Interactions between *Bifidobacterium* and *Bacteroides* species in co-fermentations are affected by carbon sources, including exopolysaccharides produced by bifidobacteria.
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Introduction

The colon is a complex microbial ecosystem dominated by obligate anaerobes that reach levels up to 10^{11} cells per gram of intestinal content. *Bacteroides* and *Bifidobacterium* coexist in this ecosystem and they account for up to 20% and 3% of the adult human microbiota respectively. Prebiotics are defined as nondigestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacterial species in the colon, thus improving host health. Bifidobacteria have traditionally been considered as the target of prebiotic action as these substrates can be directly metabolized by these microorganisms; however, some *in vitro* and *in vivo* evidences indicate that the effects of prebiotics also involve other members of the human colon microbiota through the utilization of these substrates in combination with bifidobacteria. The most well studied prebiotics to date are inulin-type fructans (1, 2). Exopolisaccharides (EPS) are complex exocellular polymers, composed of several units of monosaccharides, produced by some bacteria. Although the synthesis of EPS *in vivo* has not been demonstrated yet and the amount of polymer released by the producing bacteria would be presumably low, our previous work indicates that bile stimulates the production of EPS by bifidobacteria under simulated gastrointestinal conditions (3, 4). In addition, EPS could act as fermentable substrates for the human colonic microbiota (5, 6). The fermentation in fecal batch cultures of low amounts of EPS and inulin (0.3% w/v) caused shifts in the synthesis of short chain fatty acids (SCFA), which were related to variations in the levels of some intestinal microbial populations such as *Bacteroides* and *Bifidobacterium* (6).
The aim of this work was to study the influence that the presence of EPS and other carbon sources (inulin and glucose) exert on the interactions between *Bacteroides* and *Bifidobacterium*.

Materials and Methods

Mono- and co-cultures of two strains from different species of *Bifidobacterium* (*B. breve* IPLA20004 and *B. longum* NB667) and *Bacteroides* (*Ba. thetaiotaomicron* DSMZ-2079 and *Ba. fragilis* DSMZ-2151) were carried out. Microbial levels were monitored by qPCR, and SCFA and organic acids were analyzed by HPLC and GC-FID/MS, respectively.
Pair-wise combinations of strains incubated with the different carbon sources were compared with the results obtained from pure cultures of the corresponding strains.

Results and Discussion

In general, the presence of bifidobacteria did not affect the growth of *Bacteroides*. The only exception to this was the delayed growth at prolonged incubation times of *Ba. thetaiotaomicron* cocultured with *B. breve* using glucose as the carbon source. Coculture with *Ba. thetaiotaomicron* did not improve the poor growth displayed by bifidobacteria in pure cultures with complex carbon sources. In contrast, the survival of *Bifidobacterium* increased in the presence of *Ba. fragilis* in most carbohydrate sources so that cocultivation of both microorganisms resulted in higher population levels of *B. breve* and *B. longum* at late stages of incubation than those obtained in the corresponding monocultures (Fig. 1).

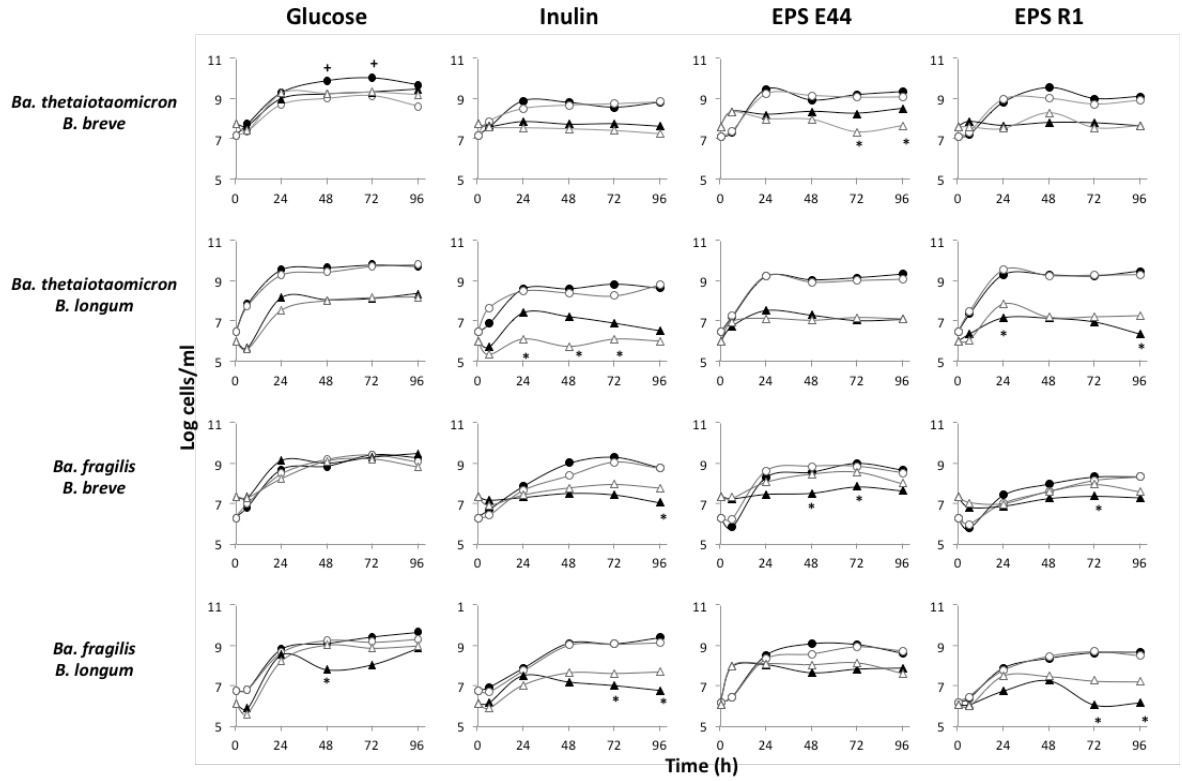

Figure 1. Growth (mean of log cells ml-1) in single culture and in coculture of *Ba. thetaiotaomicron* DSMZ 2079 or *Ba. fragilis* DSMZ 2151 with *B. longum* NB667, or *B. breve* IPLA 20004 in a basal medium supplemented with 0.3% of glucose, inulin, EPS E44 or EPS R1 as carbon source added. •, *Bacteroides* strain growing in single culture; o, *Bacteroides* strain growing in coculture; ▲, *Bifidobacterium* strain growing in single culture; Δ, *Bifidobacterium* strain growing in coculture. The coefficient of variation [SD*100/mean] of data obtained from the three replicates was about 4.2-5.5 %. +, indicates significant differences ($P < 0.05$) of *Bacteroides* counts reached in coculture as compared to the corresponding monoculture. *, indicates significant differences ($P < 0.05$) of *Bifidobacterium* counts reached in coculture as compared to the corresponding monoculture.

The metabolic contribution of each microorganism in coculture was inferred from the level of specific metabolic end-products of carbohydrate fermentation (SCFA and organic acids) corresponding exclusively to each microorganism as well as from the levels of common metabolites synthesized by both bacteria. With glucose as the carbon source, the metabolic activity of *Ba. thetaiotaomicro*n in the presence of *B. breve* was impared as a results of its growth inhibition whereas it seems to remain unaffected in the presence of *B. longum,* promoting in such case a shift towards more formic acid formation at the expense of lactic acid synthesis by *B. longum*. In complex carbon sources, the metabolic activity of *Bacteroides* seems not to be affected by the presence of bifidobacteria. In addition, for all

pair combinations with inulin as carbon source as well as for bifidobacteria and *Ba. thetaiotaomicron* with EPS, the profile of metabolites formed point out to a predominant contribution of *Bacteroides* over bifidobacteria to the formation of SCFA and organic acids in such conditions. In contrast, in cocultures of both bifidobacteria and *Ba. fragilis* DSM-2151 with EPS as the carbon source, the metabolic profile of cocultures as compared to monocultures suggests a joint contribution of bifidobacteria and *Ba. fragilis* to the formation of SCFA and organic acids (Table1).

Table 1. SCFA and organic acid concentrations (mM), in uncontrolled-pH cocultures of *Bifidobacterium* and *Bacteroides* strains, at 72 hours of incubation with glucose (initial levels 12.04 ± 1.38 mM), inulin, EPS E44 or EPS R1 as carbon sources. ↑Ba and ↓Ba indicate significantly higher or lower levels ($P<0.05$), respectively, of a given metabolite in coculture than in the corresponding monoculture of the *Bacteroides* strain. ↑B and ↓B indicate significantly higher or lower levels ($P<0.05$), respectively, of a given metabolite in coculture than in the corresponding monoculture of the *Bifidobacterium* strain. Glucose consumption is indicated for cocultures with this sugar as carbon source.

Carbon source		Control (0h)	*Ba. thetaiotaomicron*		*Ba. fragilis*	
			B. breve	*B. longum*	*B. breve*	*B. longum*
Glucose	Glucose consumption	0	11.20±1.43	12.91±1.83	7.55±0.71 $^{\downarrow B}$	11.33±0.81 $^{\uparrow Ba}$
	Acetic acid	3.77±1.29	24.16±3.15 $^{\uparrow Ba}$	18.20±4.63 $^{\downarrow B}$	19.61±2.24 $^{\uparrow Ba\downarrow B}$	20.88±2.92 $^{\uparrow Ba\downarrow B}$
	Propionic acid	0.48±0.01	0.83±0.26 $^{\downarrow Ba}$	3.89±0.50	6.37±0.62 $^{\downarrow Ba}$	5.83±0.47 $^{\downarrow Ba}$
	Lactic acid	0.08±0.13	0.27±0.10	3.05±1.94 $^{\downarrow B}$	0.00±0.00 $^{\downarrow B}$	5.71±1.18 $^{\downarrow B}$
	Formic acid	0.05±0.07	8.83±1.27 $^{\uparrow Ba}$	2.48±0.19 $^{\uparrow Ba\uparrow B}$	4.75±0.66 $^{\uparrow Ba\downarrow B}$	1.86±0.21 $^{\uparrow Ba}$
	Succinic acid	0.09±0.01	1.04±0.68 $^{\downarrow Ba}$	6.88±0.74	2.95±0.29	2.45±0.30 $^{\downarrow Ba}$
Inulin	Acetic acid	2.02±0.49	3.79±0.34	4.81±0.92	8.21±1.40 $^{\uparrow B}$	8.48±1.10 $^{\uparrow B}$
	Propionic acid	0.46±0.01	1.41±0.06	2.55±0.46	5.34±0.42 $^{\downarrow Ba}$	9.31±1.10
	Succinic acid	0.09±0.02	0.95±0.15	1.25±0.14	1.05±0.18	1.37±0.04
EPS E44	Acetic acid	1.29±0.14	8.52±2.16 $^{\uparrow B}$	8.47±2.47 $^{\uparrow B}$	9.03±1.66 $^{\uparrow Ba\uparrow B}$	6.96±0.99 $^{\uparrow Ba\uparrow B}$
	Propionic acid	0.45±0.00	4.78±1.97	7.24±1.81	6.07±0.85	5.96±1.94
	Succinic acid	0.10±0.02	1.44±0.06 $^{\downarrow Ba}$	2.12±0.22	1.55±0.14	1.52±0.32
EPS R1	Acetic acid	1.33±0.22	7.49±1.99	7.78±1.67 $^{\uparrow B}$	4.81±0.47 $^{\uparrow Ba\uparrow B}$	4.91±0.76 $^{\uparrow Ba\uparrow B}$
	Propionic acid	0.45±0.00	3.51±0.94	6.57±1.00	3.39±0.49	4.07±0.77
	Succinic acid	0.10±0.03	0.96±0.80	1.99±0.17	0.96±0.35	1.44±0.22

The results presented here stress the importance of considering specific species and strains, and not simply high taxonomic divisions, in the relationship among intestinal microbial populations. Variations at the level of species or strain-composition among individuals or human population groups could condition a different response of their intestinal microbiota to specific diets or probiotic and prebiotic interventions.

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