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Strain level analysis of BIFIDOBACTERIA in the microbiome of early intervention formula-fed infants in the first year of life

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introduction

Strain-level resolution picture of the microbiome modulation in the infant gut

Gut-residing bacteria from the *Bifidobacterium* genus play an important role in infant metabolism and health. It is known that human milk oligosaccharides (HMOs), from breast milk, are essential for the growth of specific microbiome taxa, including *Bifidobacterium* members. Although many studies have explored, via 16S sequencing, the presence and abundance of the *Bifidobacterium* genus in the infant gut, there is no data on specific *Bifidobacterium* strains and their possible differential role in the infant gut. In the present study, we analyzed strain level of *Bifidobacterium* genus in the infant gut, and how specific strains are related to the mode of feeding.

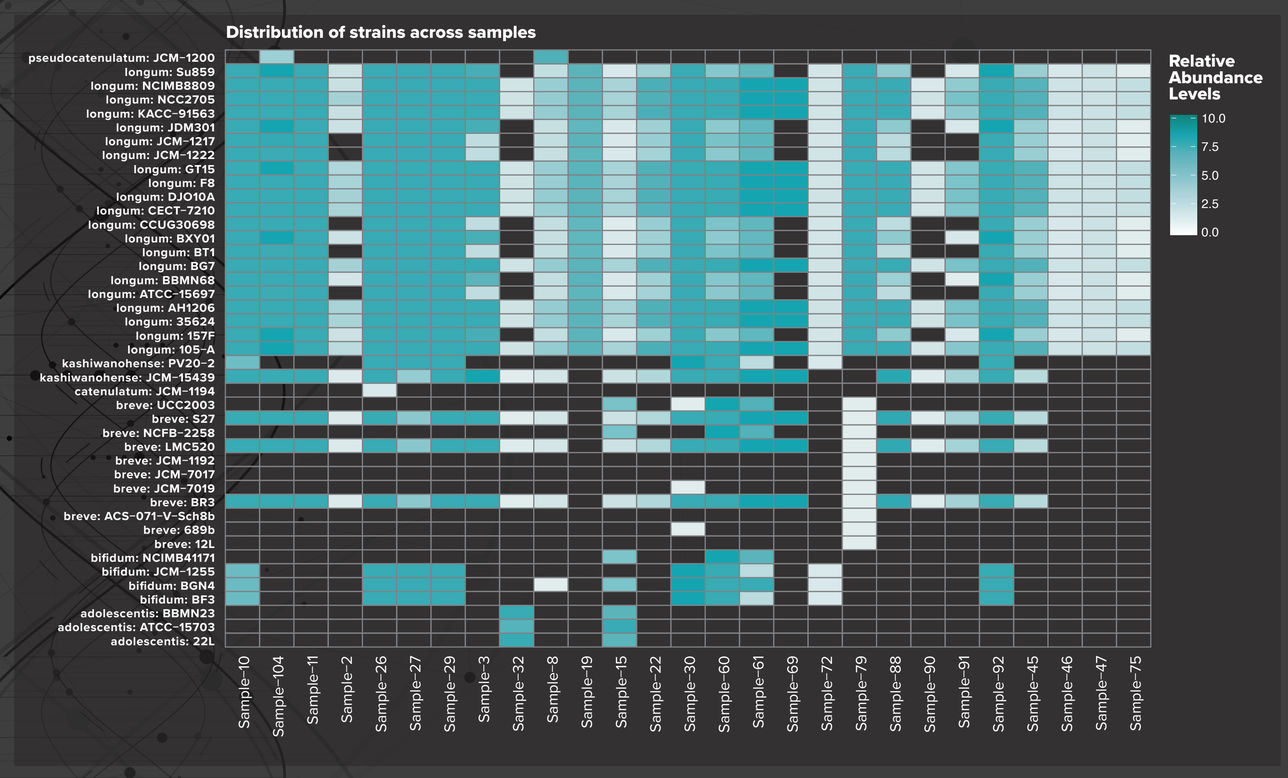

Figure 1. Detection and quantitative analysis of 55 Bifidobacterium strains in the infant gut
Heat map analysis showing relative levels of most highly expressed *Bifidobacterium* strains in infant feces. The analysis includes samples from all 3 feeding methods (breastfed, unsuplemmented formula, and supplemented formula), as well as all 4 ages (1, 3, 7, and 12, months).

methods

Beyond 16S: high resolution sequencing approach to microbiomics

Using novel, high resolution quantitative sequencing method, we analyzed the presence and abundance of specific *Bifidobacterium* strains, in the feces of infants that were either breastfed (bf, n=29), fed with formula (f- n=37) or with bifido-supplemented formula (f+ n=38). Samples came from infants at 1, 3, 7, and 12 months of age. Bioinformatics analysis was done using a curated database and the *BESTFIT* algorithm, which utilizes BLAST and a tiered approach combining percent identity and alignment overlap cutoffs.

results

55 *Bifidobacterium* strains resolved, with breastfed infants having higher strain diversity and abundance than formula-fed infants

With our sequencing method were were able to detect 55 *Bifidobacterium* strains across all samples **(Figure 1)**. In line with previously published analyses of the *Bifidobacterium* genus level, we showed that breastfed infants have higher number and abundance of *Bifidobacterium* strains, compared to the formula-fed (f-) infants. Interestingly, we also observed that the breastfed infants had higher number and abundance of *Bifidobacterium* strains compared to the infants fed with bifid-supplemented formula (f+) **(Figure 2a and 2b)**. 21 of the detected strains, with prominent abundance, belonged to the *B. longum* species **(Figure 3a)**. The most abundant among *B. longum* were: *NCC2705, KACC-91563, GT15, DJO10A, BG7, and 105-A*. Additionally, there were 11 highly abundant strains which belonged to the *B. breve* species **(Figure 3b)**. Most prominent ones were: *S27, LMC520, and BR3. B. Breve BR3* strain was added as a supplement in the f+ formula, thus the high abundance in the f+ cohort was expected. Interestingly, there were clear differences in the levels of individual strains depending on the mode of feeding. For example, *B. longum AH1206 and NCC2705* are more highly expressed than *B. longum CCUG30698* in the f- cohort.

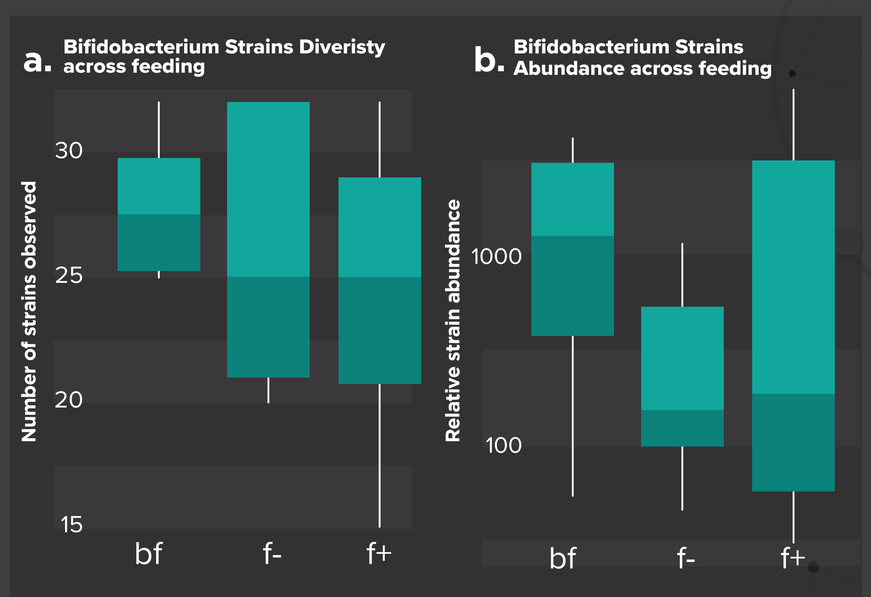

Figure 2. Breastfed infants present higher number and abundance of *Bifidobacterium* strains compared to formula-fed infants. a) Box plot comparing number of observed strains in breastfed (bf), unsupplemented formula-fed (f-) and Bifido-supplemented formula-fed (f+) infants. Although difference is not statistically significant (p=0.4), trend is observed where bf infants have higher number of strains than f- infants. **b)** Box plot comparing relative abundance of *Bifidobacterium* strains in bf vs. f+ vs. f- samples. Although difference is not significant (p=0.08), trend is observed where bf infants present higher *Bifidobacterium* strain abundance than f-. Both box plots include samples from all 4 ages (1,3,7, and 12).

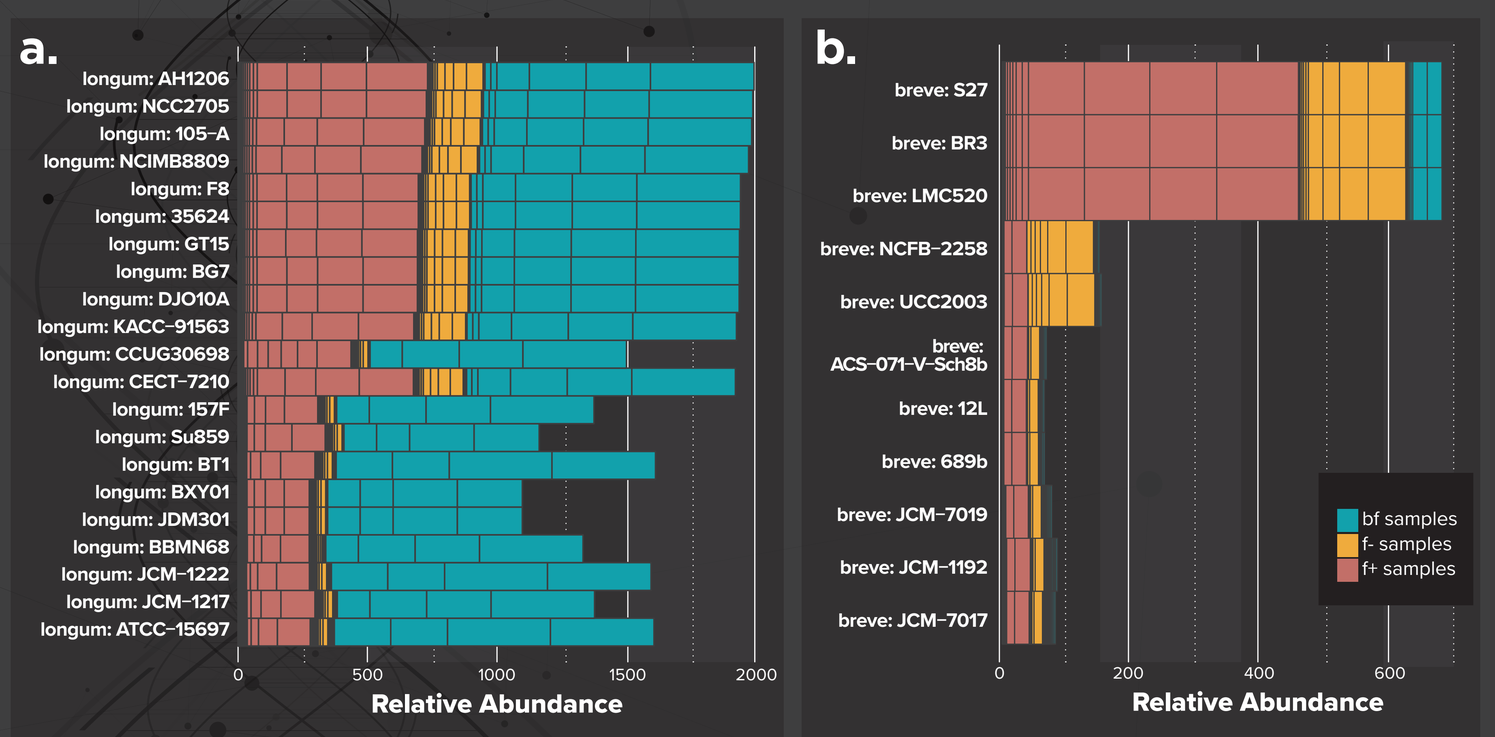

Figure 3. 21 *B. longum* and 11 *B. breve* strains were identified across samples. Stacked bar graph showing relative abundance of B. longum strains (a) and B. breve strains (b) across bf, f+, and f- samples. Each square in the graph represents a sample and its width represents relative abundance of a particular strain present in that sample.

conclusion and next steps

Strain level monitoring of the modulatory effect of highly diverse MAC formulations on the infant gut microbiota

This study presents a novel approach to microbiomics, enabling the first strain level analysis of Bifidobacteria in an infant microbiome. Our methodology permits for deeper look at specific *Bifidobacterium* strains in the human gut, and how they may be differentially modulated across different sample cohorts. Given the importance of these bacteria in human health and their direct link with the HMOs, this could serve as an important tool for dissecting the dynamics of *Bifidobacterium* in relation to infant nutrition and metabolism. This high-resolution tool for the profiling of *Bifidobacterium* strains can be used to further evaluate the effects of our GnuBiotics MACs formulations that contain over 130 HMO structural mimics.