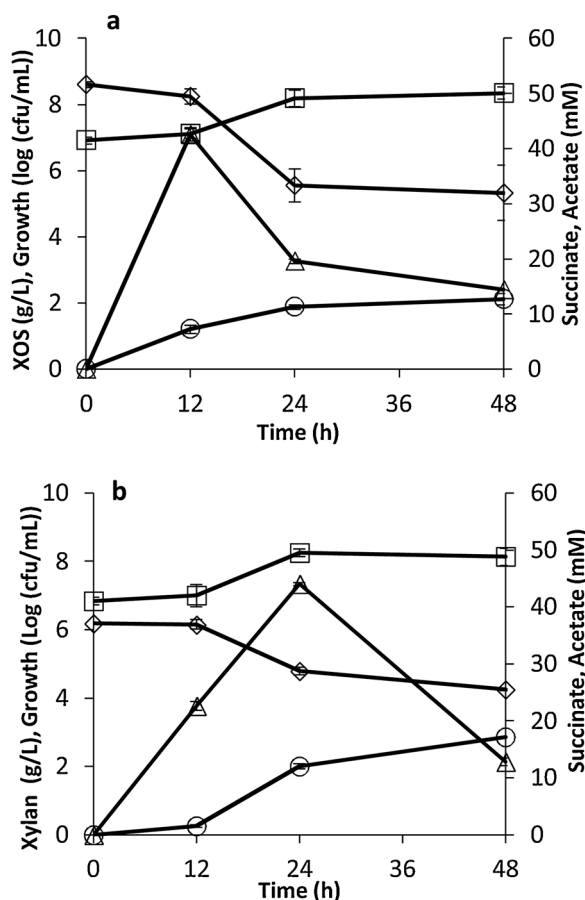| Organism	xylose		XOS		beechwood xylan		corncob xylan	
	OD_{600}	pH	OD_{600}	pH	OD_{600}	pH	OD_{600}	pH
Bact. xylanisolvens	0.47	5.20	0.87	5.21	0.32	5.23	0.47	5.26
Bact. ovatus	0.59	5.04	1.04	5.06	0.62	5.59	0.66	5.07
B. animalis subsp. *lactis*	–	6.89	0.57	4.87	–	6.96	–	6.99
B. bifidum	–	6.99	–	6.93	–	6.97	–	6.94
B. breve	–	6.93	–	6.83	–	6.95	–	6.95
B. infantis	–	6.94	–	6.96	–	6.98	–	6.96
B. longum	–	6.97	–	6.91	–	6.91	–	6.93

–: no growth.

Fig. 3. Growth, carbohydrate consumption and metabolite production in the pure culture of *Bacteroides xylanisolvens* with XOS (a) and beechwood xylan (b) as the carbon source; (square: growth; diamond: carbohydrate; triangle: acetate; circle: succinate).

of XOS (Figs. 2 and 3). Both organisms were also tested using corncob alkali extract as the sole carbon source. The extract was composed of 57 % xylose and 8 % arabinose (w/w). The carbohydrate consumption, growth, and metabolite concentration profiles were similar to those obtained with beechwood xylan (data not shown).

B. animalis subsp. *lactis* could grow only in cultures with XOS but not with beechwood or corncob xylan (Table 1). It consumed 3.7 g/l XOS in 24 h, and the cell concentration increased by 1.7 log (Fig. 4). XOS consumption was similar to that of *Bact. ovatus*, however, the growth
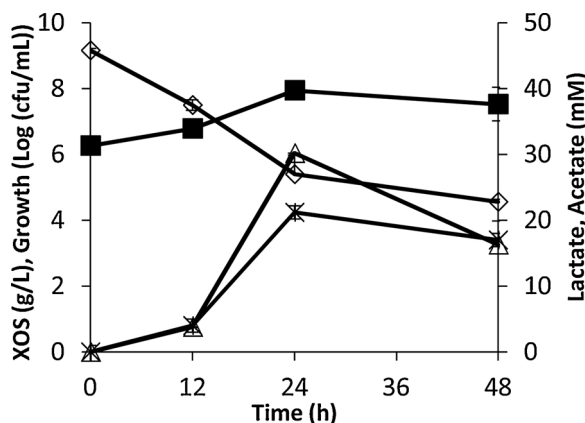

Fig. 4. Growth and carbohydrate consumption and metabolite production in the pure culture of *Bifidobacterium animalis* subsp. *lactis* on XOS; (square: growth; diamond: XOS; triangle: acetate; star: lactate).

was lower. Unlike *Bacteroides* species, *B. animalis* subsp. *lactis* produced lactate but not succinate, in addition to acetate (Fig. 4).

3.2. Co-cultures

Although *B. animalis* subsp. *lactis* did not grow on beechwood and corncob xylans, it could grow for 24 h when co-cultured with either *Bact. ovatus* or *Bact. xylanisolvens*. In *Bact. ovatus* - *B. animalis* subsp. *lactis* co-culture cell numbers increased by 2.0 and 1.1 log, respectively (Fig. 5a). The utilization of beechwood xylan in this co-culture was similar to that observed in the pure culture of *Bact. ovatus* (Figs. 5a and 2 b). The co-culture of *Bact. ovatus* and *B. animalis* subsp. *lactis* growing on beechwood xylan produced acetate, lactate, and succinate (Fig. 5a). The acetate was substantially lower than observed in pure cultures of both species (Figs. 2b and 3 b). Similar to the previous culture, *B. animalis* subsp. *lactis* when co-cultured with *Bact. xylanisolvens* could proliferate on xylan. The growth kinetics and the metabolic profiles were also similar (Fig. 5a and b). Both co-cultures were also tested using corncob xylan as the sole carbon source. Xylan consumption, growth and metabolite concentration profiles were similar to those obtained with beechwood xylan. The other *Bifidobacterium* species were also tested in co-cultures with the *Bacteroides* species. In those cultures, only the growth of *Bacteroides* species was observed.

4. Discussion

The pure culture studies showed that the ability of *Bifidobacterium* species to assimilate xylose and XOS was different. Among the species tested only *B. animalis* subsp. *lactis* was capable of growing on XOS, whereas none could utilize xylose. This is in line with the "resource partitioning" phenomenon among *Bifidobacterium* species in the utilization of different forms of xylan based oligosaccharides. Rivière et al. (2018) observed that *Bifidobacterium* strains originated from a single individual displayed different ability to degrade AXOS as well as inulin-type fructans. Falony, Lazidou et al. (2009) also observed variations among *Bifidobacterium* species in the degradation of inulin-type fructans. The probable differences in the genetic potential of *Bifidobacterium* strains in synthesis and localization of glycosidases, transporters and sugar metabolic pathways can explain the diversity in the utilization of xylan type oligosaccharides as well as other carbohydrates. It should be noted that the substitutions and linkages on xylans and xylan derived oligosaccharides could affect their catabolism. Previous studies showed interspecies and interstrain differences among Bifidobacteria for xylose utilization as well (Hopkins, Cummings, & Macfarlane, 1998; Palframan, Gibson, & Rastall, 2003; Rivière et al., 2014). Previous studies have also reported that *B. animalis* subsp. *lactis* was capable of utilizing XOS as a carbon source but could not ferment xylan, arabinoxylan, or xylose (Crittenden et al., 2002; Gilad et al., 2010). The fact that *B. animalis* subsp. *lactis* can utilize XOS while it does not grow well on xylose shows that it imports XOS without being hydrolyzed and has no effective membrane transport mechanism for free xylose (Crittenden et al., 2002). In *in-vitro* and *in-vivo* studies, a similar increase in *B. animalis* subsp. *lactis* count was observed on XOS (Gibson et al., 2010; Hansen et al., 2013), indicating that XOS is selectively fermented by this species. In Bifidobacteria XOS transport is mediated by ABC-type sugar transport system(s) following binding to a sugar-binding protein on the cell surface (Gilad et al., 2010; Palframan et al., 2003). Internally, XOS is hydrolyzed the xylose by endo-1,4-β-xylanase and a β-xylosidase.

The proliferation of *B. animalis* subsp. *lactis* in co-cultures can be ascribed to metabolite or substrate cross-feeding, which is defined as the utilization of the end products of bacterial metabolism such as organic acids and the hydrolysis products of polysaccharides as a carbon source by the other members of the microbiota (Belenguer et al., 2006; Moens, Weckx, & De Vuyst, 2016). In the co-cultures, the ability of *Bacteroides* species to hydrolyze xylan may have released XOS, which
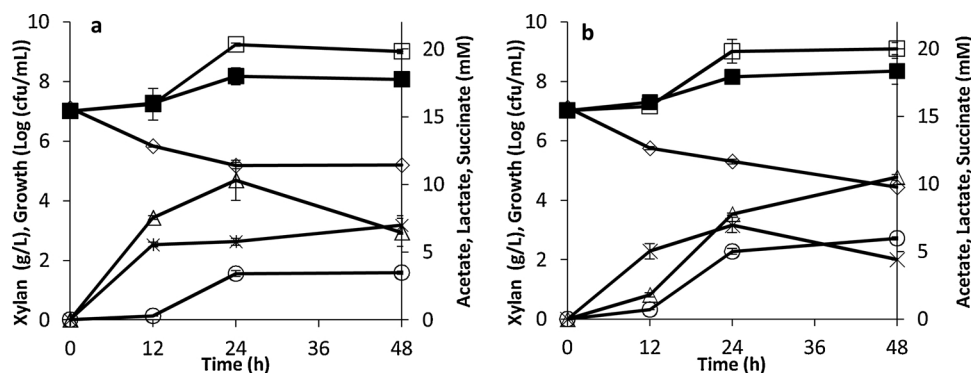
Fig. 5. Growth and carbohydrate consumption and metabolite production in the co-culture of *Bifidobacterium animalis* subsp. *lactis* with *Bacteroides ovatus* (a) and with *Bacteroides xylanisolvens* (b) on beechwood xylan (open square: *Bacteroides* growth; closed square: *Bifidobacterium animalis* subsp. *lactis* growth; diamond: carbohydrate; triangle: acetate; circle: succinate; star: lactate).

could be assimilated by *B. animalis* subsp. *lactis,* so that the latter species could proliferate in the co-culture. This can also explain the inability of *B. bifidum, B. longum, B. infantis,* and *B. breve* to grow in the co-cultures in the presence of xylan as the sole carbon source. Since these species could not utilize XOS, the xylan degradation products released by *Bacteroides* species did not support their growth.

Similar substrate cross-feeding was shown by Rogowski et al. (2015) in a co-culture of *B. adolescentis* and *Bact. ovatus* on three different forms of xylan. *B. adolescentis* was able to grow in the co-culture with xylans from birch and wheat, but not in the pure cultures. Falony, Calmeyn et al. (2009) tested the same phenomenon for *B. breve* Yakult and *B. adolescentis* on inulin in a co-culture of each with *Bact. thetaiotaomicron*. These *Bifidobacterium* species were unable to utilize inulin but fructooligosaccharides or fructose in pure cultures. The ability of *Bacteroides uniformis* to utilize agarose oligosaccharide allowed *B. infantis* and *B. adolescentis* to grow on this carbon source in co-cultures (Li et al., 2014).

In line with the substrate cross-feeding phenomenon, the culture broth was analyzed for XOS. There was no detectable amount of XOS in the samples, which can be explained by the rapid utilization of XOS released by the *Bacteroides* species by *B. animalis* subsp. *lactis*, as well as the *Bacteroides* species. In *Bacteroides* species, the xylan-type polysaccharides are degraded into oligosaccharides by the enzymes that are localized on the outer cell membrane (encoded by the polysaccharide utilization loci (PUL-XylS and PUL-XylL)) (Dodd et al., 2011; Rogowski et al., 2015). The xylan-derived oligosaccharides are degraded into their components in the periplasm and transported into the cytoplasm (Koropatkin, Cameron, & Martens, 2012; Martens, Koropatkin, Smith, & Gordon, 2009; Terrapon & Henrissat, 2014). In co-cultures, those oligosaccharides released as a result of the enzymatic action on the bacterial surface may have been used as a carbon source by the *Bifidobacterium* species. Metabolic cross-feeding was also observed among colonic microbiota. In co-cultures of *B. adolescentis* and *Faecalibacterium prausnitzii*, the concentration of butyrate was increased by means of *F. prausnitzii* converting the acetate to butyrate (Rios-Covian, Gueimonde, Duncan, Flint, & de Los Reyes-Gavilan, 2015). The lactate produced by some bacterial species stimulated sulfide production by *Desulfovibrio piger* (Marquet, Duncan, Chassard, Bernalier-Donadille, & Flint, 2009). *Bifidobacterium* and *Bacteroides* species are unable to produce butyrate (Klijn, Mercenier, & Arigoni, 2005), thus no butyrate was detected in the samples from either pure cultures or co-cultures. Acetate was observed as the main fermentation end product in all cultures. *In-vivo*, acetate can be a potential source for butyrate synthesis by other colonic bacteria. *F. prausnitzii* and *Anaerostipes, Eubacterium,* and *Roseburia* species are known to convert acetate to butyrate (Louis & Flint, 2009). This way, xylan can exhibit a butyrogenic effect, in addition to its bifidogenic effect.

The other fermentation end product detected in the *Bacteroides* pure cultures was succinate, whereas in *B. animalis* subsp. *lactis* pure culture, lactate was produced. The presence of both lactate, and succinate, in addition to acetate in the co-cultures, indicated that both *Bacteroides*

and *Bifidobacterium* species were actively growing. The succinate and lactate in the co-cultures should have been released as a result of the fermentative metabolism of *Bacteroides* and *Bifidobacterium* species, respectively. The absence of lactate and succinate in *Bacteroides* and *Bifidobacterium* cultures, respectively, was also reported in previous studies (Chassard, Delmas, Lawson, & Bernalier-Donadille, 2008; Louis & Flint, 2009; Macfarlane & Macfarlane, 2003). The absence of propionate in the pure and co-cultures in this study can be explained by the availability of carbon source throughout the cultures, which may have hindered conversion of succinate into propionate by *Bacteroides* (Macfarlane & Macfarlane, 2003; Van der Meulen, Makras, Verbrugghe, Adriany, & De Vuyst, 2006).

The additional benefit of xylan lies in its slower utilization by the colon microbiota compared to other well established prebiotic carbohydrates (Crittenden et al., 2002; Karppinen, Liukkonen, Aura, Forssell, & Poutanen, 2000). Generally, carbohydrate fermentation takes place in the proximal part. As a result of fermentation, SCFAs and lactic and succinic acids are formed and the pH of the colon decreases. In contrast, protein degradation occurs in the distal part of the colon and as a result of this, ammonia, polyamines, polyphenols, and other metabolites are formed. Arabinoxylan, xylan, and xylan based long-chain oligosaccharides can be degraded towards the distal part of the column because the fermentations are suppressed by the other easier-to-use substrates (Grootaert et al., 2007; Sanchez et al., 2009). The increase in carbohydrate fermentation in the distal colon is thought to reduce the risk of colon cancer (Hooper, Midtvedt, & Gordon, 2002).

5. Conclusion

The prebiotic activity of some of the complex dietary carbohydrates requires symbiosis of bacterial species. Elucidation of the association of different species can provide a better understanding of the health benefits ascribed to prebiotics and can offer novel prebiotics and symbiotics. In this study, possible cooperation between *Bifidobacterium* and *Bacteroides* species for the bifidogenic effect of xylan was sought, and it is suggested that bifidobacteria can benefit from the ability of *Bacteroides* species to hydrolyze xylan. *B. animalis* subsp. *lactis* could proliferate in a co-culture with either *Bact. xylanisolvens* or *Bact. ovatus* in the presence of xylan as the sole carbon source. The end product profiles were also an indication of the activity of *Bifidobacterium* species in the co-cultures. These can be ascribed to the ability of *B. animalis* subsp. *lactis* to utilize XOS as a carbon source. However, not all *Bifidobacterium* species are eligible for the cross-feeding due to their inability to utilize xylan hydrolysis products. This reflects the current perspective suggesting the specialization of the bacteria towards different forms of the available carbohydrates. Xylan is obtained from agricultural and forestry wastes so that it is an abundant and cost-effective natural carbohydrate. Revealing its potential prebiotic activity as well as its hydrocolloidal properties can enable xylan to find uses in food and nutraceutical industries.

Funding

CRediT authorship contribution statement

Nuket Zeybek: Investigation, Data curation, Writing - original draft, Validation. **Robert A. Rastall:** Conceptualization, Supervision, Methodology. **Ali Oguz Buyukkileci:** Conceptualization, Methodology, Writing - original draft, Supervision.

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