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Mother-to-Child Transmission of and Multiple-Strain Colonization by *Bacteroides fragilis* in a Cohort of Mothers and Their Children▽†

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Bacteroides fragilis **represents an early infant colonizer with important host interactions. Our knowledge about the diversity, transmission, and persistence of this bacterium, however, is limited. Here, we addressed these questions using a combination of multilocus sequence typing (MLST) and variable-number tandem repeat (VNTR) sequence analyses. We used both culture-dependent and -independent typing. We genotyped *B. fragilis* in fecal samples from a cohort of 93 mothers and their children, with samples taken from the mothers and from the children at the ages 1 to 10 days, 4 months, 1 year, and 2 years. By MLST we found two main *B. fragilis* groups, which we denoted clades A and B. Direct typing of stool samples using the *icd* gene revealed seven sequence types, five within clade A and two within clade B. A single clade A sequence type, however, represented 79% of all the sequences. This sequence type was further subtyped using VNTR. VNTR subtyping revealed 16 different VNTR types. Based on the distribution patterns of these, we show mother-to-child transmission and multiple-strain colonization. We argue that negative host selection promotes the coexistence of multiple strains. The significance of our findings is that we have started unraveling the transmission and persistence patterns of one of the most important human gut colonizers.**

Bacteroides fragilis is both an important pathogen and a commensal bacterium within the human gut. *B. fragilis* is highly prevalent in the human population and does not have other known niches than the gut of mammals (21) Despite its importance, our knowledge about the transmission and persistence of commensal *B. fragilis* within a healthy human population is very limited. Most of the research attention until now has been on disease-associated strains. These strains include enterotoxigenic strains (28) in addition to nonenterotoxigenic strains associated with anaerobic bacteremia (3) and a range of opportunistic diseases (23).

The aim of the present work was therefore to use a newly developed multilocus sequence typing (MLST) directly on feces (4) in combination with a variable-number tandem repeat (VNTR) sequence approach (35) to determine the *B. fragilis* transmission, persistence, and correlation to allergen-specific IgE in an unselected cohort of mothers and their children. This was done using material collected from the IM-PACT cohort, which is a part of the large prospective Prevention of Allergy among Children in Trondheim (PACT) study in Norway. PACT focuses on the impacts of systematic and structured interventions on childhood allergy, while IM-PACT is the immunology and microbiology (IM) part of PACT (20).

B. fragilis contacts the epithelial mucosa, and it demonstrates a closer host interaction than the other *Bacteroides* species (16). *B. fragilis* is able to modulate the surface structure, and 28 polysaccharide loci have been identified (22). The production of these is switched on and off by a reversible inversion of promoter-containing DNA segments (12). Recent data indicate that phase-variable surface structure is important for the persistence of *B. fragilis* within the gut (14). With respect to bacteria-bacteria competition, it has been shown that *B. fragilis* can produce a variety of bacteriocins which are important for suppressing closely related species (25).

There is relatively strong evidence that *B. fragilis* has immune-modulating properties, which is important for maturation of the human immune system (27). For the IM-PACT cohort, we have recently shown that, at the age of 1 and 2 years, the levels of *B. fragilis* are higher in children with high specific IgE (sIgE) (32). IgE plays a central role in asthma and allergy (8). Other studies have shown a correlation of *B. fragilis* with asthma (34) and pollen allergy (19).

The main findings presented demonstrate mother-to-child transmission of *B. fragilis* and indicate that multiple strains can colonize a single child. We argue that the multiple-strain coexistence is promoted by negative mucosal selective pressure.

MATERIALS AND METHODS

Test material. In this study we analyzed 483 fecal samples from a total of 93 mothers and their children in connection with the IM-PACT study, which was started in Trondheim in 2001. Stool samples were taken from mothers around gestational weeks 13 and 38 and from their children at ages 1 to 10 days, 4 months, 1 year, and 2 years. The stool samples were immediately frozen in Cary-Blair transport medium (0.5% NaCl, 0.11% Na_2HPO_4, 0.15% $HSCH_2COONa$, 0.09% $CaCl_2$, pH 8.4) (18) at −20°C and transferred to −80°C for long-term storage. Venous blood samples from the children were taken at 2 years of age for specific IgE measurements to determine allergic sensitization, defined as an sIgE of >0.35 kU/ml for at least one allergen (32). IM-PACT is an extension of the PACT study of allergy in children in Trondheim. PACT began in 2000 with the objective of reducing the increase in the incidence of asthma and allergy and is described in detail elsewhere (31). Participants in the IM-PACT trial were recruited from the main study control cohort (20).

Cultivation of *B. fragilis* from stool samples. Cultivation of *B. fragilis* was carried out using selective medium (*Bacteroides* bile esculin agar with amikacin;

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BD Diagnostic Systems, Heidelberg, Germany) with incubation at 37°C for 48 h. Incubation took place under anaerobic conditions created by an Oxoid AnaeroGen system (Oxoid Ltd., Hampshire, England). Single colonies were then picked and transferred to 200 μl of Cary-Blair transport medium and frozen at −80°C.

Isolation of DNA from stool samples. For sequencing analyses, we used DNA purified with paramagnetic beads in accordance with an optimized and automated protocol (29). For VNTR sequence analyses, DNA was reisolated from stool samples using a QIAamp DNA Stool Mini Kit (Qiagen, Hilden, Germany). The protocol for isolation of DNA from stool samples for pathogen detection was followed.

Isolation of DNA from bacterial isolates. Bacterial cell pellets from five strains of *B. fragilis* from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ; Braunschweig, Germany) were rehydrated in 500 μl of Tris-EDTA (TE) buffer. For DNA isolation we used a DNeasy Blood and Tissue Kit (Qiagen), following the protocols for pretreatment of Gram-negative bacteria. For isolation of DNA from growing colonies of *B. fragilis*, we used PrepMan Ultra Sample Preparation Reagent according to the manufacturer's recommendations (Applied Biosystems, Foster City, CA).

Primer design. CLC Main Workbench (version 4.1.1) (CLCbio, Aarhus, Denmark) was used for the design of both the MLST and VNTR primers and analysis of sequenced fragments.

Construction of the MLST primers was based on the seven published MLST genes for *Escherichia coli* (24). These were identified in the sequenced genomes of *B. fragilis* strain NCTC 9343 and *B. fragilis* YHC46. Aligned sequences were used as templates for the design of forward and reverse primers in conserved regions using standard criteria for primer design. Subsequent to design, primer pairs for each of the housekeeping genes were examined for specificity using the Basic Local Alignment Search Tool (BLAST) in the NCBI database (blast.ncbi.nlm.nih.gov/Blast).

The five *B. fragilis* strains from DSMZ were used in the evaluation. Optimal annealing for PCR was chosen on the basis of visualization of PCR products by agarose gel electrophoresis. Only primers that gave consistent amplification were included in the final work (see Table S1 in the supplemental material).

To identify VNTR sequences, the mreps web server (bioinfo.lifl.fr/mreps/mreps.php) was used. mreps is a software program designed to identify tandem repeats in a DNA sequence. This program has no restrictions on the size of the repeated pattern and can therefore be used to detect all types of tandem repeats (11). The two sequenced and annotated genomes of *B. fragilis* strain NCTC 9343 (2) and *B. fragilis* YCH46 (13) were analyzed by the program with the error margin set to zero and no filtering for maximum or minimum length of the tandem repetitive sequence or the number of repetitions. The VNTR sequences found in these *B. fragilis* strains were further sorted using Excel to find matching regions. Sequences flanking the regions of matching VNTR sequences found in the two *B. fragilis* strains were used as templates for the design of VNTR primers using the same criteria and evaluation as for the MLST primers. PCR primer pairs for 12 VNTR sequences were constructed and evaluated, while only the primer pair that gave variation and consistent amplification for all the strains evaluated was used in the screening (see Table S2 in the supplemental material).

PCR amplification and DNA sequencing. PCRs were performed in a 25-μl reaction volume, containing 1 μl of DNA solution as template, 1× Phusion HF buffer (providing 1.5 mM $MgCl_2$) (Finnzymes, Espoo, Finland), 200 μM each deoxynucleoside triphosphate (dNTP) (Thermo Scientific, Waltham, MA) 0.2 μM each primer (forward and reverse) (Invitrogen, OR), and 0.02 U/μl Phusion Hot Start High-Fidelity DNA Polymerase (Finnzymes).

For MLST, the following amplification protocol was used: an initial denaturation at 98°C for 10 s, followed by 35 cycles at 98°C for 10 s, 54°C for 30 s, and 72°C for 30 s, with a subsequent extension at 72°C for 7 min. For the VNTR analyses the same protocol was used, changing the annealing temperature to 53°C and using 5′ 6-carboxyfluorescein (FAM)-labeled forward primers, while the *cepA* and *cfiA* PCR analyses were performed as previously published (9).

DNA sequencing was used to verify amplification products and for MLST genotyping of samples. To remove single-stranded, unincorporated oligonucleotides (DNA primers) before sequencing, 0.5 μl of PCR product (reaction) was treated with 0.4 U of exonuclease I ([Exo I] New England Biolabs, Ipswich, MA) in a volume of 5 μl containing 1× BigDye sequencing buffer (Applied Biosystems, Foster City, CA). The samples were incubated at 37°C for 30 min, followed by 80°C for 15 min to inactivate the exonuclease. For sequencing reactions, 0.5 μl of 5× sequencing buffer (Applied Biosystems, Foster City, CA), 1.0 μl of BigDye Terminator, version 1.1 (Applied Biosystems), and 0.32 μM forward or 0.32 μM reverse sequencing primer was added to the Exo I-treated PCR products in a total volume of 10 μl.

The cycle sequencing reaction took place under the following thermocycling conditions: 96°C for 1 min, followed by 25 cycles at 95°C for 15 s, 50°C for 5 s, and 60°C for 4 min. In order to purify and stabilize the sequencing reaction products, 45 μl of SAM Solution (Applied Biosystems) and 10 μl of XTerminator Solution (Applied Biosystems) were added, and the reaction mixtures were shaken (mini-shaker for immunology PSU-2T; BioSan, Riga, Latvia) at maximum speed (1,000 rpm) for 1 h in a microtiter plate. Detection of sequences was performed on an ABI 3130xl Genetic Analysis workstation (Applied Biosystems, Foster City, CA).

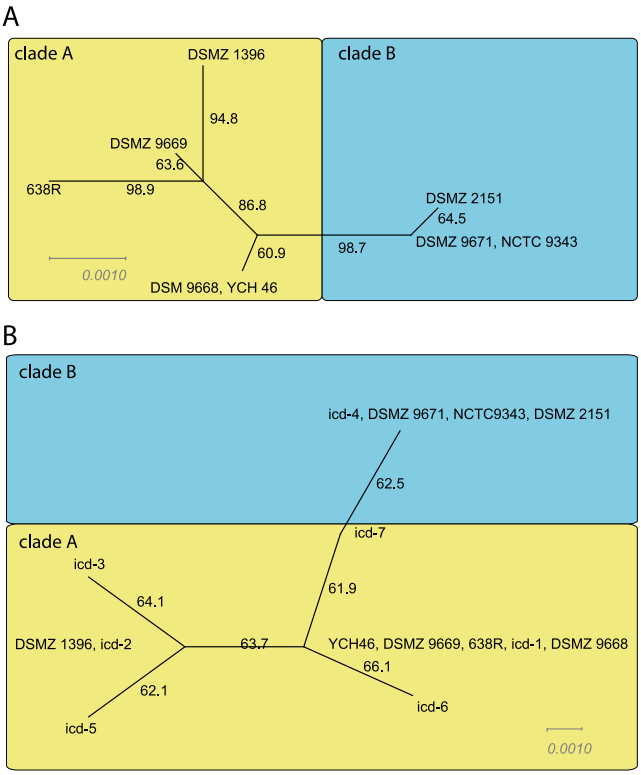

FIG. 1. SplitsTree for full MLST and *icd* genotype data. (A) The full MLST was determined for the pure strains included in this work, in addition to the genome-sequenced strains. (B) All identified *icd* genotypes were included in the tree. Numbering at the branches shows the bootstrap support in percentage. Distance bars represent uncorrected *p*-distances for neighbor-joining trees.

For the full MLST, complementary sequences from forward and reverse sequencing reactions were assembled into a consensus sequence for each sample. Discrepancies in the software selection and interpretation of the incorporated nucleotide were manually checked and corrected if necessary using the information from the electropherograms.

VNTR amplicon analyses by capillary gel electrophoresis. One microliter of 6-FAM-labeled PCR product was added to 9.3 μl of deionized formamide (HiDi Formamide; Applied Biosystems) to lower the melting temperature, and 0.3 μl of Gene Scan Rox500 (Applied Biosystems, Warrington, United Kingdom) was added as an internal size standard. The samples were centrifuged for 10 s at 1,650 × *g* (IEC Centra CL3; Thermo Electron Corporation), denatured at 95°C for 2 min, and cooled on ice. Capillary gel electrophoresis was run on an ABI 3130xl Genetic Analysis workstation (Applied Biosystems).

The data from the VNTR capillary electrophoresis fragments were analyzed by using GeneMapper software, version 4.0 (Applied Biosystems). Allele sizes were determined by the size standard and the number of tandem repeating units (mono-, di-, tri-, and tetranucleotide repeats). Preset analysis parameters for tetranucleotide repeats were used. A bin window size of ±1.70 was employed for the detection of alleles, and homozygotes required a minimum peak of 200, while this cutoff was 100 for heterozygotes.

Phylogenetic and statistical analyses. Sequences were aligned using the Muscle algorithm for alignment (CLC Main Workbench), and SplitsTree, version 4, was used for phylogenetic reconstruction (10). The basic principle of split networks is that incongruent phylogenetic relations are also visualized, as would be

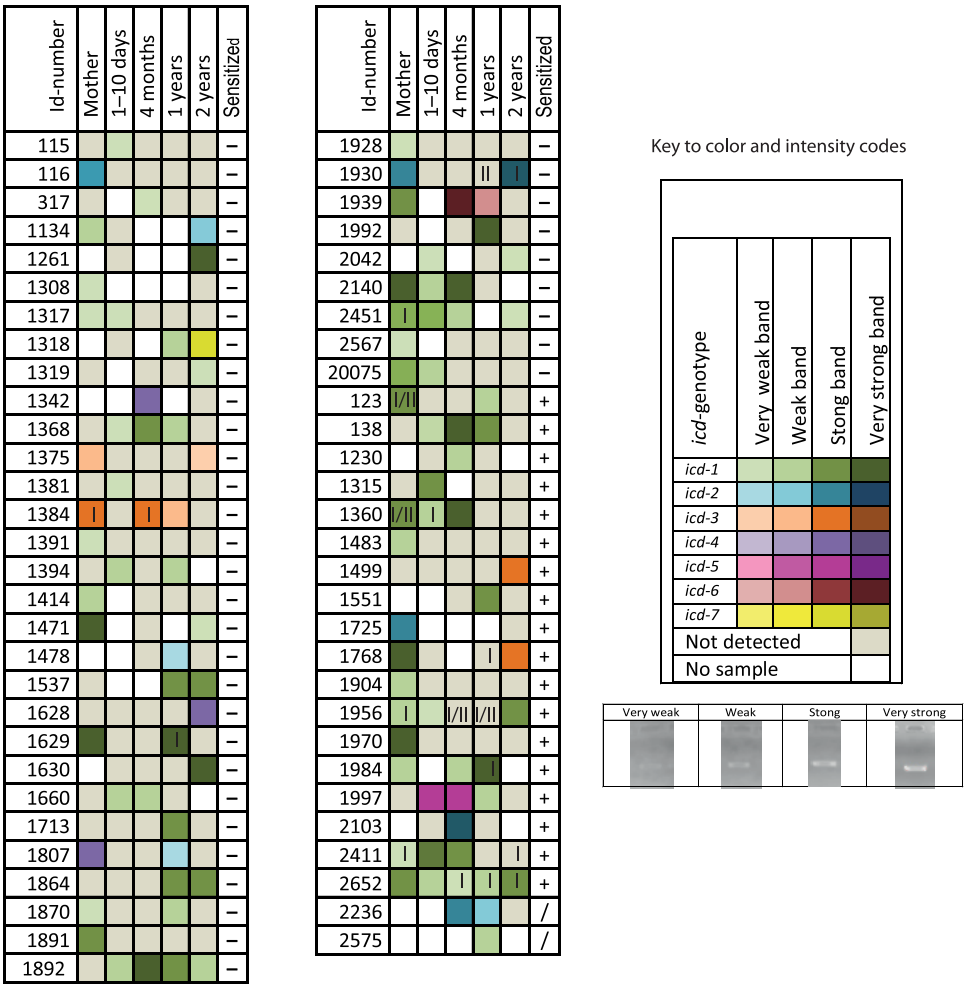

FIG. 2. Distribution of *icd* genotypes among mothers and infants analyzed in this study. A color code and intensity scale are used to indicate genotype and signal intensity, respectively. The Roman numbers (I and II) indicate whether *cepA*- or *cfiA*-positive strains were isolated from the samples, respectively. IgE sensitization information is given for children 2 years of age: +, sensitized; −, nonsensitized; /, lack of information about sensitization. The 8-bit pixel values for the inverse band signal intensities were defined as follows: high, >200 (maximum, 256); medium, 200 to 170; weak, 170 to 150; and very weak, <150. The background signals were about 120. Id, identification.

the case of recombination and horizontal gene transfer between the four genes in the full MLST.

Basic statistical analyses were performed using Tibco Spotfire S+, version 8.2, software (Palo Alto, CA). Cross-tables were analyzed for significance by using Fisher's exact test, while distributions were analyzed using the nonparametric Wilcoxon rank sum test.

Nucleotide sequence accession numbers. Sequences generated in this work were deposited in GenBank with the accession numbers JF803602 to JF803629. Details about the sequences are given in Tables S3 and S5 in the supplemental material.

RESULTS

Diversity and transmission patterns determined by MLST. The first analyses performed evaluated whether consistent phylogenetic reconstruction for the strains analyzed could be derived for the four housekeeping genes included in this work. This was accomplished using SplitsTree (10) analysis for the full MLST, which showed congruent phylogenetic relations for all the strains evaluated. These analyses revealed two main clades, A ($n = 4$) and B ($n = 3$), with clade A being apparently more diverse than B (Fig. 1A).

The *icd* gene was chosen for the direct culture-independent screening of the fecal samples. The rationale for this choice was that this gene gave the most robust amplification and contained the highest level of polymorphic sites. We obtained *icd* amplification products of the expected sizes from 160 out of 483 stool samples. Of these, 129 were confirmed as *icd* by DNA sequencing. These represented a total of seven sequence types. None of the direct sequences were mixed, indicating a single dominant sequence type. The sequence types from the direct sequencing formed the same clades as for the full MLST, with five clade A and two clade B sequence types (Fig. 1B).

A semiquantitative investigation of the individual distribution of the identified sequence types was performed by combining the information from the band intensity of the PCR-amplified *icd* fragment with sequence type information (Fig. 2). These analyses revealed relatively large fluctuations in *B. fragilis* levels and a single dominant sequence type (icd-1). With respect to prevalence we found that icd-1 represented 79% of all the sequences. Interestingly, the age-related distributions of the sequence types showed an apparent reduced

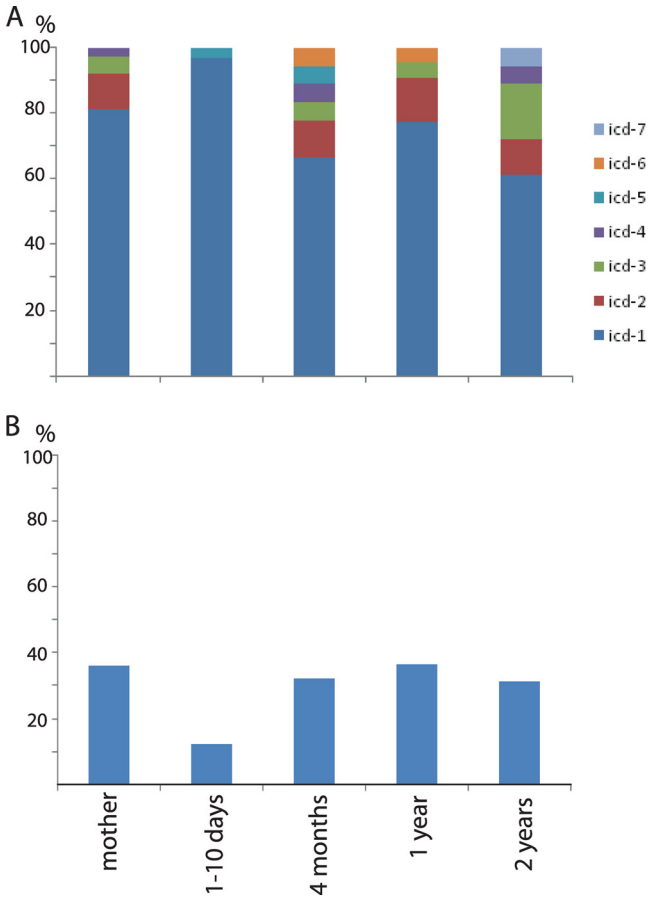

FIG. 3. Relative *icd* genotype composition (A) and proportion of *B. fragilis*-positive samples (B) for the different age categories investigated. The representations are summaries of the data presented in Fig. 2.

diversity at the age of 1 to 10 days, with a nearly complete dominance of icd-1 (Fig. 3A). The reduced diversity was also correlated to a decreased prevalence of *B. fragilis* at 1 to 10 days (Fig. 3B).

Transmission patterns were tested by Fisher's exact test. With respect to mother-to-child transmission, the icd-1 sequence type was at the border of significance at 1 to 10 days. Sequence type icd-3, on the other hand, showed a significant association with that of the mother at both 4 months and 1 year (Table 1).

Subtyping by VNTR analyses. Due to the very high prevalence of icd-1, we chose to search for the more polymorphic VNTR markers for resolving this sequence type. Potential hypervariable VNTR sequences were identified by comparison and analysis of the genome-sequenced strains using the web-based mreps program. We identified a set of 12 candidate VNTR regions while only one region (TRS1) showed size variation and consistent amplification of all five reference strains included in our work (see supplemental text for details).

VNTR genotyping was conducted for TRS1 using a selection of seven sensitized and six nonsensitized children. From the direct, culture-independent VNTR analyses, we routinely observed amplification as expected from the *icd* genotyping. Most samples yielded one allele per sample, but in some samples up to four alleles were detected, with the highest diversity at 2 years (Table 2). For the culture-dependent analyses, on the other hand, we observed the highest diversity in mothers (Table 3). In total, we detected 16 TRS1 alleles in this study, with 9 identified both by direct analyses and by cultivation (see Table S4 in the supplemental material). Three of the VNTR sequences were detected only in stool, while four were detected only by cultivation. Identity of the different TRS1 alleles was confirmed by DNA sequencing (see Table S5).

For the direct analyses of feces, we found that the TRS1 genotype diversity was highest in mothers and 2-year-old children. From 1 to 10 days to 1 year there was a relative increase in the prevalence of the 252 genotype (Fig. 4). As determined by Fisher's exact test, the dependencies between the mothers' and the children's TRS1 genotypes were highly significant for all age categories. There was, however, a decrease in significance with age from a P value of 10^{-6} at 1 to 10 days to 0.0005 at 2 years (see Table S6 in the supplemental material).

Typing of cultured isolates. A total of 77 colonies were recovered from the samples analyzed. Of these, 55% ($n = 42$) were *cepA* positive, 8% ($n = 6$) were *cfiA* positive, 70% ($n = 54$) were *icd* positive, and 71% ($n = 55$) were VNTR positive (Table 4). The multiple VNTR genotypes for eight colonies

TABLE 1. The association between the dominant *icd* genotypes in mothers and their children

Sequence type	Age of child	No. of instances of sequence type for the indicated mother/child condition[a]				P value[b]
		Present/present	Absent/present	Present/absent	Absent/absent	
icd–1	1–10 days	8	8	9	27	0.074
	4 mos	6	5	15	36	0.108
	1 yr	5	9	8	31	0.217
	2 yr	4	5	16	36	0.328
icd–2	1–10 days	0	0	3	44	1
	4 mos	0	1	2	60	0.9999
	1 yr	0	1	2	57	1
	2 yr	1	1	2	59	0.094
icd–3	1–10 days	0	0	2	54	1
	4 mos	1	0	0	61	0.016
	1 yr	1	0	1	57	0.034
	2 yr	1	2	1	60	0.092

[a] The presence or absence of the sequence type is given in respective order for mothers and children.
[b] Fisher's exact test.

TABLE 2. TRS1 genotypes identified directly from stool samples

Identification no.	Sensitized	TRS1 genotype by sample group[a]				
		Mother	Child at:			
			1–10 days	4 mos	1 yr	2 yr
1317	−	272	244		**252**	
		280	252		284	
1384	−	236	236	236	NA	NA
1629	−	268	252	252	244	244
1930	−	276	252	NA	252	196
					276	236
						252
						276
2451	−	224	240	240		
		240				
20075	−	268	268	NA	268	252
						268
						272
						284
123	+	NA	252	272	236	268
1360	+	252	252	252	252	252
1768	+	**268**	**244**		252	**252**
		252	252			196
			268			
1956	+	244	244	NA	NA	236
1984	+	196		196	196	
2411	+	272	272	272	240	256
						272
2652	+	236	NA	NA	236	236

[a] Dominant sequence types are in boldface. NA, not amplified; empty cell, no sample taken.

indicate that these represented mixed strains. Generally, *cepA*- and *cfiA*-positive strains were mutually exclusive while *icd*-positive strains covered both groups.

icd sequencing showed a dominance of icd-1. Interestingly, two out of four icd-3 strains were recovered from mother 1384, which also showed dominance for icd-3 in the direct stool analyses. A new sequence type, icd-8, dominated among *cfiA*-positive strains. This sequence type is distantly related to the other sequence types detected by the direct stool analyses, supporting the contention that it belongs to *B. fragilis* division II, with the other icd sequence types belonging to division I (9).

TABLE 3. TRS1 genotypes identified from colonies

Identification no.	Sensitized	TRS1 genotype by sample group[a]				
		Mother	Child at:			
			1–10 days	4 mos	1 yr	2 yr
1384	−	220		288	236	
		236		236	272	
1629	−	232			244	
1930	−				NA	NA
2451	−	240				
		224				
20075	−	236				
		272				
123	+	NA			236	
1360	+	252	252			
		248				
		264				
1768	+				272	NA
1956	+	252			NA	
		240				
		220				
		244				
1984	+				196	
2411	+	256	272			272
		272				
		280				
2652	+			236	236	236
					252	

[a] NA, not amplified; empty cell, no sample taken.

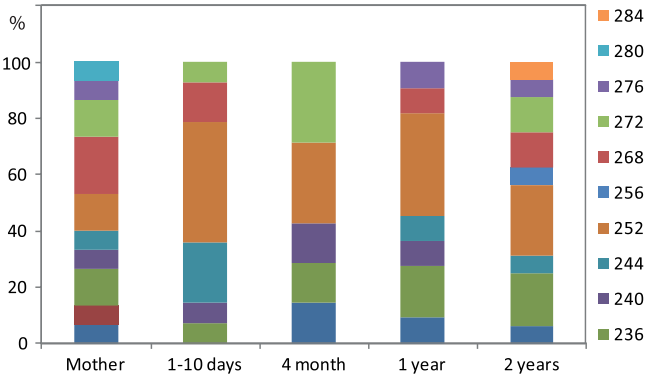

FIG. 4. Relative TRS1 composition directly from the stool samples for the different age categories investigated. The percentages of the different TRS1 types identified are shown.

Correlation to IgE sensitization. Using Fisher's exact test, we explored whether there was a significant association between the *icd* genotypes and allergic sensitization. When a Bonferroni correction was taken into account in these analyses, no significant associations were found. Likewise, we found no significant association between TRS1 genotypes and sensitization. With respect to TRS1 diversity, however, two out of four nonsensitized 2-year-old children with samples for that point had four different TRS1 alleles, while none of the six nonsensitized children harbored more than two alleles. We tested whether this represented a significant overrepresentation by use of a nonparametric Wilcoxon rank sum test with the null hypothesis that four alleles were equally distributed among sensitized and nonsensitized children. This test suggested that there was a significant overrepresentation of multiple alleles for the nonsensitized children ($P = 0.03$).

DISCUSSION

Our data support mother-to-child transmission of *B. fragilis*, followed by persistence of multiple closely related strains within a single child. We found the long-term persistence of multiple strains particularly intriguing. From ecological theory one can explain multiple-strain coexistence by (i) negative external selection pressure, (ii) mutualism, (iii) equilibrium interactions, (iv) physical separation, or (v) occupancy of different biochemical niches. The fact that *B. fragilis* has phase-variable surface epitopes (15) suggests that this bacterium could be under negative external selection pressure either by the host's adaptive immune system (6, 26), predation (36), or bacteriophage infections (22, 37). Thus, it is likely that negative external selection pressure (i) drives multiple-strain coexistence (30). With respect to physical separation (iv), it has been proposed that

TABLE 4. Characteristics of colonies recovered from the stool samples

Identification no.	Sample source (age)	Isolate profile[a]			
		cepA	*cfiA*	icd sequence type	TRS1 type
1384	Mother	−	−	−	−
	Mother	+	−	3	236
	Mother	+	−	1	272
	Mother	+	−	3	−
	Child (4 mos)	+	−	ND	236
	Child (1 yr)	−	−	−	236/272
	Child (1 yr)	−	−	−	236
1629	Mother	−	−	−	244
	Mother	−	−	−	−
	Child (1 yr)	+	−	1	244
	Child (1 yr)	+	−	3	−
	Child (1 yr)	+	−	1	272
	Child (1 yr)	+	−	1	244
1930	Child (1 yr)	−	−	−	−
	Child (1 yr)	−	−	−	−
	Child (1 yr)	−	+	8	236
	Child (1 yr)	−	−	1	236/272
	Child (2 yr)	−	−	−	−
	Child (2 yr)	−	−	−	−
	Child (2 yr)	+	−	ND	252
2451	Mother	+	−	1	240
20075	Mother	−	−	−	−
	Mother	−	−	1	280
	Mother	−	−	−	−
123	Mother	−	−	−	−
	Mother	−	−	ND	272
	Mother	−	+	8	−
	Child (1 yr)	+	−	1	236
1360	Mother	+	+	ND	252
	Mother	+	−	1	272
	Mother	−	−	1	−
	Mother	+	−	1	248/264
	Mother	+	−	3	236
	Mother	−	−	−	236
	Child (1–10 days)	+	−	1	252
	Child (1–10 days)	+	−	1	236
	Child (1–10 days)	+	−	1	252
	Child (1–10 days)	+	−	1	252
	Child (1–10 days)	+	−	1	196
1768	Child (1 yr)	+	−	8	272
	Child (1 yr)	−	−	ND	−
	Child (1 yr)	+	−	1	252
	Child (2 yr)	−	−	−	−
	Child (2 yr)	−	−	−	−
	Child (2 yr)	−	−	−	236
1956	Mother	+	−	1	220/244
	Child (4 mos)	−	+	8	252
	Child (4 mos)	+	−	1	196
	Child (4 mos)	+	−	1	252
	Child (1 yr)	−	+	8	−
	Child (1 yr)	−	+	8	−
	Child (1 yr)	+	−	1	240
1984	Child (1 yr)	+	−	1	196
	Child (1 yr)	−	−	−	288
2411	Mother	+	−	1	256/272
	Mother	−	−	1	272
	Mother	+	−	1	252
	Mother	−	−	−	−
	Child (1–10 days)	−	−	−	252
	Child (2 yr)	+	−	1	272
	Child (2 yr)	+	−	ND	224/240
	Child (2 yr)	−	−	−	−
	Child (2 yr)	−	−	8	−
	Child (2 yr)	+	−	1	272
2652	Child (2 yr)	+	−	8	220/236
	Child (2 yr)	+	−	1	244
	Child (2 yr)	+	−	1	252
	Child (2 yr)	−	−	−	−
	Child (2 yr)	+	−	ND	272
	Child (2 yr)	−	−	−	272
	Child (2 yr)	+	−	1	272
	Child (2 yr)	−	−	−	236
	Child (2 yr)	+	−	1	236
	Child (1 yr)	+	−	1	236/252
	Child (1 yr)	+	−	1	244
	Child (4 mos)	+	−	1	236
	Child (4 mos)	+	−	1	244

[a] +, amplification; −, no amplification.
[b] icd types are indicated by numbers, where 8 is a new sequence type detected only from colonies. ND, no readable sequence was recovered.

the mucosal microbiota has mosaic structure (5). Given that there is a flux of mucosa-associated bacteria to feces, this would lead to multiple-strain coexistence. Therefore, physical separation in conjunction with negative selection could be the underlying factor forcing diversity. We find mutualism, equilibrium interactions, and occupancy of different niches less likely explanations of the multiple-strain coexistence observed in this study. These mechanisms would imply structured interactions, which we did not observe.

Independently, we have found that high levels (32) and low diversity (this work) of *B. fragilis* were correlated to sensitization. Mucosal protection, however, is highly complex, relying on a balance between innate and adaptive responses (6, 17). The mechanisms (if any) for the correlations detected here cannot, therefore, easily be deduced.

Previous studies have indicated a relatively high global diversity for *B. fragilis* (1, 9). Our finding of the high prevalence of a single group of closely related phylotypes suggests that the strains colonizing the infants in the IM-PACT cohort represent a *B. fragilis* subpopulation. The ecological drivers for the subpopulation still remain unknown. However, since there was a relative increase in the dominant phylotype right after birth, this suggests an enrichment of this phylotype during the birth process.

On one hand, *B. fragilis* is an opportunistic pathogen (3, 28) while, on the other hand, this bacterium is important for human infant immune development and maturation (33). It will be very important in the future to determine whether the mother-to-child transmission is host selected, commensal, pathogenic, or symbiotic.

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REFERENCES

1. **Antunes, E. N., et al.** 2004. Non-toxigenic pattern II and III *Bacteroides fragilis* strains: coexistence in the same host. Res. Microbiol. **155:**522–524.
2. **Cerdeno-Tarraga, A. M., et al.** 2005. Extensive DNA inversions in the *B. fragilis* genome control variable gene expression. Science **307:**1463–1465.
3. **Cheng, C. W., et al.** 2009. Clinical significance of and outcomes for *Bacteroides fragilis* bacteremia. J. Microbiol. Immunol. Infect. **42:**243–250.
4. **de Muinck, E. J., and T. Øien, et al.** 2011. Diversity, transmission and persistence of *Escherichia coli* in a cohort of mothers and their infants. Environ. Microbiol. Rep. **3:**352–359.
5. **Eckburg, P. B., et al.** 2005. Diversity of the human intestinal microbial flora. Science **308:**1635–1638.
6. **Edelman, S. M., and D. L. Kasper.** 2008. Symbiotic commensal bacteria direct maturation of the host immune system. Curr. Opin. Gastroenterol. **24:**720–724.
7. Reference deleted.
8. **Gould, H. J., and B. J. Sutton.** 2008. IgE in allergy and asthma today. Nat. Rev. Immunol. **8:**205–217.
9. **Gutacker, M., C. Valsangiacomo, M. V. Bernasconi, and J. C. Piffaretti.** 2002. *recA* and *glnA* sequences separate the *Bacteroides fragilis* population into two genetic divisions associated with the antibiotic resistance genotypes *cepA* and *cfiA*. J. Med. Microbiol. **51:**123–130.
10. **Huson, D. H., and D. Bryant.** 2006. Application of phylogenetic networks in evolutionary studies. Mol. Biol. Evol. **23:**254–267.
11. **Kolpakov, R., G. Bana, and G. Kucherov.** 2003. mreps: efficient and flexible detection of tandem repeats in DNA. Nucleic Acids Res. **31:**3672–3678.
12. **Krinos, C. M., et al.** 2001. Extensive surface diversity of a commensal microorganism by multiple DNA inversions. Nature **414:**555–558.
13. **Kuwahara, T., et al.** 2004. Genomic analysis of Bacteroides fragilis reveals extensive DNA inversions regulating cell surface adaptation. Proc. Natl. Acad. Sci. U. S. A. **101:**14919–14924.
14. **Liu, C. H., S. M. Lee, J. M. Vanlare, D. L. Kasper, and S. K. Mazmanian.** 2008. Regulation of surface architecture by symbiotic bacteria mediates host colonization. Proc. Natl. Acad. Sci. U. S. A. **105:**3951–3956.
15. **Nakayama-Imaohji, H., et al.** 2009. Identification of the site-specific DNA invertase responsible for the phase variation of SusC/SusD family outer membrane proteins in *Bacteroides fragilis*. J. Bacteriol. **191:**6003–6011.
16. **Namavar, F., et al.** 1989. Epidemiology of the Bacteroides fragilis group in the colonic flora in 10 patients with colonic cancer. J. Med. Microbiol. **29:**171–176.
17. **Neish, A. S.** 2009. Microbes in gastrointestinal health and disease. Gastroenterology **136:**65–80.
18. **Neumann, D. A., M. W. Benenson, E. Hubster, and N. Thi Nhu Tuan.** 1972. Cary-Blair, a transport medium for *Vibrio parahaemolyticus*. Am. J. Clin. Pathol. **57:**33–34.
19. **Odamaki, T., et al.** 2007. Fluctuation of fecal microbiota in individuals with Japanese cedar pollinosis during the pollen season and influence of probiotic intake. J. Investig. Allergol. Clin. Immunol. **17:**92–100.
20. **Oien, T., O. Storro, and R. Johnsen.** 2006. Intestinal microbiota and its effect on the immune system—a nested case-cohort study on prevention of atopy among small children in Trondheim: the IMPACT study. Contemp. Clin. Trials **27:**389–395.
21. **Pamer, E. G.** 2007. Immune responses to commensal and environmental microbes. Nat. Immunol. **8:**1173–1178.
22. **Patrick, S., et al.** 2010. Twenty-eight divergent polysaccharide loci specifying within- and amongst-strain capsule diversity in three strains of *Bacteroides fragilis*. Microbiology **156:**3255–3269.
23. **Patrick, S., S. Houston, Z. Thacker, and G. W. Blakely.** 2009. Mutational analysis of genes implicated in LPS and capsular polysaccharide biosynthesis in the opportunistic pathogen *Bacteroides fragilis*. Microbiology **155:**1039–1049.
24. **Perez-Losada, M., et al.** 2006. Population genetics of microbial pathogens estimated from multilocus sequence typing (MLST) data. Infect. Genet. Evol. **6:**97–112.
25. **Pumbwe, L., C. A. Skilbeck, and H. M. Wexler.** 2006. The *Bacteroides fragilis* cell envelope: quarterback, linebacker, coach—or all three? Anaerobe **12:**211–220.
26. **Rissing, J. P., T. B. Buxton, and H. T. Edmondson.** 1979. Detection of specific IgG antibody in sera from patients infected with *Bacteroides fragilis* by enzyme-linked immunosorbent-assay. J. Infect. Dis. **140:**994–998.
27. **Round, J. L., and S. K. Mazmanian.** 2009. The gut microbiota shapes intestinal immune responses during health and disease. Nat. Rev. Immunol. **9:**313–323.
28. **Sears, C. L.** 2009. Enterotoxigenic *Bacteroides fragilis*: a rogue among symbiotes. Clin. Microbiol. Rev. **22:**349–369.
29. **Skanseng, B., M. Kaldhusdal, and K. Rudi.** 2006. Comparison of chicken gut colonisation by the pathogens *Campylobacter jejuni* and *Clostridium perfringens* by real-time quantitative PCR. Mol. Cell. Probes **20:**269–279.
30. **Skanseng, B., et al.** 2007. Co-infection dynamics of a major food-borne zoonotic pathogen in chicken. PLoS Pathog. **3:**e175.
31. **Storro, O., T. Oien, C. K. Dotterud, J. A. Jenssen, and R. Johnsen.** 2010. A primary health-care intervention on pre- and postnatal risk factor behavior to prevent childhood allergy. The Prevention of Allergy among Children in Trondheim (PACT) study. BMC Public Health **10:**443.
32. **Storro, O., et al.** 13 July 2011. Temporal variations in early gut microbial colonization are associated with allergen-specific immunoglobulin E but not atopic eczema at 2 years of age. Clin. Exp. Allergy. doi: 10.1111/j.1365-2222.2011.03817.x.
33. **Troy, E. B., and D. L. Kasper.** 2010. Beneficial effects of Bacteroides fragilis polysaccharides on the immune system. Front. Biosci. **15:**25–34.
34. **Vael, C., V. Nelen, S. L. Verhulst, H. Goossens, and K. N. Desager.** 2008. Early intestinal *Bacteroides fragilis* colonisation and development of asthma. BMC Pulm. Med. **8:**19.
35. **van Belkum, A., S. Scherer, L. van Alphen, and H. Verbrugh.** 1998. Short-sequence DNA repeats in prokaryotic genomes. Microbiol. Mol. Biol. Rev. **62:**275–293.
36. **Wildschutte, H., D. M. Wolfe, A. Tamewitz, and J. G. Lawrence.** 2004. Protozoan predation, diversifying selection, and the evolution of antigenic diversity in *Salmonella*. Proc. Natl. Acad. Sci. U. S. A. **101:**10644–10649.
37. **Zitomersky, N. L., M. J. Coyne, and L. E. Comstock.** 2011. Longitudinal analysis of the prevalence, maintenance, and IgA response to species of the order *Bacteroidales* in the human gut. Infect. Immun. **79:**2012–2020.