

Sequence based high-resolution profiling of *Bacteroides* species and strains in the microbiome of caesarean section and vaginally delivered infants

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introduction

Proper establishment of infant gut microbiome plays an essential role in the foundations of human health. Mode of delivery (vaginal birth vs. c-section), as well as mode of feeding (breastfeeding vs. formula) have been shown to greatly influence the presence and abundance of different taxa in the infant gut. Previous studies showed that bacteria from the *Bacteroides* genus (Bact) are among the first to populate the infant gut. However, 16S technology that is commonly used for these microbiota analyses can detect organisms mostly only at the taxonomic levels of genus and higher. In addition, it has been recently shown that 16S technology has produced a lot of false results (up to 88% false positives). Aim of this study was to analyze, with high fidelity, high resolution quantitative sequencing methodology *Bacteroides* species and strains in the infant gut microbiome, and examine how their presence correlates with the mode of delivery as well as feeding methods.

methods

A world beyond 16S: A high resolution sequencing approach to microbiomics

Using novel, high resolution quantitative sequencing method, we analyzed the presence of 14 specific Bact species, in the feces of infants born vaginally (n=51) vs. c-section (n=53), at 1, 3, 7, and 12 months of age. These samples also differed in the feeding method: breastfed (bf, n=29), unsupplemented formula (f-, n=37), and *Bifidobacterium*-supplemented formula (f+, n=38). Further, the same high resolution quantitative method was used to detect and analyze 4 specific Bact strains. For bioinformatics analysis, blast was used as the alignment tool, comparing each of the query datasets to each of the sample libraries, and setting parameters for optimal, tight alignments favoring identity matching versus homology matching.

results

Detection of specific *Bacteroides* species and strains in infant stool

Out of 14 targeted *Bacteroides* species, 5 were detected at Month 1, 7 at month 3, 7 at month 7, and 8 at Month 12 (Figure 1). *B. vulgatus* and *B. fragilis* appeared to be the most prevalent species across the samples. In accordance with previously published data, we showed that c-section delivery and exclusive breastfeeding are associated with the decrease of *Bacteroides* in the infant stool: Bact species were detected in 73% vaginal and 16% c-section samples (Figure 2a); and when analyzed by the feeding method, there were more Bact species present in formula-fed infants (both unsupplemented and bifid-supplemented formula) than in breastfed infants. Additionally, we were able to identify more than 15 specific bacteroides strains, 4 of which were predominantly expressed: *B. fragilis* YCH46, *B. fragilis* S14, *B. distasonis* ACCT 8503, and *B. vulgatus* ATCC 8482. (Figure 3). Interestingly, growth of *B. vulgatus* ATCC 8482, *B. fragilis* S14, and *B. fragilis* YCH46 was particularly stimulated in the formula-fed infants, while bifidobacteria-supplemented formula appeared to decrease the levels of specific *Bacteroides* strains (S14 and YCH46).

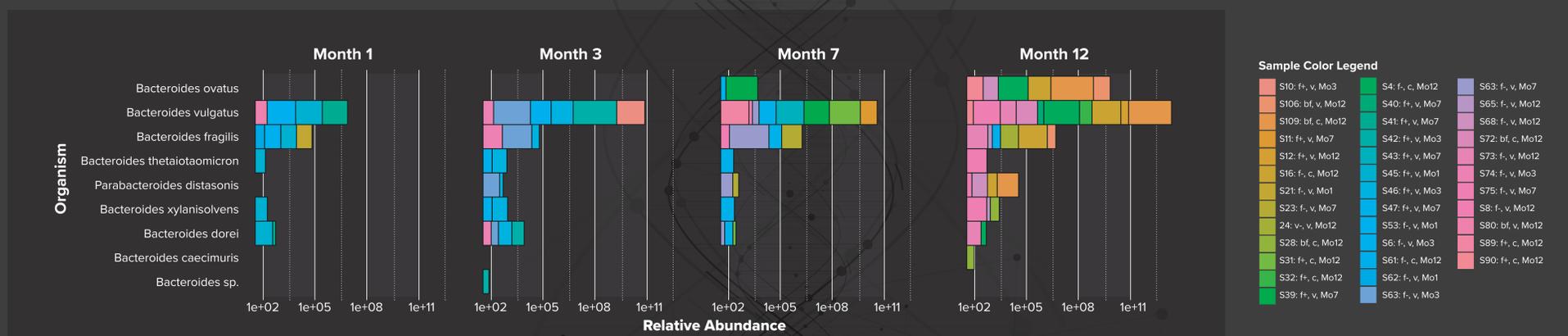


Figure 1. Detection of specific *Bacteroides* species in the infant gut during the first 12 months. Relative abundance of fecal *Bacteroides* at 1, 3, 7, and 12 months of life. Relative abundance levels are normalized and plotted in log scale for cross sample comparison.

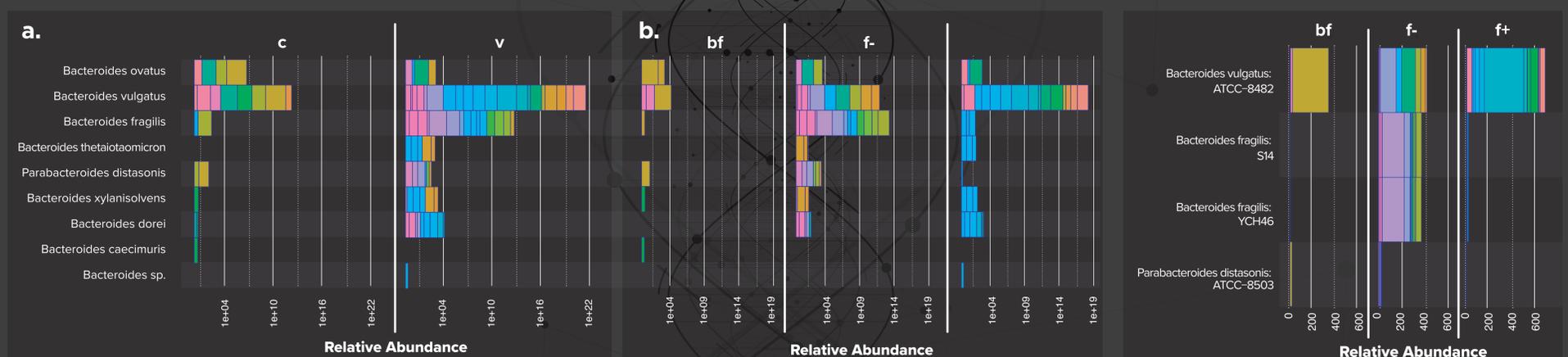


Figure 2. C-section as well as exclusive breastfeeding are associated with a decrease in *Bacteroides* species. a) Relative abundance of fecal *Bacteroides* species in vaginally born (v) vs. c-section (c) born infants. Samples from all 4 time points, and 3 methods of feeding are included in the analysis. b) Relative abundance of fecal *Bacteroides* species in infants that were breastfed (bf) vs. formula fed (either unsupplemented formula f-, or bifido-supplemented formula f+). Samples from all time points and modes of birth were included in the analysis. Relative abundance levels are normalized and plotted in log scale for cross sample comparison.

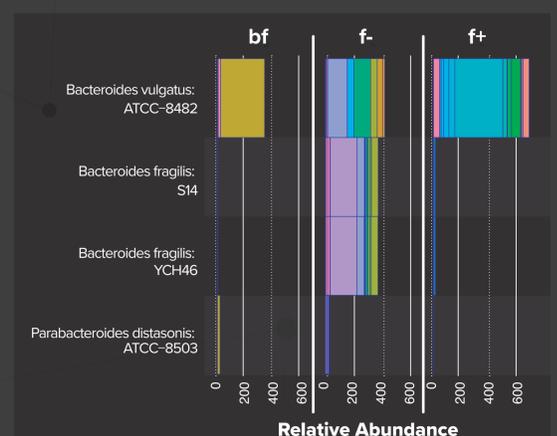


Figure 3. Abundance of four prevalent *Bacteroides* strains differs depending on the feeding method. Relative abundance of fecal *Bacteroides* strains in infants that were breastfed (bf) vs. formula fed (f-). Formula supplemented with bifidobacteria was also tested (f+). Samples from all time points and modes of birth were included in the analysis. Relative abundance levels are normalized for cross sample comparison.

conclusion

Strain level resolution can be used to monitor the modulatory effects of high diversity MACs on the growth of *Bacteroides* in C-Section babies

This study represents the first species and strain level analysis of *Bacteroides* in the infant microbiome. Considering that the absence of *Bacteroides* species is associated with gastrointestinal symptoms in infants, and that potentiating the growth of these bacteria may be of clinical importance, being able to monitor dynamics of individual *Bacteroides* species and strains within the microbiome would be of great importance. Human milk oligosaccharides (HMOs) from mother's milk are known to be consumed by *Bacteroides* thus supplementation of formula with highly diverse HMO-mimcs may potentiate the growth of *Bacteroides*.