

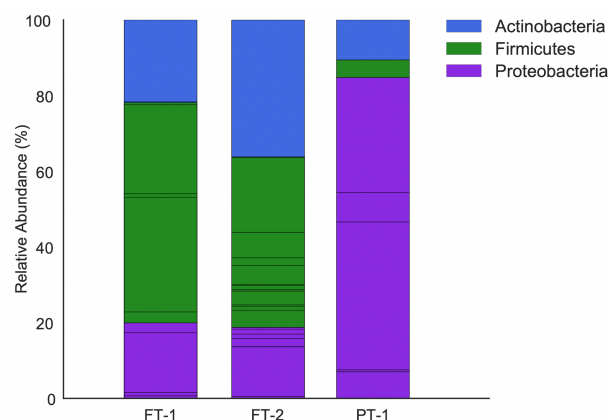


Figure S1. Community compositions of the three infant stool inocula. The x-axis represents the stool inocula. Bar height represents normalized species relative abundance, and bars are colored by phylum. Sections of the same color with horizontal black lines correspond to individual species of the same phylum.

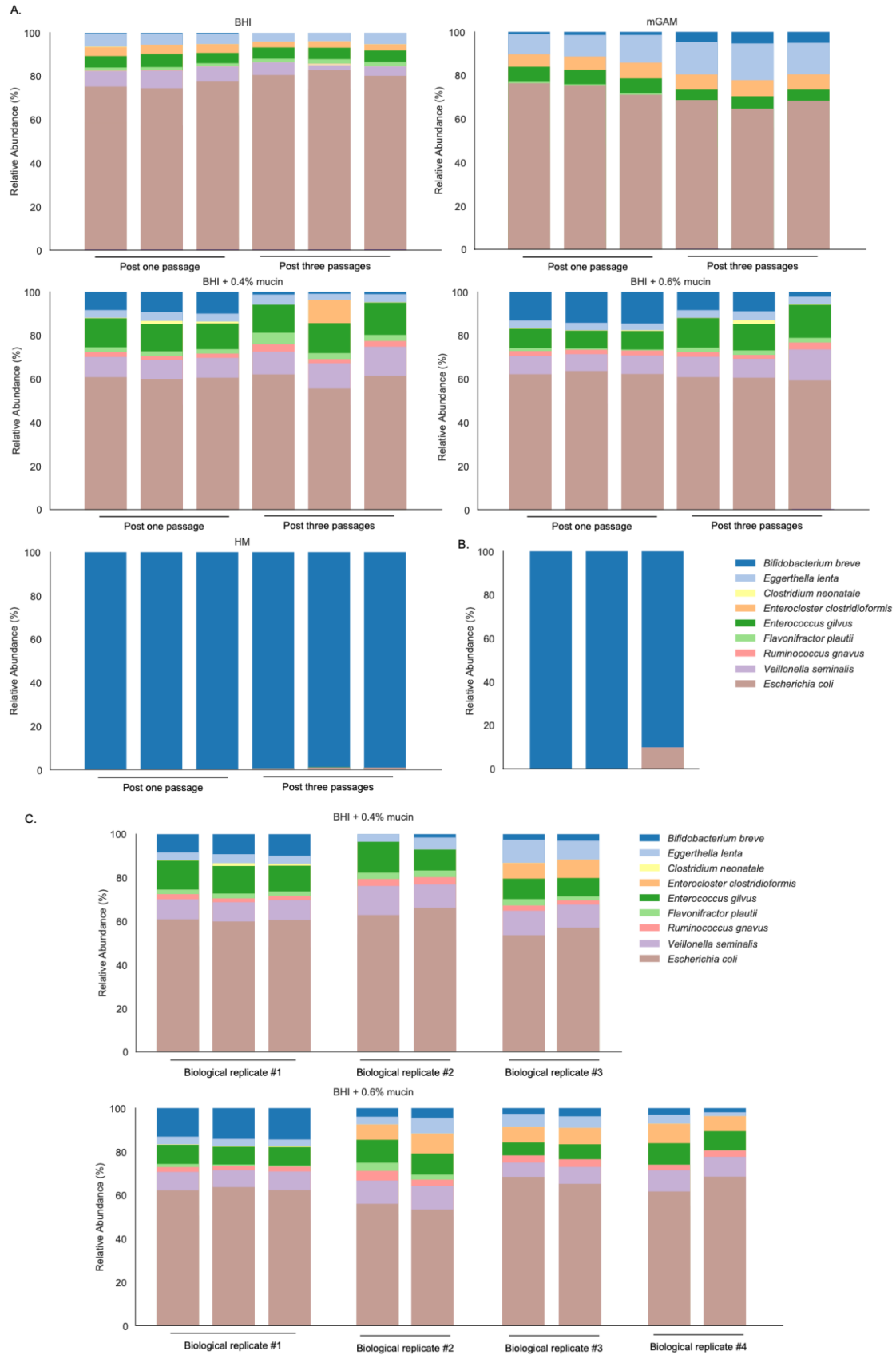


Figure S2. Stability and Reproducibility of the FT-1 *in vitro* microbiomes.

A) FT-1 in different growth media, and the compositions are compared between the first and the third passaging (x-axis, n=3). Bar height represents normalized species relative abundance, and bars are colored by species.

B) A HM-enrichment glycerol inoculum was grown in BHI + 0.6% mucin (n=3).

C) Reproducible FT-1 microbiome cultivation with BHI + 0.4% or 0.6% mucin. FT-1 inocula were inoculated in BHI + 0.4% or 0.6% mucin in three or four independent experiments (n=2 or 3 for each independent experiment).

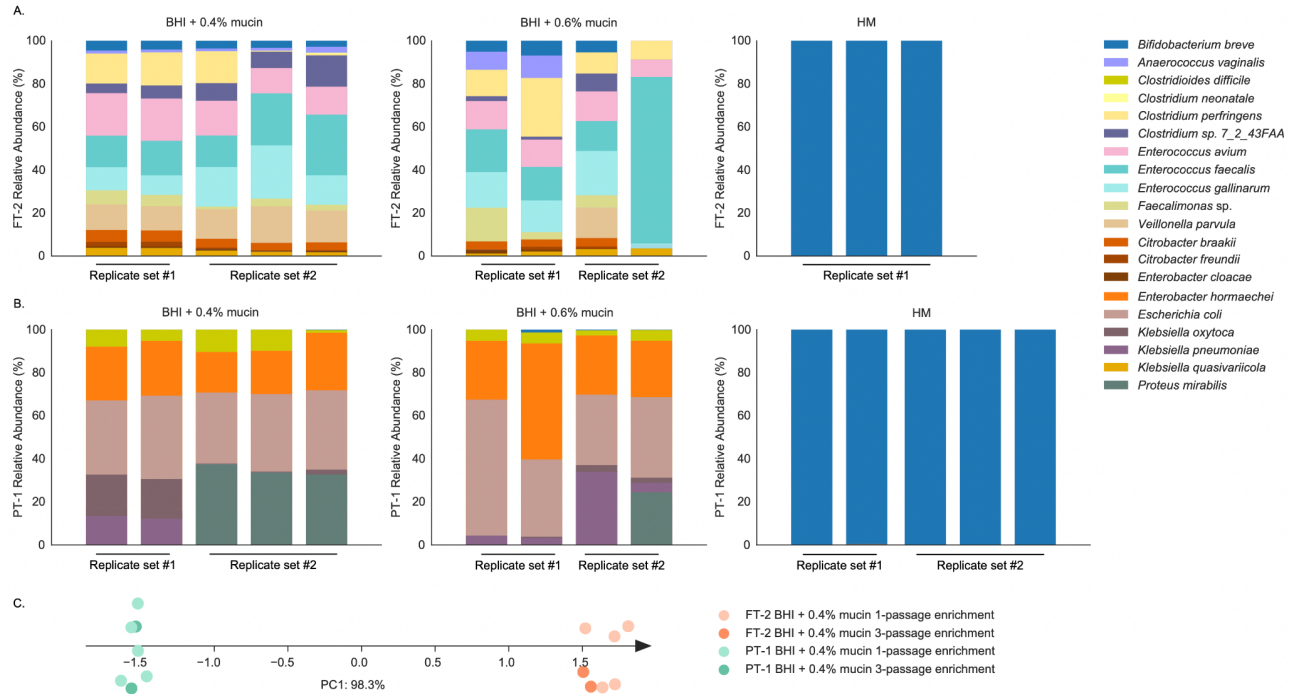


Figure S3. FT-2 and PT-1 microbiome cultivation with BHI + 0.4% or 0.6% mucin

A-B) S2 (A) and S3 (B) inocula were inoculated in BHI + 0.4%, BHI + 0.6% mucin, or HM. Bar height represents normalized species relative abundance, and bars are colored by species. The x-axis represents technical replicates ($n=2$ or 3 for each independent experiment).

C) PCA calculated using the weighted UniFrac Distances comparing FT-2 (green) and PT-1 (orange) communities grown in BHI + 0.4% mucin over three passages. Each circle represents a replicate ($n=2$ or 3 for each independent experiment).

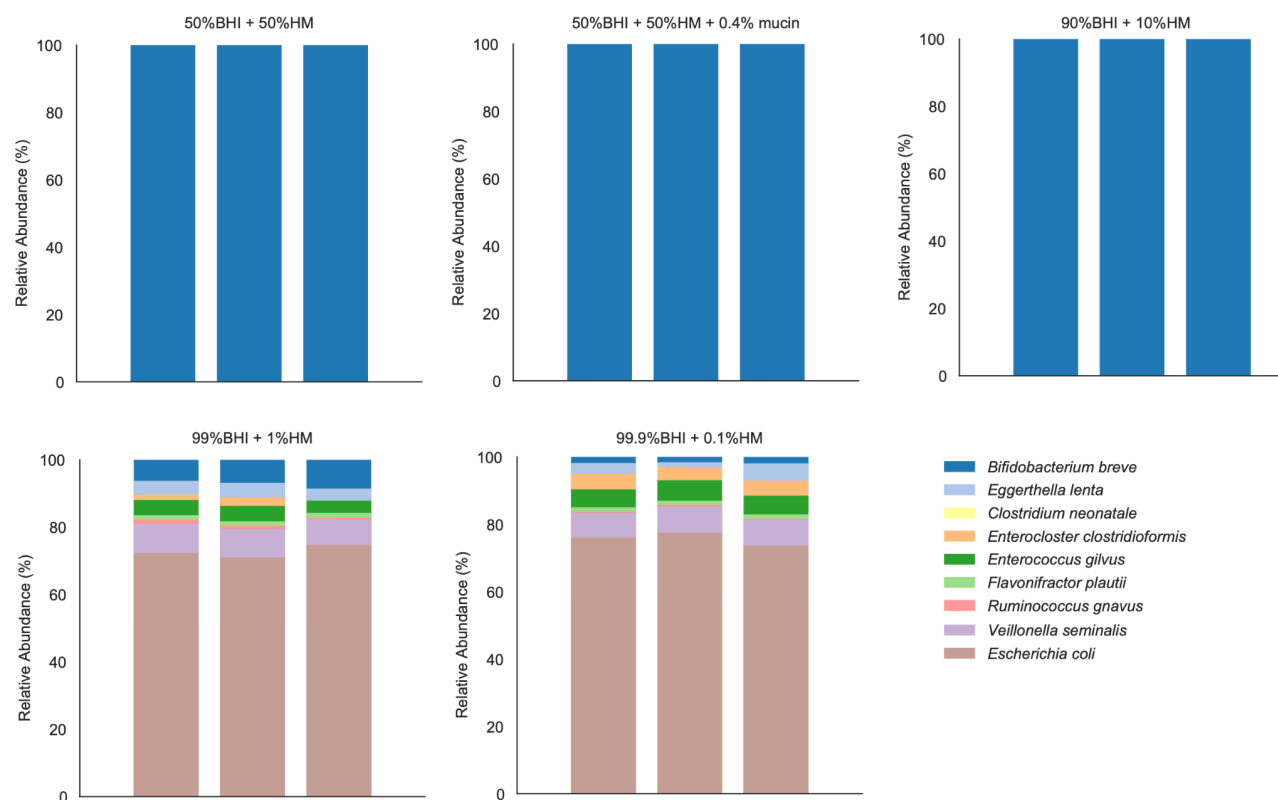


Figure S4. FT-1 microbiome compositions in BHI-HM mixtures. FT-1 inocula were inoculated in five BHI-HM mixtures. Bar height represents normalized species relative abundance, and bars are colored by species. The x-axis represents all replicates (n=3).

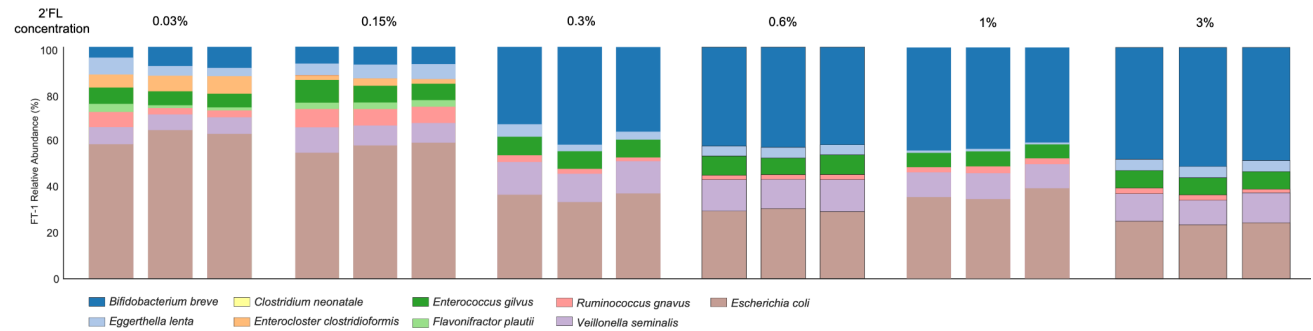


Figure S5. The FT-1 microbiome grown in different concentrations of 2'FL

The compositions of the FT-1 enrichments grown in BHI-mucin supplemented with varying concentrations of 2'FL (0.03%-3%) are shown in stacked bar plots. Bar height represents normalized species relative abundance, and bars are colored by species. The x-axis represents the growth media, and all replicates (n=3) are shown.

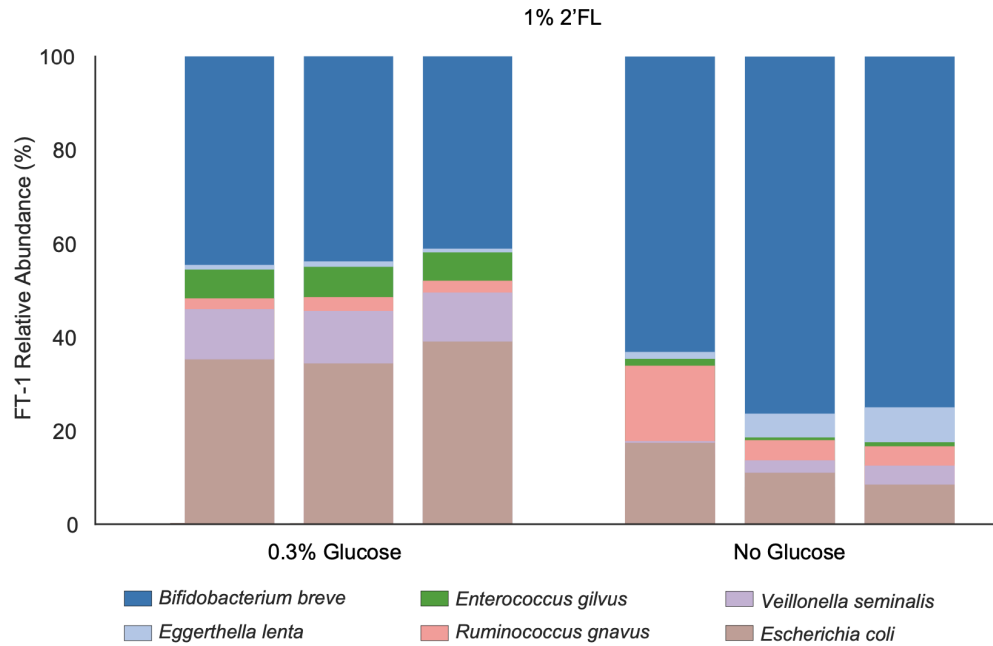


Figure S6. Glucose concentrations influence the FT-1 microbiome composition

The compositions of the FT-1 enrichments grown in BHI-mucin supplemented with 1% 2'FL with (left) and without (right) glucose. Bar height represents normalized species relative abundance, and bars are colored by species. The x-axis represents the growth media, and all replicates (n=3) are shown.

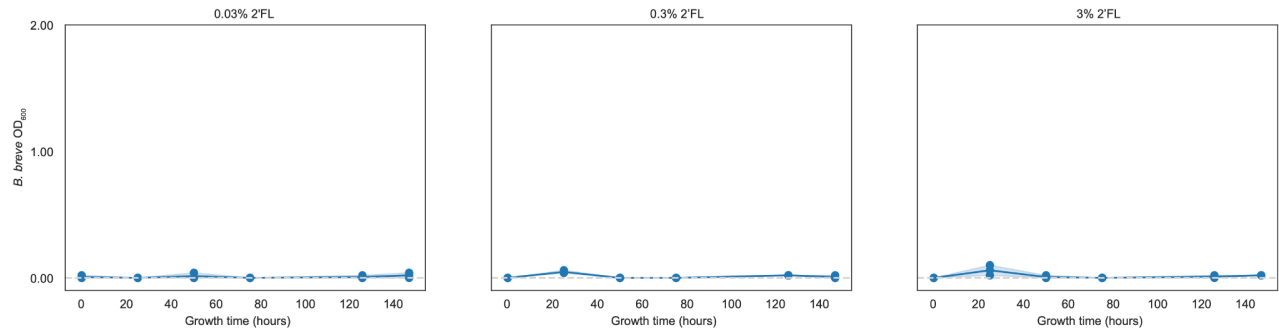


Figure S7. *B. breve* monoculture growth in 2'FL. *B. breve* (blue) grew in media in which 0.03% (left), 0.3% (middle), or 3% (right) 2'FL was added as the only carbohydrate source. The x-axis represents the growth time (in hours). The y-axis represents OD₆₀₀. Each circle represents a replicate sample, and the line represents the average of all replicates (n=3) at each time point; error bands indicate the 95% confidence interval.