A symbiont-derived glycosphingolipid enhances aerotolerance to enable neonatal gut colonization

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Abstract

Early-life gut colonization is a major ecological bottleneck for host-associated microbes because the neonatal intestine remains transiently oxygenated before becoming fully anaerobic. Although *Bacteroides fragilis* is a strict anaerobe, it displays strong fitness during neonatal colonization, suggesting the existence of stage-specific adaptive mechanisms. Here, we identify a species-specific glycosphingolipid, alpha-galactosylceramide (BfaGC), as a key determinant of this early-life fitness. Using genome-wide transposon sequencing during vertical transmission in gnotobiotic mice, we found that neonatal colonization depends on oxidative respiration-related pathways and on *agcT*, the gene required for BfaGC biosynthesis. Deletion of *agcT* selectively impaired neonatal colonization, whereas heterologous expression of *agcT* conferred a competitive advantage in early life to a non-BfaGC-producing *Bacteroides* strain. Mechanistically, BfaGC was induced by oxygen exposure and promoted aerotolerance by stabilizing membrane bioenergetics, preserving proton motive force, and supporting oxygen-dependent respiration. In addition to enhancing microbial fitness, neonatal BfaGC production was associated with host immune modulation, linking bacterial adaptation to developmental host-microbe interactions. Together, these findings identify BfaGC as a stage-specific colonization factor that enables *B. fragilis* to overcome transient oxygen stress in the neonatal gut and provide a molecular framework for host-microbiome co-adaptation in early life.

BfaGC is required for early colonization

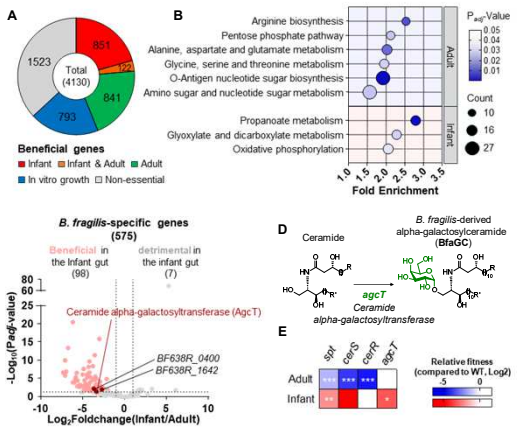


Figure 3. Genetic determinants of *Bacteroides fragilis* colonization in the neonatal gut.
(A) Classification of beneficial genes based on Tn-seq revealed condition-specific requirements for *B. fragilis* colonization in the adult and neonatal gut, **(B)** with subsequent KEGG pathway enrichment analysis highlighting distinct metabolic pathways engaged at each developmental stage (right). **(C)** Among *B. fragilis*-specific genes, those beneficial in the infant gut are indicated in pale red, and a subset previously shown to be oxygen-induced is marked in deep brown. *agcT*, encoding ceramide alpha-galactosyltransferase **(panel D)**, is one of only three genes belonging to both categories, uniquely positioning it as a key oxygen-responsive determinant of neonatal colonization. **(E)** Relative fitness of transposon-insertion mutant of core ceramide synthesis genes (*spt*, *cerR*, *cerS*) and *agcT* was determined compared to WT in both adult and infant gut. (*p-value<0.05, **p-value<0.005, and ***p-value<0.001).

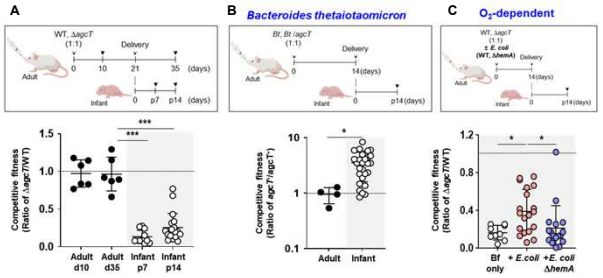


Figure 4. *BfaGC enhances B. fragilis colonization in the neonatal gut by promoting aerotolerance*
(A) Pregnant germ-free Swiss-Webster mice were orally gavaged with a 1:1 mixture of wild-type and Δ*agcT B. fragilis*, and strain abundances were quantified from the intestines of neonatal (at the postnatal day 7 and 14) and adult mice. **(B)** Pregnant germ-free mice were colonized with a 1:1 mixture of *B. thetaiotaomicron* strains lacking (*agcT*⁻) or expressing (*agcT*⁺) *agcT* gene. **(C)** Pregnant germ-free mice were co-colonized with wild-type and Δ*agcT B. fragilis*, along with either wild-type *E. coli* or an *E. coli* Δ*hemA* mutant, and offspring were analyzed at P14. Competitive fitness was calculated as the ratio of mutant to wild-type strain abundance. Data represent individual animals; bars indicate mean ± SD. (*p-value<0.05 and ***p-value<0.001).

Observation & Experimental Design

● ***Bacteroides fragilis*, an early colonizer of the infant gut**

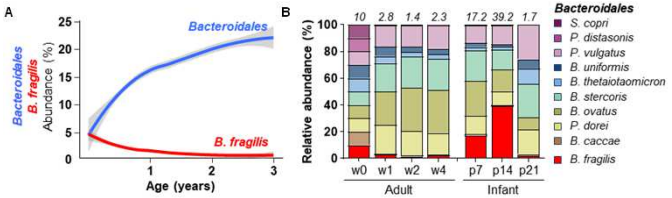


Figure 1. *Bacteroides fragilis* is an early-colonizing *Bacteroidales* species
Longitudinal relative abundance of **(A)** the order *Bacteroidales* and its member species *B. fragilis* in the infant human microbiota (TEDDY cohort) and **(B)** a defined consortium of 10 *Bacteroidales* species colonized in germ-free Swiss-Webster mice. The abundance of *B. fragilis* is described in the graphs. Data represent the mean of n = 6–7 mice per time point.

● **Genome-wide screen for neonatal colonization factors in *B. fragilis***

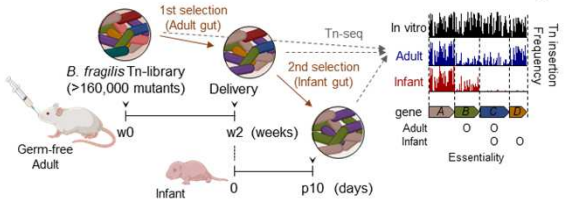


Figure 2. Transposon sequencing (Tn-seq) workflow for identifying genes essential for colonization.
A high-density transposon mutant library of *B. fragilis* (~160,000 mutants) was introduced into germ-free mice, enabling natural vertical transmission to neonates and stage-specific in vivo selection. The initial pool and bacterial populations recovered from adult and infant mice were analyzed by insertion sequencing. Genes with significantly reduced insertion frequencies under specific conditions were classified as beneficial for colonization in that environment.

BfaGC enhances aerobic respiration by limiting proton leakage

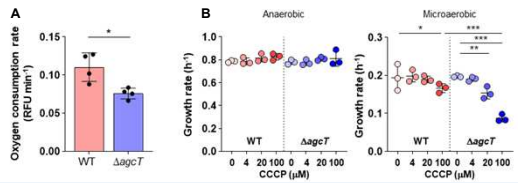


Figure 5. BfaGC enables aerobic respiration by maintaining proton motive force in *B. fragilis*
(A) Normalized oxygen consumption rate of WT and Δ*agcT* under microaerobic condition. **(B)** Growth rates of WT and Δ*agcT* was measured in PYG medium in the presence of indicated amounts of CCCP under anaerobic and microaerobic condition. Bars indicate mean ± SD. (*p-value<0.05, **p-value<0.005, and ***p-value<0.001).

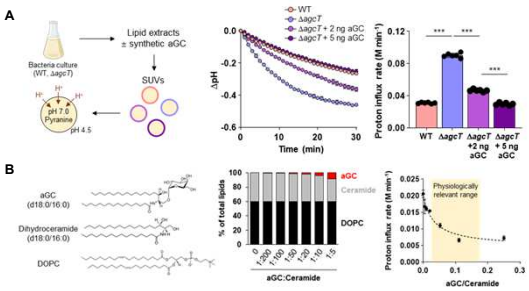


Figure 6. BfaGC reduces proton permeability across biological membranes and synthetic lipid vesicles.
(A) Proton permeability was assessed using small unilamellar vesicles (SUVs) composed of lipid extracts from wild-type, Δ*agcT*, and Δ*agcT* supplemented with synthetic BfaGC (2 or 5 ng). SUVs were loaded with the pH-sensitive dye pyranine (2 mM), equilibrated at pH 7.0, and transferred to pH 4.5. Intravesicular pH changes were monitored over 30 minutes via fluorescence (excitation: 410/454 nm; emission: 510 nm), and proton influx rates were calculated by linear regression after inverse log transformation. **(B)** The assay was repeated with SUVs composed of synthetic lipids (DOPC, aGC, and dihydroceramide) at physiological aGC/ceramide ratios observed in *B. fragilis* (0.02 in exponential phase and up to 0.18 in stationary phase or under oxygen-exposed condition). Bars indicate mean ± SD. (***p-value<0.001).

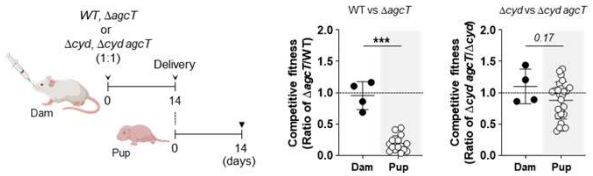


Figure 7. BfaGC promotes neonatal colonization in an aerobic respiration-dependent manner
Competitive colonization assay of Δ*cyd* and Δ*agcT cyd B. fragilis* in neonatal mice. Germ-free mice were co-colonized with either a 1:1 mixture of WT and Δ*agcT* or a mixture of Δ*cyd* and Δ*cyd agcT*. Competitive fitness of the agcT gene was determined by calculating the ratio of Δ*agcT* to WT or Δ*cyd agcT* to Δ*cyd*. Shown are the means and SD. (***p-value<0.001).

Oxygen-induced BfaGC drives temporal immunomodulation by *B. fragilis*

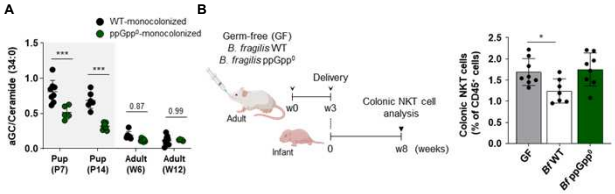
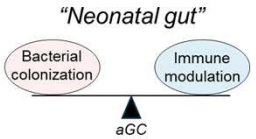


Figure 8. Oxygen-responsive BfaGC mediates immune modulation by *B. fragilis* in the neonatal gut
(A) Quantification of aGC levels in wild-type and ppGpp⁰ mutant *B. fragilis* strains under neonatal (postnatal day 7 and 14) and adult (at 6- and 12-weeks old) gut conditions. **(B)** Flow cytometry analysis of colonic NKT cell frequency in germ-free (GF) mice and gnotobiotic mice monocolonized with either wild-type *B. fragilis* (WT) or ppGpp⁰ mutant at 8 weeks of age. Data represent means ± SD. (*p-value<0.05 and ***p-value<0.001).

Conclusions

1. BfaGC is a **stage-** and **species-specific** sphingolipid produced by *B. fragilis*.
2. BfaGC enhances **neonatal colonization** by promoting aerobic respiration.
3. BfaGC stabilizes the **proton motive force** to support aerobic respiration.
4. Oxygen-responsive regulation of BfaGC controls **colonic NKT cell development**.



→**Temporally aligned mutual benefits** for microbe and host reveal **BfaGC** as a **co-evolved adaptation to the neonatal gut.**

Acknowledgement

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