AEM
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Interactions between *Bifidobacterium* and *Bacteroides* Species in Cofermentations Are Affected by Carbon Sources, Including Exopolysaccharides Produced by Bifidobacteria

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Cocultures of strains from two *Bifidobacterium* and two *Bacteroides* species were performed with exopolysaccharides (EPS) previously purified from bifidobacteria, with inulin, or with glucose as the carbon source. *Bifidobacterium longum* NB667 and *Bifidobacterium breve* IPLA20004 grew in glucose but showed poor or no growth in complex carbohydrates (inulin, EPS E44, and EPS R1), whereas *Bacteroides* grew well in the four carbon sources tested. In the presence of glucose, the growth of *Bacteroides thetaiotaomicron* DSM-2079 was inhibited by *B. breve*, whereas it remained unaffected in the presence of *B. longum*. *Ba. fragilis* DSM-2151 contributed to a greater survival of *B. longum*, promoting changes in the synthesis of short-chain fatty acids (SCFA) and organic acids in coculture with respect to monocultures. In complex carbohydrates, cocultures of bifidobacterium strains with *Ba. thetaiotaomicron* did not modify the behavior of *Bacteroides* nor improve the poor growth of bifidobacteria. The metabolic activity of *Ba. fragilis* in coculture with bifidobacteria was not affected by EPS, but greater survival of bifidobacteria at late stages of incubation occurred in cocultures than in monocultures, leading to a higher production of acetic acid than in monocultures. Therefore, cocultures of *Bifidobacterium* and *Bacteroides* can behave differently against fermentable carbohydrates as a function of the specific characteristics of the strains from each species. These results stress the importance of considering specific species and strain interactions and not simply higher taxonomic divisions in the relationship among intestinal microbial populations and their different responses to probiotics and prebiotics.

The colon is a complex microbial ecosystem dominated by obligate anaerobes that reach levels up to 10^{11} cells per gram of intestinal content (1, 2). In spite of the huge diversity of strains, up to 87% of the microbial inhabitants of the human colon belong to only two bacterial phyla, *Bacteroidetes* and *Firmicutes*. *Actinobacteria* and other phyla are present at lower levels (3). Within the group of intestinal *Bacteroidetes*, *Bacteroides* spp. account for up to 20% of the human colon microbiota (4). Although a great variety of *Bacteroides* species has been reported among individuals, *Bacteroides thetaiotaomicron* always seems to be present (5, 6). This species is considered a human symbiont that stabilizes the colon ecosystem, but the genus also harbors some notorious opportunistic and pathogenic species, as is the case of *Bacteroides fragilis* (7). Members of the *Bacteroides* genus are saccharolytic microorganisms producing succinic, acetic, lactic, and propionic acids, but they are also capable of proteolytic fermentation (8). Bifidobacteria account for approximately 3% of the adult human microbiota (9) and are frequently identified as probiotics, based on the implied health-promoting benefits attributed to some strains (10). *Bifidobacterium longum* is one of the predominant species in adult humans. This species and *Bifidobacterium breve* are also abundant in the intestine of infants. Bifidobacteria produce lactic and acetic acids as the main metabolic end products of carbohydrate fermentation and smaller amounts of formic acid and ethanol (11). 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 (12). Many of the health-promoting effects attributed to prebiotic substrates are due to their suitability to be fermented by the colonic microbiota producing short-chain fatty acids (SCFA). Bifidobacteria have traditionally been considered 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 could also indirectly 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 (13–15). Some researchers have previously demonstrated different degradation mechanisms of oligofructose and inulin-like fructans by *Bifidobacterium* and *Bacteroides* species in pure cultures, as well as in cocultures (14–16).

Some bifidobacteria are able to produce exopolysaccharides (EPS), which are complex polymers composed of several units of monosaccharides (17). EPS from bifidobacteria may be released *in situ* by microorganisms of this species inhabiting the human colon or may be produced by probiotics present as adjunct cultures in fermented dairy products. Although the synthesis of EPS *in vivo* has not been demonstrated 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 in *in vitro* simulated gastrointestinal conditions (17,

Received 29 July 2013 Accepted 20 September 2013
Published ahead of print 27 September 2013
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doi:10.1128/AEM.02545-13

18). In addition, EPS could act as fermentable substrates for the human colonic microbiota (19, 20). The fermentation in fecal batch cultures of small amounts of EPS and inulin (0.3%, wt/vol) caused shifts in the synthesis of SCFA related to variations in the levels of some intestinal microbial populations, such as *Bacteroides* and *Bifidobacterium* (20). Therefore, in the present work, we selected strains from two species of *Bifidobacterium* (*B. breve* and *B. longum*) and two species of *Bacteroides* (*Ba. thetaiotaomicron* and *Ba. fragilis*) as a model of study, in order to gain an insight into the influence that the presence of EPS and other carbon sources could exert on the interactions between members of these two intestinal microbial groups, by growing them separately and together.

MATERIALS AND METHODS

Bacterial strains. Two *Bacteroides* and two *Bifidobacterium* strains belonging to different species were used in monocultures and cocultures in this study. *Ba. thetaiotaomicron* DSM-2079 and *Ba. fragilis* DSM-2151 strains were obtained from the DSMZ bacterial culture collection (Braunschweig, Germany). *B. longum* NB667 was from the NIZO food research culture collection (Ede, The Netherlands), and *B. breve* IPLA20004 (also named *B. breve* BM 12/11) was isolated from breast milk (21) and is held in the IPLA's culture collection. Species identity was confirmed by partial amplification of the 16S rRNA gene using primers plb16 and mlb16 (22) and by sequencing and alignment with sequences from reference strains held in the GenBank database. Strains from frozen stocks were reactivated in Gifu anaerobic medium (GAM) broth (Nissui Pharmaceutical Co, Tokyo, Japan) and in MRS broth (BioKar Diagnostics, Beauvais, France) supplemented with 0.25% (wt/vol) of L-cysteine (Sigma Chemical Co., St. Louis, MO) (named GAMc and MRSc) for *Bacteroides* and *Bifidobacterium*, respectively. Strains were incubated overnight at 37°C in an anaerobic cabinet (Mac 1000; Don Whitley Scientific, West Yorkshire, United Kingdom) under a 10% H_2, 10% CO_2, and 80% N_2 atmosphere. To prepare the inoculum stocks, 10 ml of modified carbohydrate-free basal medium (mCFBM; composition specified below) with 1% (wt/vol) glucose was inoculated (1% [vol/vol]) with cultures of *Bifidobacterium* and *Bacteroides* strains and incubated for 16 to 18 h, as indicated before. Cultures were then centrifuged at 12,000 × *g* for 10 min and resuspended in the same volume of mCFBM without a carbon source. Inocula were frozen under liquid N_2 and stored at −80°C until use.

EPS isolation. EPS fractions produced by *Bifidobacterium animalis* subsp. *lactis* IPLA R1, a dairy origin strain (23), and by *B. longum* subsp. *longum* IPLA E44, a fecal isolate from a healthy adult (24), were isolated and purified from the cellular biomass harvested from agar-MRSc agar plates as specified by Ruas-Madiedo et al. (23).

Batch culture fermentation. Uncontrolled-pH batch cultures were performed in the nondefined peptone and yeast extract containing CFBM, previously described by Salazar et al. (20). For the present work, it was modified by the addition of vitamin B_{12} (10 mg liter^{-1}), vitamin K (2 mg liter^{-1}), vitamin B_1 (2 mg liter^{-1}), pyridoxal (1 mg liter^{-1}), calcium pantothenate (2 mg liter^{-1}), folic acid (1 mg liter^{-1}), riboflavin (1 mg liter^{-1}), biotin (1 mg liter^{-1}), nicotinic acid (3 mg liter^{-1}), para-aminobenzoic acid (1 mg liter^{-1}), and a solution (2 ml liter^{-1}) of ferrous citrate (25 mM) and trisodium citrate (75 mM) (mCFBM). The final pH of the medium ranged between 6.7 and 7.0.

Pairwise combinations of *Bifidobacterium* and *Bacteroides* strains, as well as monocultures of strains, were performed in mCFBM with an added 0.3% (wt/vol) of glucose, inulin, or purified EPS E44 or EPS R1 fractions. The corresponding frozen inocula were added (1% [vol/vol]) to 3.5 ml of the culture medium. Trials of cocultures and the corresponding monocultures in different carbon sources were run in triplicate for a period of 72 h at 37°C under anaerobic conditions. Samples were obtained at fixed times for microbial counts and SCFA and organic acid analyses.

The ability to utilize lactic acid by the two *Bacteroides* strains considered in this work was tested in mCFBM, with 0.15% lactic acid (vol/vol) added as the carbon source. Additionally, the ability of *Bacteroides* strains to use the organic nitrogen compounds present in the culture medium was assessed in mCFBM by determining growth and the ability to produce branched-chain fatty acids (BCFA). Cultures were incubated for 72 h in anaerobic conditions as indicated above. At the end of incubation, optical density at 600 nm was determined in cultures, and samples were taken for SCFA and organic acid analyses.

Estimation of bacterial growth by qPCR. Quantification (cell counts ml^{-1}) of *Bifidobacterium* and *Bacteroides* species growing in monoculture and coculture was performed throughout fermentations by quantitative PCR (qPCR) using DNA isolated from batch cultures. Standard curves were obtained by converting 16S rRNA gene copies to cell counts obtained in pure cultures of each strain growing in MRSc in the case of *Bifidobacterium* and GAMc for *Bacteroides*. Primers and conditions were those previously described (25).

Analysis of SCFA, organic acids, and glucose. Cell-free supernatants from cultures were filtered through 0.2-μm-pore-size filters. Identification and quantification of SCFA and BCFA were carried out by gas chromatography-mass spectrometry/flame ionization detector (MS/FID), using a system composed of a 6890N gas chromatograph (Agilent Technologies Inc., Palo Alto, CA, USA) connected with an FID and an MS 5973N detector (Agilent), as described previously (19, 26). A high-performance liquid chromatography (HPLC) system composed of an Alliance 2695 separation module, a photodiode array (PDA) detector (Waters 996), a refractive index detector (Waters 2414), and Empower software (Waters, Milford, MA) was employed. The PDA detector was used for quantification of organic acids at 210 nm, whereas the amount of glucose was analyzed with the refractive index detector. Chromatographic conditions were those indicated previously by Salazar et al. (27). Results of SCFA, BCFA, and organic acids were expressed in millimolar concentrations.

Calculation of carbon recovery. Carbon recovery (CR), expressed in percentages, was calculated by comparing the total amount of carbon recovered in the metabolites analyzed to the total amount of glucose consumed. For *Bacteroides* strains, the production of one mole of CO_2 for every mole of acetic acid formed (+ 1 × [acetic acid] in the equation below) was considered, as well as the uptake of one mole of CO_2 for every mole of succinic acid produced (− 1 × [succinic acid] in the equation) (16, 28–30).

The following equations were used: CR of bifidobacteria ≥ 100 × (3 × [lactic acid] + 2 × [acetic acid] + 1 × [formic acid]/6 × [glucose consumed]) and CR of bacteroides ≥ 100 × (2 × [acetic acid] + 3 × [propionic acid] + 4 × [succinic acid] + 1 × [formic acid] +1 × [acetic acid] − 1 × [succinic acid]/6 × [glucose consumed]).

Statistical analysis. Statistical analyses were performed using the SPSS-PC software, version 19.0 (SPSS Inc., Chicago, IL, USA). One-way analysis of variance (ANOVA) tests were run in monocultures of *Bacteroides* and *Bifidobacterium* for the different SCFA and organic acids. Strains were used as factors, with two categories corresponding to the different species of each genus analyzed. One-way ANOVA was also performed to compare the results of the different parameters by using cocultures versus monocultures and time of incubation as factors. When necessary, a *post hoc* least significant difference (LSD) comparison test was applied to determine statistical differences between categories.

RESULTS AND DISCUSSION

Behavior of *Bifidobacterium* species growing in pure culture. In pure culture, *B. longum* NB667 was able to grow in glucose, inulin, and EPS ($P < 0.05$), whereas *B. breve* IPLA20004 displayed significant growth only in cultures with glucose but not with EPS or inulin (Fig. 1). In monocultures of bifidobacteria, the pH decreased by about 2.5 units throughout fermentation when glucose was the carbon source, whereas it showed little variation in complex carbohydrates (data not shown).

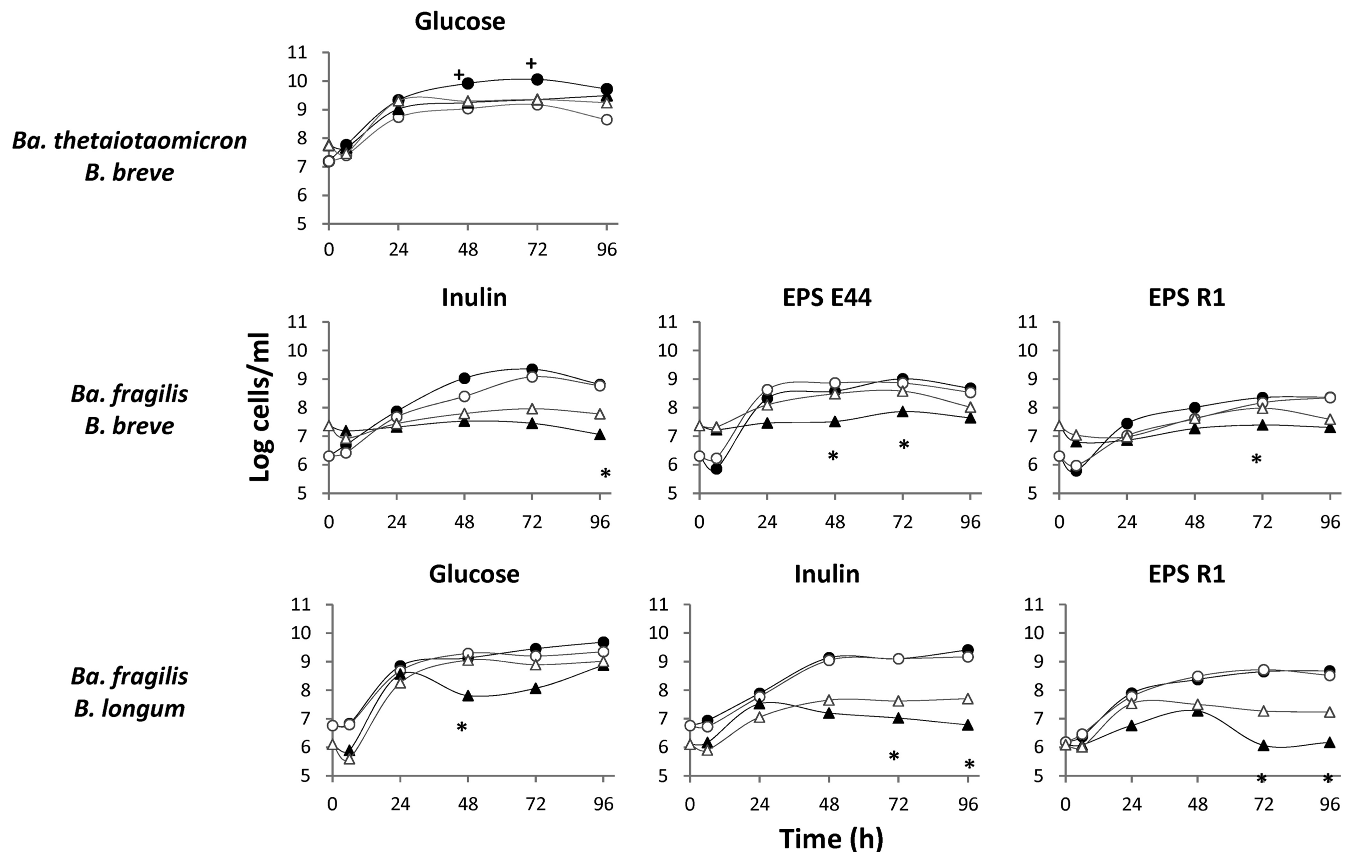

FIG 1 Growth (mean of log cells ml^{-1}) in single culture and in coculture of *Ba. thetaiotaomicron* DSMZ 2079 or *Ba. fragilis* DSMZ 2151 with *B. longum* NB 667 or *B. breve* IPLA 20004 in a basal medium supplemented with 0.3% glucose, inulin, EPS E44, or EPS R1 as a carbon source. ●, *Bacteroides* strain growing in single culture; ○, *Bacteroides* strain growing in coculture; ▲, *Bifidobacterium* strain growing in single culture; △, *Bifidobacterium* strain growing in coculture. The coefficient of variation (standard deviation × 100/mean) of data obtained from the three replicates was about 4.2 to 5.5%. +, significant differences ($P < 0.05$) of *Bacteroides* counts reached in coculture compared to the corresponding monoculture; *, significant differences ($P < 0.05$) of *Bifidobacterium* counts reached in coculture compared to the corresponding monoculture. Among all the possible culture combinations of *Bacteroides* and *Bifidobacterium* strains, the sole combination showing significant variation in the growth of *Bacteroides* and those enhancing significantly the survival of *Bifidobacterium* ($P < 0.05$) are presented.

Glucose was consumed almost completely after 72 h of fermentation in mCFBM cultures of both strains, with a carbon recovery above 90%. Although acetic acid was the most abundant metabolite formed, clear differences between the metabolic profiles of both *Bifidobacterium* strains were found (Table 1). Thus, whereas *B. longum* NB667 formed considerable amounts of lactic acid and smaller amounts of formic acid, *B. breve* IPLA20004 produced more formic than lactic acid.

Glucomannans from yeast extract present in the culture medium interfere with the detection and quantification of EPS and inulin (19), and, therefore, the calculation of the polymer consumed for cultures with added EPS was not possible in our experimental conditions. The fermentation pattern in cultures with complex carbohydrates by *Bifidobacterium* differed from those obtained with glucose and led mainly to the formation of small amounts of acetic acid in cultures of both strains (Table 1). Several authors have previously demonstrated a metabolic shift in the glycolytic pathway toward more acetic and formic acids and ethanol production at the expense of lactic acid in bifidobacteria when growth slows (31–33). In this way, less readily fermentable energy sources lead to more ATP formed per mole of sugar consumed (14, 15, 34). The predominant acetic acid production together with the limited or no increase of *B. breve* and *B. longum* population levels in cultures with EPS and inulin support the conditions of limited access to energy from these carbohydrates.

Behavior of *Bacteroides* species growing in pure culture. *Ba. thetaiotaomicron* DSM-2079 and *Ba. fragilis* DSM-2151 grew well in the carbon sources tested (Fig. 1). The pH during incubation decreased more in glucose (1.5 to 1.9 units) than in cultures with EPS (0.3 to 0.7 units). In inulin as the carbohydrate source, *Ba. fragilis* was able to promote a more pronounced pH decrease than *Ba. thetaiotaomicron* (1 pH unit compared to 0.1), which is in line with the higher SCFA production by *Ba. fragilis* than by *Ba. thetaiotaomicron* (Table 1). About 60 to 70% of the glucose was consumed after 72 h of fermentation in pure cultures of both strains (Table 1). In spite of this, CR at this time was nearly 100% in cultures of *Ba. thetaiotaomicron* and higher than this value in cultures of *Ba. fragilis*. A reason for this may be the fermentation of carbohydrates different from glucose and/or organic nitrogen compounds present in the culture medium. In this respect, it is known that members of the genus *Bacteroides* can ferment proteins and amino acids producing BCFA (35–37). We corroborated that *Ba. thetaiotaomicron* DSM-2079 and *Ba. fragilis* DSM-2151 grew slowly in mCFBM, producing BCFA (mainly isobutyric and

TABLE 1 SCFA and organic acid concentrations and glucose consumption obtained in uncontrolled-pH monocultures of *Bifidobacterium* and *Bacteroides* species at 72 h of incubation with glucose, inulin, EPS E44, and EPS R1 as carbon sources[a]

Carbon source	Species	Glucose consumption (mM)	Concn (mM)					Carbon recovery (%)
			Acetic acid	Propionic acid	Lactic acid	Succinic acid	Formic acid	
Glucose	Control (0 h)	0	3.77 ± 1.29	0.48 ± 0.01	0.08 ± 0.13	0.09 ± 0.01	0.05 ± 0.07	NA
	Ba. thetaiotaomicron	8.91 ± 1.41	10.94 ± 1.81	3.74 ± 0.50*	–	6.18 ± 0.73*	1.83 ± 0.31	97 ± 12
	Ba. fragilis	7.08 ± 1.24	12.58 ± 0.77	10.28 ± 1.00	–	3.24 ± 0.30	1.33 ± 0.20	132 ± 27
	B. breve	11.29 ± 1.22	29.24 ± 4.65	–	1.96 ± 0.55*	–	9.50 ± 1.26*	97 ± 14
	B. longum	12.88 ± 2.38	27.21 ± 4.31	–	10.15 ± 0.85	–	1.75 ± 0.13	102 ± 10
Inulin	Control (0 h)	NA	2.02 ± 0.49	0.46 ± 0.01	–	0.09 ± 0.02	–	NA
	Ba. thetaiotaomicron	NA	4.86 ± 0.80*	2.25 ± 0.40*	–	1.26 ± 0.12	–	NA
	Ba. fragilis	NA	9.55 ± 1.44	9.66 ± 1.47	–	1.22 ± 0.21	–	NA
	B. breve	NA	2.95 ± 0.51	–	–	–	–	NA
	B. longum	NA	3.10 ± 0.76	–	–	–	–	NA
EPS E44	Control (0 h)	NA	1.29 ± 0.14	0.45 ± 0.00		0.10 ± 0.02	–	NA
	Ba. thetaiotaomicron	NA	7.40 ± 1.59	6.29 ± 1.92		1.79 ± 0.32	–	NA
	Ba. fragilis	NA	6.12 ± 1.09	6.50 ± 0.32		1.72 ± 0.30	–	NA
	B. breve	NA	3.74 ± 1.06*	–	–	–	–	NA
	B. longum	NA	1.97 ± 0.18	–	–	–	–	NA
EPS R1	Control (0 h)	NA	1.33 ± 0.22	0.45 ± 0.00		0.10 ± 0.03	–	NA
	Ba. thetaiotaomicron	NA	7.02 ± 1.74*	5.58 ± 1.96		1.45 ± 0.79	–	NA
	Ba. fragilis	NA	3.71 ± 0.51	4.09 ± 0.93		1.45 ± 0.24	–	NA
	B. breve	NA	3.08 ± 0.61*	–	–	–	–	NA
	B. longum	NA	1.68 ± 0.23	–	–	–	–	NA

[a] Initial glucose level in the culture medium was 12.04 ± 1.38 mM. *, significant differences between strains from the same genus ($P < 0.05$); –, no detection or detection below the quantification limit; NA, not applicable.

isovaleric acids) and SCFA (experimental data not shown). This provides a rationale for the high CR values obtained in pure cultures of *Ba. thetaiotaomicron* and *Ba. fragilis*.

Different fermentation patterns were evidenced between *Ba. thetaiotaomicron* DSM-2079 and *Ba. fragilis* DSM-2151 regarding the production of SCFA (Table 1). Although acetic acid was the most abundant metabolite produced from glucose, in cultures of *Ba. thetaiotaomicron*, it was followed in abundance by succinic and then propionic acids, whereas *Ba. fragilis* produced clearly more propionic than succinic acid (Table 1). In complex carbon sources (inulin and EPS), the metabolic profile also differed between *Bacteroides* strains (Table 1). Propionic acid was the most abundant metabolite produced by *Ba. fragilis*, followed by acetic acid, whereas *Ba. thetaiotaomicron* produced more acetic than propionic acid. Previous studies by other authors indicated that the fermentation product profile from carbohydrates by *Bacteroides* greatly differed depending on the substrates. Succinic acid was generally the main metabolite produced at short generation times, whereas the proportions of acetic and propionic acids increased at long generation times or with less readily fermentable carbohydrates (28, 30, 38). Our results confirm these observations, as the proportion of propionic to succinic acid was higher in complex carbon sources than in glucose.

Interaction of *Bacteroides* and *Bifidobacterium* species in coculture. Decreases in pH paralleled the increases in SCFA concentrations in cocultures of *Bifidobacterium* and *Bacteroides*. In inulin, cocultures with *Ba. fragilis* reached higher concentrations of SCFA and succinic acid than cocultures with *Ba. thetaiotaomicron* (Table 2), thus leading to more pronounced pH decreases in the former.

Bacterial levels and metabolite production by pairwise combinations of *Bifidobacterium* and *Bacteroides* strains incubated with the different carbon sources were compared with the results obtained from pure cultures of the corresponding strains. In general, *Bacteroides* reached higher population levels than *Bifidobacterium* in cocultures, and the presence of bifidobacteria seems not to affect the growth of *Bacteroides* (data not shown). The only exception to this was the delayed growth at prolonged incubation times of *Ba. thetaiotaomicron* cocultured with *B. breve* when glucose was used as the carbon source (Fig. 1). A possible explanation for this inhibition could be the production under such conditions of antimicrobial compounds by *B. breve* (39) or the outcompetition at prolonged incubation times of bifidobacteria by using carbon sources still available in the culture medium and not consumed by *Ba. thetaiotaomicron*. Relating to this, we have previously reported on the inhibition by *B. longum* of other Gram-positive bacteria growing in combined culture (40, 41). Coculture with *Ba. thetaiotaomicron* did not improve the poor growth displayed by bifidobacteria in pure cultures with complex carbon sources (data not shown). 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).

Both *Bifidobacterium* and *Bacteroides* species can produce acetic and formic acids. *Bacteroides* is able to form succinic acid, whereas this compound is not synthesized or is produced in very small amounts by bifidobacteria in any condition. Finally, while *Bacteroides* is a propionic acid producer, the metabolic pathway for the synthesis of propionic acid is not present in bifidobacteria

TABLE 2 SCFA and organic acid concentrations in uncontrolled-pH cocultures of *Bifidobacterium* with *Bacteroides* strains at 72 h of incubation with glucose (initial levels of 12.04 ± 1.38 mM), inulin, EPS E44, or EPS R1 as carbon sources[a]

Carbon source	Sugar consumption or SCFA and organic acid formation	Concn (mM) of glucose consumed or SCFA and organic acids formed				
			Ba. thetaiotaomicron		*Ba. fragilis*	
		Control (0 h)	*B. breve*	*B. longum*	*B. breve*	*B. longum*
Glucose	Glucose consumption	0	11.20 ± 1.43	12.91 ± 1.83	7.55 ± 0.71 $^{\downarrow \mathrm{B}}$	11.33 ± 0.81 $^{\uparrow \mathrm{Ba}}$
	Acetic acid	3.77 ± 1.29	24.16 ± 3.15 $^{\uparrow \mathrm{Ba}}$	18.20 ± 4.63 $^{\downarrow \mathrm{B}}$	19.61 ± 2.24 $^{\uparrow \mathrm{Ba} \downarrow \mathrm{B}}$	20.88 ± 2.92 $^{\uparrow \mathrm{Ba} \downarrow \mathrm{B}}$
	Propionic acid	0.48 ± 0.01	0.83 ± 0.26 $^{\downarrow \mathrm{Ba}}$	3.89 ± 0.50	6.37 ± 0.62 $^{\downarrow \mathrm{Ba}}$	5.83 ± 0.47 $^{\downarrow \mathrm{Ba}}$
	Lactic acid	0.08 ± 0.13	0.27 ± 0.10	3.05 ± 1.94 $^{\downarrow \mathrm{B}}$	0.00 ± 0.00 $^{\downarrow \mathrm{B}}$	5.71 ± 1.18 $^{\downarrow \mathrm{B}}$
	Formic acid	0.05 ± 0.07	8.83 ± 1.27 $^{\uparrow \mathrm{Ba}}$	2.48 ± 0.19 $^{\uparrow \mathrm{Ba} \uparrow \mathrm{B}}$	4.75 ± 0.66 $^{\uparrow \mathrm{Ba} \downarrow \mathrm{B}}$	1.86 ± 0.21 $^{\uparrow \mathrm{Ba}}$
	Succinic acid	0.09 ± 0.01	1.04 ± 0.68 $^{\downarrow \mathrm{Ba}}$	6.88 ± 0.74	2.95 ± 0.29	2.45 ± 0.30 $^{\downarrow \mathrm{Ba}}$
Inulin	Acetic acid	2.02 ± 0.49	3.79 ± 0.34	4.81 ± 0.92	8.21 ± 1.40 $^{\uparrow \mathrm{B}}$	8.48 ± 1.10 $^{\uparrow \mathrm{B}}$
	Propionic acid	0.46 ± 0.01	1.41 ± 0.06	2.55 ± 0.46	5.34 ± 0.42 $^{\downarrow \mathrm{Ba}}$	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 $^{\uparrow \mathrm{B}}$	8.47 ± 2.47 $^{\uparrow \mathrm{B}}$	9.03 ± 1.66 $^{\uparrow \mathrm{Ba} \uparrow \mathrm{B}}$	6.96 ± 0.99 $^{\uparrow \mathrm{Ba} \uparrow \mathrm{B}}$
	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 $^{\downarrow \mathrm{Ba}}$	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 $^{\uparrow \mathrm{B}}$	4.81 ± 0.47 $^{\uparrow \mathrm{Ba} \uparrow \mathrm{B}}$	4.91 ± 0.76 $^{\uparrow \mathrm{Ba} \uparrow \mathrm{B}}$
	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

[a] ↑ 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 the carbon source.

(15, 28, 29, 32). Therefore, the metabolic contribution of microorganisms in coculture was inferred from the levels of propionic and succinic acids produced by *Bacteroides*, as well as from the levels of other common metabolites from carbohydrate fermentation (SCFA and organic acids) synthesized by both bacteria. With glucose as the carbon source, acetic acid reached levels in cocultures of *Ba. thetaiotaomicron* and *B. breve* similar to those in pure cultures of the bifidobacteria, whereas considerably smaller amounts of propionic and succinic acids were obtained from cocultures than in the monocultures of *Bacteroides* (Table 2). This suggests an impairment of the metabolic activity of *Ba. thetaiotaomicron* in the presence of *B. breve* as a consequence of its growth inhibition. In the remaining *Bacteroides* and *Bifidobacterium* combinations, acetic acid attained intermediate levels between the lower concentration reached by the monocultures of *Bacteroides* and the higher level of the monocultures of bifidobacteria. Specifically, in cocultures of *Ba. thetaiotaomicron* DSM-2079 and *B. longum* NB667, the production of propionic and succinic acids was similar to that in the monoculture of *Bacteroides*, suggesting that the metabolic activity of *Ba. thetaiotaomicron* probably remained unaffected under such conditions; however, in combined cultures of *Ba. fragilis* and bifidobacteria, lower propionic concentrations, and similar or lower levels of succinic acid than in *Bacteroides* monocultures, were obtained. This pointed to a probable slowdown of the metabolic activity of *Ba. fragilis* when bifidobacteria were present. On the other hand, lower lactic acid levels were obtained in most cocultures in glucose with respect to the monocultures of the corresponding *Bifidobacterium* strain, as was previously reported in coculture fermentations of bifidobacteria and bacteroides with inulin-type fructans (16). *Ba. thetaiotaomicron* DSM-2079 and *Ba. fragilis* DSM-2151 growing alone contributed scarcely both to the consumption of lactic acid present in the culture medium (14 to 16%) and to the production of formic acid (experimental data not shown). These findings, together with the increase of formic acid in cocultures of *Ba. thetaiotaomicron* and *B. longum* with respect to the corresponding monocultures, point to shifts in the metabolism of lactic and formic acids by one or both microorganisms when they are growing together.

With complex carbon sources, acetic acid was generally the most abundant metabolite produced in cocultures, followed by propionic acid and smaller amounts of succinic acid. Propionic and succinic acid levels in cocultures were similar to levels attained in the corresponding monocultures of *Bacteroides* for most pairwise combinations of strains, indicating that the metabolism of *Bacteroides* was probably not affected by the presence of bifidobacteria. In the presence of EPS, cocultures with *Ba. thetaiotaomicron* displayed levels of acetic acid close to the concentrations reached by monocultures of *Bacteroides*. In contrast, higher production of acetic acid was obtained in cocultures of bifidobacteria and *Ba. fragilis* DSM-2151 with EPS as the carbon source than in the corresponding monocultures of *Bifidobacterium* and *Bacteroides*, thus indicating an enhancement of the production of this acid in cocultures with *Ba. fragilis*. Therefore, the behavior of bifidobacterium strains in the same substrate appears to be influenced by the growth and metabolic characteristics of the *Bacteroides* strain present in the same environment. Falony et al. (16) indicated that the capacity of several *Bifidobacterium* strains to compete with *Ba. thetaiotaomicron* for the use of inulin-type fructans was dependent on the ability of the bifidobacteria to degrade fructose and oligofructose in addition to inulin. Using germfree mice colonized with *Ba. thetaiotaomicron* and *B. longum*, an expansion in the diversity of polysaccharides targeted for degradation by *B. thetaiotaomicron* has been observed in the presence of the bifidobacteria, demonstrating an adaptation for substrate utilization by both species in response to one another (42).

In short, differences in growth and metabolic characteristics of *Bifidobacterium* and *Bacteroides* strains can influence their joint behavior against EPS and other fermentable carbohydrate sources available in the growth environment. 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.

ACKNOWLEDGMENTS

This work was financially supported by the project AGL2010-16525 from the Spanish Ministry of Economy and Competitiveness (MINECO). David Rios-Covian was funded by a predoctoral fellowship from MINECO (Formación de Personal Investigador Program [FPI]), and Silvia Arboleya was supported by a predoctoral Junta de Ampliación de Estudios Program (JAE) fellowship from CSIC, Spain.

The excellent technical assistance of María Fernández-García and Lidia Aláez is greatly appreciated.

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