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Enhanced butyrate formation by cross-feeding between *Faecalibacterium prausnitzii* and *Bifidobacterium adolescentis*

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One sentence summary: The article provides the first experimental demonstration of enhanced butyrate formation by a cross feeding mechanisms betweeen *Faecalibacterium prausnitzii*, a comensal bacteria of the human colon, with *Bifidobacterium adolescentis*, one of the most abundant bifidobacteria from the adult's intestinal microbiota.

Editor: Michael Cox

ABSTRACT

Cross-feeding is an important metabolic interaction mechanism of bacterial groups inhabiting the human colon and includes features such as the utilization of acetate by butyrate-producing bacteria as may occur between *Bifidobacterium* and *Faecalibacterium* genera. In this study, we assessed the utilization of different carbon sources (glucose, starch, inulin and fructooligosaccharides) by strains of both genera and selected the best suited combinations for evidencing this cross-feeding phenomenon. Co-cultures of *Bifidobacterium adolescentis* L2–32 with *Faecalibacterium prausnitzii* S3/L3 with fructooligosaccharides as carbon source, as well as with *F. prausnitzii* A2–165 in starch, were carried out and the production of short-chain fatty acids was determined. In both co-cultures, acetate levels decreased between 8 and 24 h of incubation and were lower than in the corresponding *B. adolescentis* monocultures. In contrast, butyrate concentrations were higher in co-cultures as compared to the respective *F. prausnitzii* monocultures, indicating enhanced formation of butyrate by *F. prausnitzii* in the presence of the bifidobacteria. Variations in the levels of acetate and butyrate were more pronounced in the co-culture with fructooligosaccharides than with starch. Our results provide a clear demonstration of cross-feeding between *B. adolescentis* and *F. prausnitzii*.

Keywords: *Faecalibacterium prausnitzii*; *Bifidobacterium adolescentis*; butyrate; acetate; starch; fructooligosaccharides (FOS); probiotics

INTRODUCTION

Nutrient cross-feeding is an important and integral component of the dynamic and functional complex microbial ecosystem found in the human colon (Belenguer *et al.* 2006; Flint *et al.* 2012). The large intestine is colonised by a dense microbial community comprised mainly of Bacteroidetes, Firmicutes and Actinobacteria. A key function of these predominantly anaerobic

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microbes is to ferment dietary residues that escape digestion by host enzymes (Flint *et al.* 2012). Collectively, the bacterial species that co-exist within this dense community possess an elaborate array of enzymes, including glycosyl-hydrolases, which allows the cells to degrade complex carbohydrates. For example, non-digestible carbohydrates such as resistant starches, non-starch polysaccharides (NSP) and oligosaccharides, including fructooligosaccharides (FOS), are major sources of energy for colonic bacteria (Flint *et al.* 2012). Intermediate carbohydrate breakdown products and certain fermentation products serve as carbon and energy sources for cross-feeding bacteria (Duncan, Louis and Flint 2004; Belenguer *et al.* 2006) and are mainly fermented to short-chain fatty acids (SCFA) and gases (Duncan *et al.* 2007). The major fermentation products detected in the colon are acetate, propionate and butyrate often in the ratio of around 10:2:1 depending on dietary intakes. Acetate is utilised by butyrate producers that employ the dominant butyryl CoA:acetate CoA transferase route (Duncan, Louis and Flint 2004; Louis *et al.* 2010). One of the most dominant bacterial species detected in the healthy human large intestine is *Faecalibacterium prausnitzii* (Flint *et al.* 2015); this microorganism is a butyrate producer that employs this route for butyrate formation and representative strains have been reported to grow poorly in the absence of acetate (Duncan *et al.* 2002). *F. prausnitzii* is considered important in health promotion, as in addition to forming butyrate it also possesses other anti-inflammatory attributes (Sokol *et al.* 2009).

It is interesting to speculate why *F. prausnitzii* is so successful in the healthy colon despite the fact that there is a diverse range of carbohydrates consumed in our diets and *F. prausnitzii* grows poorly on non-digestible carbohydrates (Lopez-Siles *et al.* 2012), which are a major carbon source for colonic anaerobes (Cummings and Macfarlane 1991). Its competitive prowess may, at least in part, be due to its ability to utilise end products of fermentation in the colon suggesting that it may therefore be a successful cross-feeder.

Some of the best studied bacteria from the human colon belong to the *Bifidobacterium* genus. *Bifidobacterium* has long been considered a beneficial microorganism and reduced *Bifidobacterium* levels have been linked to different disease states (Tojo *et al.* 2014). Therefore, there is a long-standing interest in promoting bifidobacteria in the colon either directly through probiotic approaches or indirectly by prebiotics (Olano-Martin, Gibson and Rastell 2002; Cardelle-Cobas *et al.* 2009; Arboleya *et al.* 2011; Tojo *et al.* 2014). Different *Bifidobacterium* species can be found in the human gut (Salazar *et al.* 2015) and *Bifidobacterium adolescentis* is one of the most frequently found in adults (Matsuki *et al.* 2004).

Furthermore, there is considerable interest in using dietary and prebiotic strategies to modulate the gut microbiota as this has the potential to influence several aspects of host health. It should be noted however that introduction of a prebiotic supplement will impact not only on one target bacterial species, which traditionally has included *Bifidobacterium* species, but also on others through cross-feeding interactions (Ramirez-Farias *et al.* 2009; Scott *et al.* 2014).

Here, we explore the distinct ability of strains belonging to the species *F. prausnitzii* and the genus *Bifidobacterium* to grow in different carbon sources with a focus on the interactions that occur between *B. adolescentis*, one of the most abundant *Bifidobacterium* species in the colon, with representative *F. prausnitzii* isolates when provided with FOS or starch in the growth medium. These investigations demonstrated clear cross-feeding and enhanced butyrate formation when certain of these strains were in co-culture.

MATERIAL AND METHODS

Bacterial strains

The study included three *F. prausnitzii* strains and five strains belonging to the genus *Bifidobacterium*. *B. adolescentis* L2–32 and *F. prausnitzii* strains A2–165, L2–6 and S3L/3 were originally isolated from adult human stool samples (Barcenilla *et al.* 2000; Louis *et al.* 2004). The strain *Bifidobacterium breve* IPLA 20006 was previously isolated from breast milk, and the strains *Bifidobacterium bifidum* IPLA 20015 and *Bifidobacterium pseudocatenulatum* IPLA 20026 from infant faeces (Solís *et al.* 2010). In addition, a *Bifidobacterium longum* strain (IPLA 20027) isolated in TOS agar medium (MERK, Germany) from the stool of a healthy 90-year-old woman was also included.

Growth media, monoculture and co-culture conditions

All strains used in this study were recovered in M2GSC medium (Miyazaki *et al.* 1997) from frozen stocks and grown overnight at 37°C under anaerobic conditions with O_2-free CO_2 using the Hungate tube method. For the substrate fermentation and SCFA growth boosting tests, strains were grown in YCFA medium (Lopez-Siles *et al.* 2012) as described above with and without SCFA supplementation. When appropriate, an SCFA solution was added to the medium obtaining a final concentration of 33 mM acetate, 9 mM propionate and 1 mM each of *iso*-butyrate, *iso*-valerate and valerate. Single carbon sources were added before autoclaving to give a final concentration of 0.2% (w/v) and the final pH was adjusted to 6.5 ± 0.2. Carbon sources used were glucose (Fischer Scientific, USA), soluble starch from potato (Sigma-Aldrich, USA), inulin from two different sources (dahlia and chicory) and FOS P95 (Beneo, Belgium). Growth in cultures was monitored spectrophotometrically by measuring the OD_{650} and growth rate (h^{-1}) was determined at exponential growth (Pirt 1975).

To study the interaction between *Bifidobacterium* and *F. prausnitzii*, co-culture combinations of selected strains in specific carbon sources were carried out. 100 μL of an M2GSC overnight culture of each strain were added to 7.5 mL of YCFA medium with and without SCFA and supplemented with the appropriate carbon source. Co-culture tubes were inoculated with 100 μL of each strain. Culture conditions were the same as described above and growth was monitored by determining OD_{650}. Samples for microbiological and SCFA analyses were taken at 0, 8 and 24 h of incubation.

Carbohydrate fermentation profiles

Fermentation profiles of *Bifidobacterium* strains were obtained in API 50 CH strips (BioMerieux, France) following the manufacturer's instructions. Strips were incubated at 37°C in an anaerobic Chamber (Mac 1000; Don Whitley Scientific, West Yorkshire, UK).

SCFA and lactate determinations

SCFA and lactate content in batch cultures were determined by capillary gas chromatography analysis following conversion to *t*-butylmethylsilyl derivatives (Richardson *et al.* 1989). The lower limit for reliable detection of SCFA changes was 0.2 mM.

One-way analysis of variance (ANOVA) using the IBM SPSS software version 22.0 (IMB, Armonk, New York, USA) was run to compare the levels of SCFA between monocultures and co-cultures at 8 and 24 h of incubation as well as between both incubation times for the same culture. *Post hoc* comparison was

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Table 1. Carbohydrate fermentation profiles obtained with API 50 CH strips of different *Bifidobacterium* strains used in this study.

Carbon source	*B. adolescentis* L2–32	*B. pseudocatenulatum* IPLA 20026	*B. bifidum* IPLA 20015	*B. longum* IPLA 20027	*B. breve* IPLA 20006
L-Arabinose	+	+	−	−	−
D-Ribose	+	+	−	+	+
D-Xylose	+	+	−	−	−
D-Mannose	−	−	−	+	+
D-sorbitol	+	−	−	−	−
MDM	−	−	−	+	−
MDG	+	+	−	+	−
NAG	−	−	+	+	−
Arbutin	+	+	−	+	−
Esculin	+	+	−	+	+
Salicin	+	+	−	+	+
D-Celobiose	+	−	−	+	−
D-Maltose	+	+	−	+	+
D-Melobiose	+	+	−	+	+
D-Sacharose	+	+	−	+	+
D-Trehalose	−	−	−	+	−
D-Rafinose	+	+	−	−	+
Starch	+	−	−	−	−
Glycogen	+	−	−	−	−
Gentiobiose	+	−	+	+	−
D-Turanose	+	+	−	−	+
D-Lyxose	−	−	−	+	−
D-Tagatose	−	−	−	+	−
5KG	−	+	−	+	−

+ Positive result; − Negative result; MDM, Methyl-αD-Mannopyranoside; MDG, Methyl-αD-Glucopyranoside; NAG, N-Acetylglucosamine; 5KG, potassium 5-Ketogluconate.

achieved when appropriate by a least significant difference test (LSD).

RESULTS

Regarding bifidobacteria, a preliminary test of the fermentation capability of different carbohydrates was carried out in API 50 CH strips, in order to ascertain whether or not they were able to ferment a range of carbohydrates. All strains fermented D-galactose, D-glucose, D-fructose and D-lactose to a variable extent whereas differential fermentation profiles were obtained among strains for the carbohydrates indicated in Table 1.

B. adolescentis L2–32 was the only one able to ferment starch whereas none of the microorganisms tested were able to ferment inulin. Further tests to confirm growth in glucose, inulin and FOS were carried out in Hungate tubes with YCFA medium with and without SCFA added (Table 2). In this medium, *B. bifidum* IPLA 20015 did not grow in any carbon source tested except glucose, whereas *B. longum* IPLA 20027 and *B. breve* IPLA 20006 displayed optimal growth with FOS P95 but grew poorly with starch. *B. pseudocatenulatum* IPLA 20026 and *B. adolescentis* L2–32 grew well on starch, and to a lesser extent in FOS P95. In view of the slightly better growth of *B. adolescentis* L2–32 with FOS P95 and starch and that the presence of SCFA did not improve its behaviour against in the later carbon source we selected *B. adolescentis* L2–32 for further experiments.

Growth of *F. prausnitzii* strains with glucose was reported to be stimulated by the presence of acetate in the growth medium, which contributes to butyrate formation via the butyryl CoA:acetate CoA-transferase route (Duncan *et al.* 2002; Duncan, Louis and Flint 2004). Table 2 shows that growth of the *F. prausnitzii* strains in different carbon sources improved when media were supplemented with SCFA (including 30 mM acetate). Growth of *F. prausnitzii* L2–6 with glucose and of S3L/3 with FOS P95 was poorer than growth of the other two *Faecalibacterium* strains with the same carbon source, as observed previously and starch promoted little or no growth of any of the three strains (Lopez-Siles *et al.* 2012). The inclusion of SCFA improved growth of *F. prausnitzii* strains with both types of inulin (dahlia or chicory origin) as carbon source.

We decided to investigate whether or not acetate supplied by a *Bifidobacterium* strain would stimulate the growth and metabolic activity of *F. prausnitzii* in co-culture. *B. adolescentis* L2–32 was able to ferment FOS P95 in pure culture, producing mainly acetate together with lower concentrations of formate and lactate (Figs 1 and 2). *F. prausnitzii* strain S3L/3 grew poorly on FOS in monoculture (Table 2). Separate co-cultures of this strain with *B. adolescentis* L2–32 resulted in an overall stimulation of bacterial growth and an increase in butyrate concentration of around 8 mM after 24 h (Figs 1 and 2); butyrate concentrations were significantly higher in such co-cultures than in the corresponding *F. prausnitzii* monoculture ($P < 0.05$). A decrease in acetate levels occurred in co-culture of *F. prausnitzii* S3L/3 with *B. adolescentis* L2–32 from 8 to 24 h of incubation and with acetate levels at 24 h being even lower than in the corresponding *B. adolescentis* monoculture ($P < 0.05$) (Fig. 2).

A similar experiment was performed involving *F. prausnitzii* A2–165 and *B. adolescentis* L2–32 with starch as substrate. *B. adolescentis* L2–32 was able to ferment this complex carbohydrate in pure culture whereas *F. prausnitzii* A2–165 did not grow in pure culture with this substrate (Table 2). Thus, we studied the ability of *F. prausnitzii* to use the acetate produced by *B. adolescentis* L2–32, which could result in enhanced growth of both microorganisms. Less butyrate was formed (2.9 mM) in the co-culture

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Table 2. Growth of *F. prausnitzii* and *B. adolescentis* strains in YC medium with (YCFA-) or without (YC-) added SCFA. Glucose (G), starch (S), inulin from dahlia (Id), inulin from chicory (Ic) or FOS (P95) were included as carbon source as indicated. Basal medium with no carbohydrates added was used for comparison.

	F. prausnitzii A2–165		*F. prausnitzii* L2–6		*F. prausnitzii* S3L/3	
Media	OD_{650} 24 h	Growth rate (h^{-1})	OD_{650} 24 h	Growth rate (h^{-1})	OD_{650} 24 h	Growth rate (h^{-1})
YCFAG	0.83 ± 0.03	0.47 ± 0.06	0.24 ± 0.08	0.46 ± 0.07	0.77 ± 0.05	0.13 ± 0.03
YCG	0.39 ± 0.05	0.38 ± 0.04	0.36 ± 0.03	0.28 ± 0.08	0.30 ± 0.01	0.36 ± 0.06
YCFAS	0.08 ± 0.00	–	0.20 ± 0.01	ND	0.19 ± 0.01	–
YCS	0.06 ± 0.01	–	0.12 ± 0.01	–	0.11 ± 0.01	–
YCFAId	0.30 ± 0.02	0.37 ± 0.03	0.32 ± 0.01	0.22 ± 0.03	0.18 ± 0.00	–
YCId	0.21 ± 0.01	0.19 ± 0.05	0.11 ± 0.01	–	0.08 ± 0.01	–
YCFAIc	0.37 ± 0.01	0.52 ± 0.11	0.34 ± 0.01	ND	0.20 ± 0.01	–
YCIc	0.09 ± 0.03	–	0.05 ± 0.00	–	0.11 ± 0.01	–
YCFAP95	0.91 ± 0.03	0.31 ± 0.05	0.63 ± 0.01	0.27 ± 0.03	0.24 ± 0.01	ND
YCP95	0.39 ± 0.08	0.16 ± 0.07	0.27 ± 0.01	0.27 ± 0.09	0.14 ± 0.00	–
YCFA	0.09 ± 0.01	–	0.20 ± 0.01	ND	0.16 ± 0.01	–
YC	0.09 ± 0.01	–	0.15 ± 0.01	–	0.06 ± 0.00	–

	B. adolescentis L2–32		*B. pseudocatenolatum* IPLA 20026		*B. bifidum* IPLA 20015		*B. longum* IPLA 20027		*B. breve* IPLA 20006	
Media	OD_{650} 24 h	Growth rate (h^{-1})	OD_{650} 24 h	Growth rate (h^{-1})	OD_{650} 24 h	Growth rate (h^{-1})	OD_{650} 24 h	Growth rate (h^{-1})	OD_{650} 24 h	Growth rate (h^{-1})
YCFAG	0.63 ± 0.05	0.76 ± 0.06	0.13 ± 0.06	–	0.61 ± 0.01	0.17 ± 0.02	0.65 ± 0.04	0.72 ± 0.08	0.22 ± 0.02	ND
YCG	0.59 ± 0.07	0.65 ± 0.06	0.16 ± 0.03	–	0.61 ± 0.01	0.27 ± 0.09	0.34 ± 0.01	0.06 ± 0.02	0.10 ± 0.02	–
YCFAS	0.63 ± 0.00	0.55 ± 0.07	0.79 ± 0.03	0.23 ± 0.04	0.00 ± 0.00	–	–0.01 ± 0.00	–	0.02 ± 0.00	–
YCS	0.97 ± 0.06	0.46 ± 0.04	0.73 ± 0.01	0.19 ± 0.04	0.01 ± 0.01	–	0.03 ± 0.01	–	0.02 ± 0.01	–
YCFAId	0.07 ± 0.03	–	0.07 ± 0.00	–	0.06 ± 0.02	–	0.08 ± 0.01	–	0.06 ± 0.01	–
YCId	0.01 ± 0.00	–	0.05 ± 0.01	–	0.02 ± 0.00	–	0.04 ± 0.02	–	0.06 ± 0.01	–
YCFAIc	0.05 ± 0.00	–	0.03 ± 0.00	–	0.03 ± 0.00	–	0.04 ± 0.00	–	0.03 ± 0.00	–
YCIc	0.05 ± 0.01	–	0.05 ± 0.01	–	0.05 ± 0.02	–	0.06 ± 0.01	–	0.03 ± 0.01	–
YCFAP95	0.44 ± 0.10	0.29 ± 0.06	0.35 ± 0.12	0.55 ± 0.06	0.06 ± 0.00	–	0.74 ± 0.05	0.42 ± 0.04	0.38 ± 0.00	ND
YCP95	0.37 ± 0.19	0.25 ± 0.10	0.33 ± 0.12	0.47 ± 0.10	0.04 ± 0.01	–	0.50 ± 0.01	0.45 ± 0.02	0.35 ± 0.02	ND
YCFA	0.06 ± 0.00	–	0.04 ± 0.00	–	0.02 ± 0.01	–	0.03 ± 0.01	–	0.00 ± 0.00	–
YC	0.05 ± 0.01	–	0.04 ± 0.02	–	0.01 ± 0.01	–	0.03 ± 0.00	–	0.04 ± 0.02	–

– means OD values lower than 0.2; ND, not done.

of *F. prausnitzii* A2–165 and *B. adolescentis* L2–32 than in the other previous strains combination using FOS P95 as fermentable substrate (Fig. 2). With starch as carbon source acetate levels in *B. adolescentis* L2–32 monoculture decreased from 22 to 18 mM in the co-culture ($P < 0.05$) (Fig. 2) but no major differences were observed in the overall growth of the co-culture with respect to the monocultures (Fig. 1).

Overall, co-cultures of *F. prausnitzii* S3L/3 and *B. adolescentis* L2–32 with FOS P95 showed a significant decrease of acetate and a concomitant increase of butyrate from 8 to 24 h of incubation ($P < 0.05$). However, both acetate and butyrate increased moderately ($P < 0.05$) between 8 and 24 h in the case of co-cultures of *F. prausnitzii* A2–165 and *B. adolescentis* L2–32 with starch.

DISCUSSION

Acetate requirement for optimal growth of *F. prausnitzii* when using glucose as carbon source has been demonstrated previously (Duncan *et al.* 2002; Duncan, Louis and Flint 2004). In the present study we have shown similar requirements of acetate when the carbon source present in the medium was FOS.

The relationship between prebiotics, bifidogenic effects and higher butyrate production has been reported previously. In this regard, several cross-feeding experiments have been described between members of the *Bifidobacterium* genus and other butyrate-producing colonic bacteria, such as members of the genera *Eubacterium*, *Anaerostipes* and *Roseburia* (Duncan, Louis and Flint 2004; Kanauchi *et al.* 1999; Belenguer *et al.* 2006; Falony *et al.* 2006), but no previous experimental evidence was available demonstrating cross-feeding between bifidobacteria and *Faecalibacterium* strains. Higher butyrate production was previously reported during *in vitro* faecal cultures when FOS was added as carbon source to the system (Vitali *et al.* 2012). On the other hand, populations of *B. adolescentis* and *F. prausnitzii* were found to increase in an intervention study, after the administration of inulin (Ramirez-Farias *et al.* 2009). Moreover, recently a computational model has been developed to predict the fluxes and SFCA production in co-cultures of *F. prausnitzii* A2–165 and *B. adolescentis* L2–32 (El-Semman *et al.* 2014). However, in spite of these previous data this is the first study providing direct experimental evidence of a potential interaction between *Bifidobacterium* and *Faecalibacterium* strains through cross-feeding mechanisms using *in vitro* co-cultures of both microorganisms.

F. prausnitzii requires a carbohydrate energy source for growth and butyrate formation, while *B. adolescentis* does not produce butyrate. The observed higher values for acetate at 8 h than at 24 h together with the butyrate production in co-cultures on

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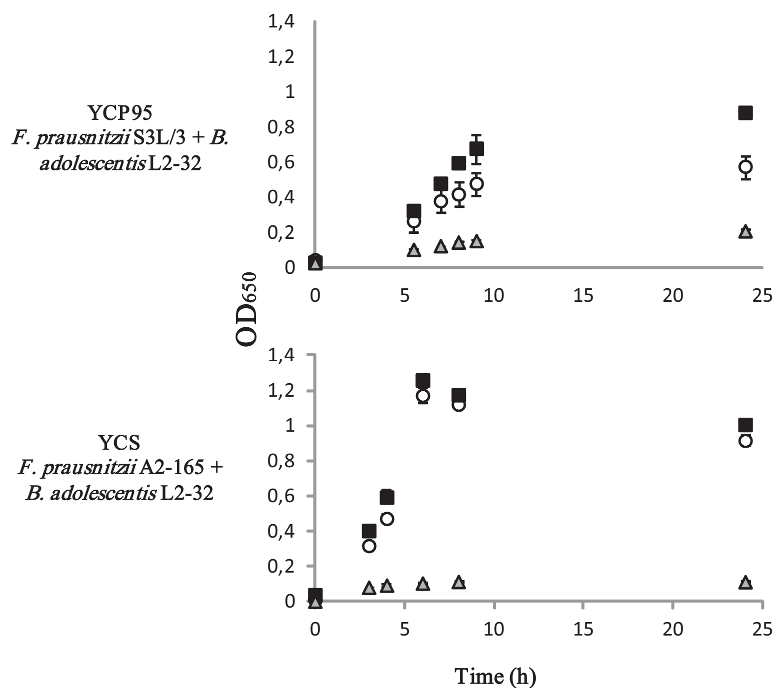

Figure 1. Growth of *F. prausnitzii* and *B. adolescentis* in monoculture and co-cultures, determined by OD_{650}: grey triangles, *F. prausnitzii* strain, white circles, *B. adolescentis* L2–32 and black squares, co-culture. Strains and carbon source used in each experiment are indicated in the left side of each panel.

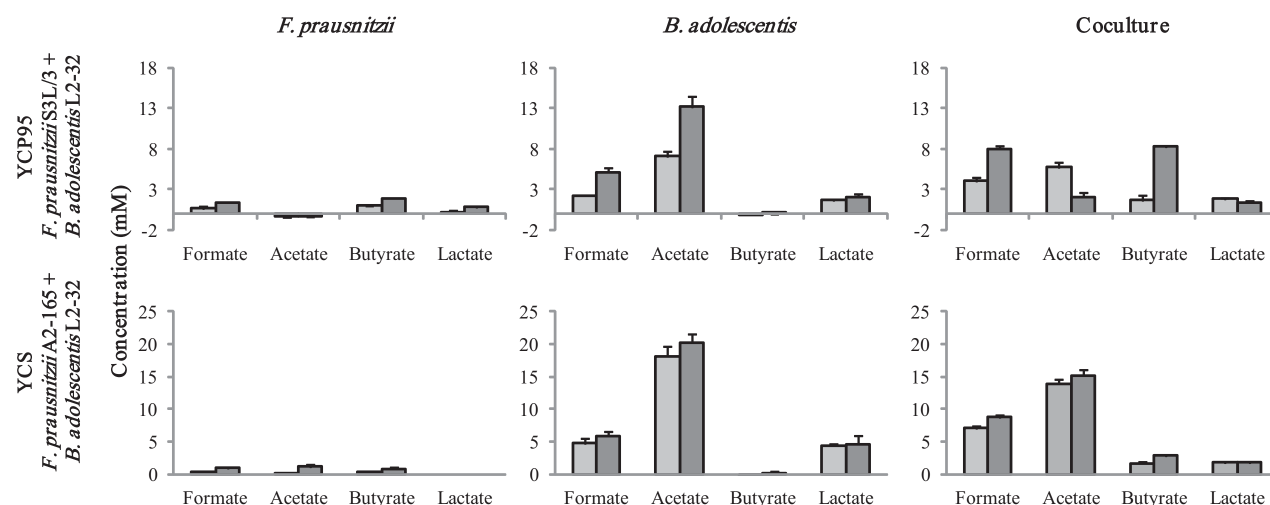

Figure 2. SCFA and lactate production by *F. prausnitzii* and *B. adolescentis* in mono-culture and co-cultures. Strains and carbon sources used in each experiment are indicated in the left side of each panel. Values are changes in concentrations calculated by subtracting the initial values at 0 h. Light grey bars, 8 h of incubation and dark grey bars, 24 h of incubation.

YCFAP95 FOS medium with no SCFA, suggest that *F. prausnitzii* growth benefits from the acetate that is being supplied by the second species, *B. adolescentis*, which is also competing for the P95 FOS present in the culture medium. In cultures of *F. prausnitzii* A2–165 using starch as carbon source, butyrate production was lower even though the production of acetate by L2–32 was higher in starch. This suggests a more limited ability of the strain *F. prausnitzii* A2–165 to compete with *B. adolescentis* for starch breakdown products as it needs available carbon source and acetate for optimal growth. The stimulation of butyrate production in the co-culture must, therefore, be partly attributed to the ability of the *F. prausnitzii* strains to compete for the substrate and the partial consumption of the acetate formed by the *Bifidobacterium* strain. This suggests that these cross-feeding mechanisms are less effective in medium containing starch as the carbon source. On the other hand, differences in the metabolism of mono and oligosaccharide constituents of FOS and starch by *B. adolescentis* and *Faecalibacterium* could also influence such cross-feeding processes.

Our results point to mechanisms of synergy that may take place between these two microorganisms in which, *F. prausnitzii* is able to use the acetate produced by *B. adolescentis* thereby boosting butyrate formation and therefore supply to the colonic mucosa. In addition, cross feeding of partial breakdown

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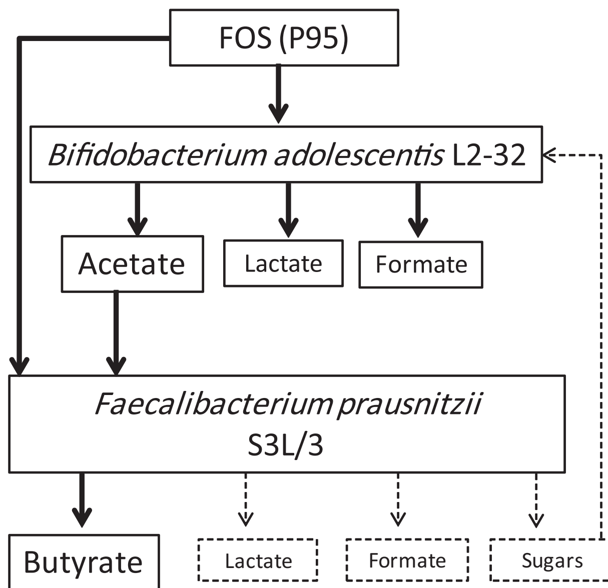

Figure 3. Schematic representation of the proposed interaction between *B. adolescentis* L2–32 and *F. prausnitzii* S3L/3 strain using FOS P95 as carbon source. FOS P95 is used by both microorganisms, but with low efficiency. The synergistic mechanism consists of consumption by *F. prausnitzii* of the acetate produced by *B. adolescentis*. The growth of *B. adolescentis* may be enhanced by carbohydrate residues released in the breakdown of the FOS chain by *F. prausnitzii*. Continuous arrows demonstrate outcomes from the present work. Discontinuous arrows indicate events that may occur but that have not been demonstrated in the present work.

products is likely to be highly significant *in vivo*. *B. adolescentis* may therefore take advantage of the breakdown products of FOS P95 formed by *F. prausnitzii* and the hypothetical mechanism of this synergy is presented in Fig. 3. Such cross feeding may benefit growth of *F. prausnitzii*, thus helping to explain the abundance of this species in the healthy human gut.

F. prausnitzii is a key player in the maintenance of intestinal and systemic host health. A decrease in *F. prausnitzii* and butyrate levels defines microbiota dysbiosis in patients suffering inflammatory bowel disease (Machiels *et al.* 2014; Lopez-Siles *et al.* 2015). Remission of inflammatory parameters has been obtained in animal models following the administration of *F. prausnitzii* or its metabolic products (Zhang *et al.* 2014; Rossi *et al.* 2015). Other studies have also highlighted the relevance of metabolic interactions between *Bacteroides thetaiotaomicron* and *F. prausnitzii* on the physiology of the colonic epithelium whereby *F. prausnitzii* is able to use the acetate produced by *B. thetaiotaomicron* with the subsequent modulation of the intestinal mucus barrier by modification of goblet cells and mucin glycosylation (Wrzosek *et al.* 2013). The relevance for the host physiology of the potential interactions between *F. prausnitzii* and probiotics or other members of the intestinal microbiota is therefore an area of great interest.

In the intestinal environment acetate is normally available at high concentrations, but this may not always be the case, for example, in special gut microenvironments, or following a period of substrate deprivation or antibiotic treatment (Hamer *et al.* 2008). Under these circumstances, the interactions revealed in the present work could have an important impact on *F. prausnitzii* populations and butyrate production in the colon. These interactions are also of interest as they suggest mechanisms by which probiotic bifidobacteria and prebiotic administration might influence gut metabolism and promote butyrate production.

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Conflict of interest. None declared.

REFERENCES

Arboleya S, Ruas-Madiedo P, Margolles A, *et al.* Characterization and *in vitro* properties of potentially probiotic *Bifidobacterium* strains isolated from breast-milk. *Int J Food Microb* 2011;**149**:28–36.

Barcenilla A, Pryde SE, Martin JC, *et al.* Phylogenetic relationships of butyrate-producing bacteria from the human gut. *Appl Environ Microb* 2000;**66**:1654–61.

Belenguer A, Duncan SH, Calder AG, *et al.* Two routes of metabolic cross-feeding between *Bifidobacterium adolescentis* and butyrate-producing anaerobes from the human gut. *Appl Environ Microb* 2006;**72**:3593–9.

Cardelle-Cobas A, Fernandez M, Salazar N, *et al.* Bifidogenic effect and stimulation of short chain fatty acid production in human faecal slurry cultures by oligosaccharides derived from lactose and lactulose. *J Dairy Res* 2009;**76**:317–25.

Cummings JH, Macfarlane GT. The control and consequences of bacterial fermentation in the human colon. *J Appl Bacteriol* 1991;**70**:443–59.

Duncan SH, Barcenilla A, Stewart CS, *et al.* Acetate utilization and butyryl coenzyme A (CoA): acetate-CoA transferase in butyrate-producing bacteria from the human large intestine. *Appl Environ Microb* 2002;**68**:5186–90.

Duncan SH, Louis P, Flint HJ. Lactate-utilizing bacteria, isolated from human feces, that produce butyrate as a major fermentation product. *Appl Environ Microb* 2004;**70**:5810–7.

Duncan SH, Belenguer A, Holtrop G, *et al.* Reduced dietary intake of carbohydrates by obese subjects results in decreased concentrations of butyrate and butyrate-producing bacteria in feces. *Appl Environ Microbiol* 2007;**73**:1073–8.

El-Semman IE, Karlsson FH, Shoaie S, *et al.* Genome-scale metabolic reconstructions of *Bifidobacterium adolescentis* L2–32 and *Faecalibacterium prausnitzii* A2–165 and their interaction. *BMC Syst Biol* 2014;**8**:41.

Falony G, Vlachou A, Verbrugghe K, *et al.* Cross-feeding between *Bifidobacterium longum* BB536 and acetate-converting, butyrate-producing colon bacteria during growth on oligofructose. *Appl Environ Microb* 2006;**72**:7835–41.

Flint HJ, Duncan SH, Scott KP, *et al.* Links between diet, gut microbiota composition and gut metabolism. *P Nutr Soc* 2015;**74**:13–22.

Flint HJ, Scott KP, Duncan SH, *et al.* Microbial degradation of complex carbohydrates in the gut. *Gut Microbes* 2012;**3**:289–306.

Hamer HM, Jonkers D, Venema K, *et al.* Review article: the role of butyrate on colonic function. *Aliment Pharm Ther* 2008;**27**:104–19.

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Kanauchi O, Fujiyama Y, Mitsuyama K, *et al*. Increased growth of *Bifidobacterium* and *Eubacterium* by germinated barley foodstuff, accompanied by enhanced butyrate production in healthy volunteers. *Int J Mol Med* 1999;**3**:175–9.

Lopez-Siles M, Khan TM, Duncan SH, *et al*. Cultured representatives of two major phylogroups of human colonic *Faecalibacterium prausnitzii* can utilize pectin, uronic acids, and host-derived substrates for growth. *Appl Environ Microb* 2012;**78**:420–8.

Lopez-Siles M, Martinez-Medina M, Abella C, *et al*. Mucosa-associated phylotype richness is reduced in inflammatory bowel disease patients. *Appl Environ Microb* 2015, DOI: 10.1128/AEM.02006-15.

Louis P, Duncan SH, McCrae SI, *et al*. Restricted distribution of the butyrate kinase pathway among butyrate-producing bacteria from the human colon. *J Bacteriol* 2004;**186**:2099–106.

Louis P, Young P, Holtrop G, *et al*. Diversity of human colonic butyrate-producing bacteria revealed by analysis of the butyryl-CoA:acetate CoA-transferase gene. *Environ Microbiol* 2010;**12**:304–14.

Machiels K, Joossens M, Sabino J, *et al*. A decrease of the butyrate-producing species *Roseburia hominis* and *Faecalibacterium prausnitzii* defines dysbiosis in patients with ulcerative colitis. *Gut* 2014;**63**:1275–83.

Matsuki T, Watanabe K, Fujimoto J, *et al*. Use of 16S rRNA gene-targeted group-specific primers for real-time PCR analysis of predominant bacteria in human feces. *Appl Environ Microbiol* 2004;**70**:7220–8.

Miyazaki K, Martin JC, Marinsek-Logar R, *et al*. Degradation and utilization of xylans by the rumen anaerobe *Prevotella bryantii* (formerly *P. ruminicola* subsp. *brevis*) B(1)4. *Anaerobe* 1997;**3**:373–81.

Olano-Martin E, Gibson GR, Rastell RA. Comparison of the in vitro bifidogenic properties of pectins and pectic-oligosaccharides. *J Appl Microbiol* 2002;**93**:505–11.

Ramirez-Farias C, Slezak K, Fuller Z, *et al*. Effect of inulin on the human gut microbiota: stimulation of *Bifidobacterium adolescentis* and *Faecalibacterium prausnitzii*. *Br J Nutr* 2009;**101**: 541–50.

Richardson AJ, Calder AG, Stewart CS, *et al*. Simultaneous determination of volatile and non volatile acidic fermentation products of anaerobes by capillary gas-charomatography. *Lett Appl Microbiol* 1989;**9**:5–8.

Pirt S. *Principles of Microbe and Cell Cultivation*. London: Blackwell Scientific Publications, 1975.

Rossi O, Khan MT, Schwarzer M, *et al*. *Faecalibacterium prausnitzii* strain HTF-F and its extracellular polymeric matrix attenuate clinical parameters in DSS-induced colitis. *PLoS One* 2015;**10**:e0123013.

Salazar N, Dewilf EM, Neyrinck AM, *et al*. Inulin-type fructans modulate intestinal Bifidobacterium species populations and decrease fecal short-chain fatty acids in obese women. *Clin Nutr* 2015;**34**:501–7.

Scott KP, Martin JC, Duncan SH, *et al*. Prebiotic stimulation of human colonic butyrate-producing bacteria and bifidobacteria, *in vitro*. *FEMS Microbiol Ecol* 2014;**87**:30–40.

Sokol H, Seksik P, Furet JP, *et al*. Low counts of *Faecalibacterium prausnitzii* in colitis microbiota. *Inflamm Bowel Dis* 2009;**15**:1183–9.

Solís G, de los Reyes-Gavilan CG, Fernández N, *et al*. Establishment and development of lactic acid bacteria and bifidobacteria microbiota in breast-milk and the infant gut. *Anaerobe* 2010;**16**:307–10.

Tojo R, Suarez A, Clemente MG, *et al*. Intestinal microbiota in health and disease: role of bifidobacteria in gut homeostasis. *World J Gastroentero* 2014;**20**:15163–76.

Vitali B, Ndagijimana M, Maccaferri S, *et al*. An *in vitro* evaluation of the effect of probiotics and prebiotics on the metabolic profile of human microbiota. *Anaerobe* 2012;**18**: 386–91.

Wrzosek L, Miquel S, Noordine ML, *et al*. *Bacteroides thetaiotaomicron* and *Faecalibacterium prausnitzii* influence the production of mucus glycans and the development of goblet cells in the colonic epithelium of a gnotobiotic model rodent. *BMC Biol* 2013;**11**:61.

Zhang MM., Qiu XY, Zhang H, *et al*. *Faecalibacterium prausnitzii* inhibits interleukin-17 to ameliorate colorectal colitis in rats. *PLoS One* 2014;**9**:e109146.

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