High-resolution evaluation of gut microbiota associated with intestinal maturation in early preterm neonates

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INTRO

- "Leaky gut" (high intestinal permeability) is the proximate cause to necrotizing enterocolitis in preterm neonates
- Intestinal barrier maturation is associated with exclusive breastfeeding, less antibiotic exposure, and altered composition of the gut microbiota
- Study objective: to define microbial biomarkers for improved intestinal barrier maturation with high taxonomic resolution to develop novel diagnostic and therapeutic strategies

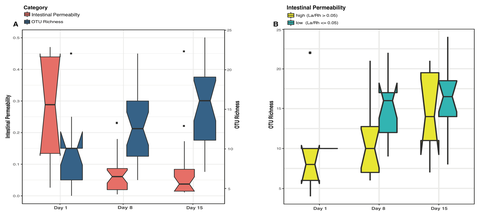
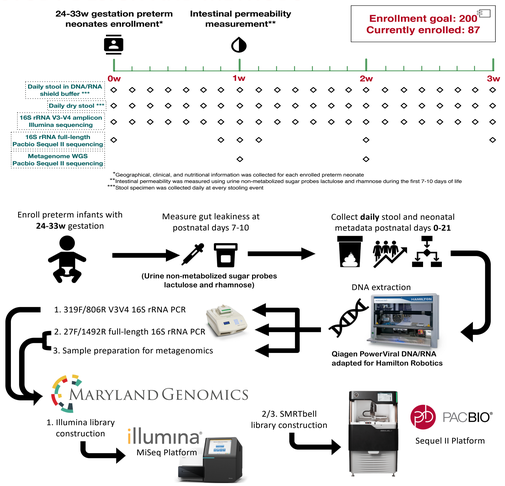


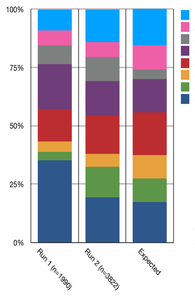
Fig. 1: Intestinal permeability as a function of microbial community composition

- At birth, majority preterm have highly leaky gut (La/Rh > 0.05)
- At postnatal day 8, intestinal barrier matured while 21.4% preterm neonates maintained elevated intestinal permeability (IP)
- IP correlated with increased community diversity and microbiota composition structure (Ma et al. 2018)

STUDY DESIGN AND METHODS



MAJOR RESULTS

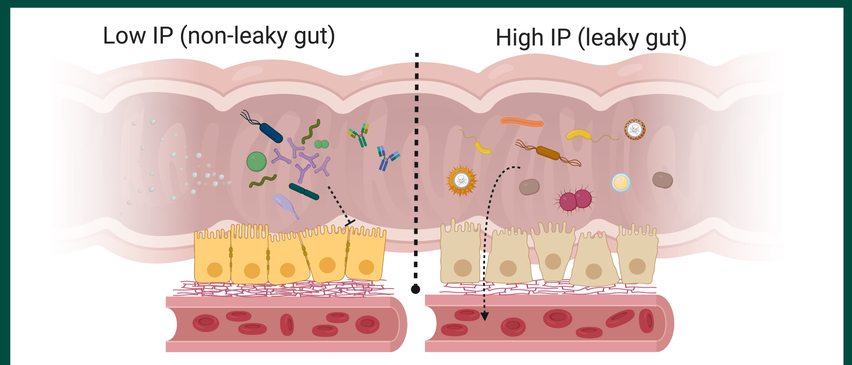


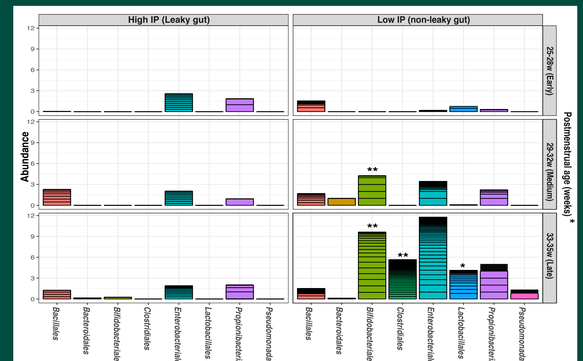
- A total of 1,360 ASVs were identified with an average sequence length of 1,462+/-14bp
- The majority of ASVs (87.6%) were assigned to 180 species, no available reference available for remaining ASVs
- 7,066 +/- 536 sequence/sample, 17.4+/-13.8 ASVs/sample and 5.3+/-3 species/sample were obtained

Sequencing the ZymoBIOMICS Microbial Community Standard, we observed unbiased and species-level taxonomic resolution

Fig. 2: ZymoBIOMICS controls sequencing on PacBio Sequel II

- Despite high abundance of *Escherichia coli* and *Klebsiella pneumoniae* in the gut microbiota, preterm neonates with ***Clostridiales*** and ***Bifidobacterium***, (***B. breve, B. longum* subsp. *infantis***, ***B. longum* subsp. *longum***) have low intestinal permeability (IP) at postnatal days 7-10.
- PacBio Sequel II closed metagenome-assembled genomes of ***B. breve*** reveal a battery of carbohydrate metabolism capabilities including "**bifid shunt**", indicating a capacity to consume and convert human milk oligosaccharides (HMO) to **short chain fatty acids** (SCFAs) with the potential to influence host physiology.





* Postmenstrual age (PMA) is calculated as gestational age at birth plus postnatal age as defined previously (Grier et al., 2017).

Fig. 3. Relative abundance of specific bacterial taxa associated with intestinal permeability

- ***Bifidobacteriales*** **and** ***Clostridiales*** **are most significantly associated with improved intestinal permeability.**

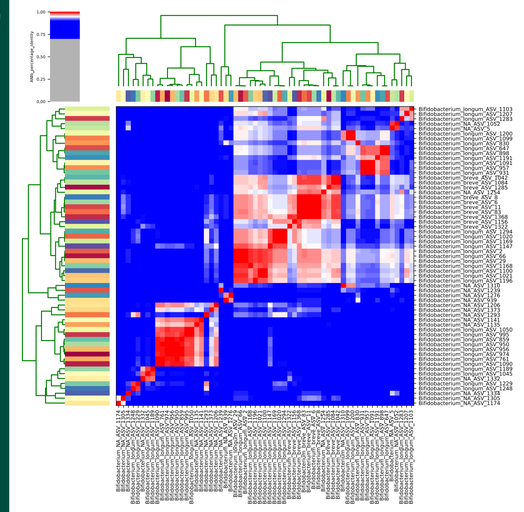


Fig. 4: ANI clustering of full-length 16S rRNA gene sequences aggregates ASVs to generate sub-species types

- At least 19 subspecies of *B. longum* and 5 *B. breve* were observed
- By comparing to 131 full-length 16S rRNA gene sequences of *B. longum*, 3 of the top most abundant *B. longum* (ASV2, 29, 66) belong to *B. longum* subspecies *infantis* and *B. longum* subspecies *longum*. These two subspecies have been previously identified to be the "champion colonizer" of the infant gut and adapted to metabolize human milk oligosaccharides and display anti-inflammatory activities
- Within *Clostridiales* and *Lactobacillales*, we observed 39 subspecies from *Clostridiales* (clusters IV , XIVa and I) in species of *Blautia faecalis, Roseburia intestinalis, Eubacterium rectale, Ruminococcus torques, etc.*, and 4 subspecies of *Lactobacillus fermentum*, 2 subspecies of *Lactobacillus salivarius*, and 4 subspecies of *Lactobacillus rhamnosus*

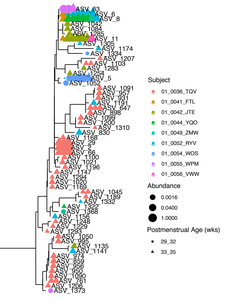


Fig. 5: Phylogenetic tree of *Bifidobacterium* subspecies in stool microbiota of cohort

- Multiple subspecies of *Bifidobacterium* can coexist in an infant
- A single subspecies of *Bifidobacterium* can be shared between several infants and that at multiple time points
- The presence of a multiple subspecies with an infant suggests strong environmental adaptive advantages and high microbial community dynamics

ONGOING

- Metagenome sequencing using long-reads PacBio Sequel II sequencing generates metagenome-assembled genomes in closed or draft forms
- Characterize genome content by sequencing metagenomes to understand strain-specific carbohydrate metabolism potentials of *Bifidobacterium* and *Clostridiales*
- Understand the genetic basis of cross-feeding interactions between *Bifidobacterium* (bifidogenic) and *Clostridiales* (butyrogenic)

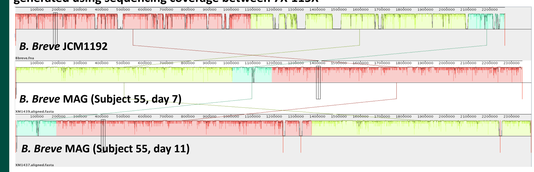
Table 1. Statistics of Sequel II sequence reads

Sample	Total Bp	#sequences	Mean	Median	Min	Max	N50
55-11.1	90,371,177	18,057	16,398	15,749	589	54,126	19,411
55-7.1	277,881,904	55,500	15,186	14,903	304	47,560	17,660

Table 2. Statistics of assembly *assembly was performed using Canu-1.8, alignment to reference *B. breve* genome JCM1192 was performed using MAUVE aligner. The statistics for the contigs aligned to reference is shown below.

Sample	Total Bp	#contig	MinLen	MedianLen	MeanLen	MaxLen	N50	N50_len	N90	N90_len
55-11.1	2392065	4	82560	144264	598016	1241394	1	1241394	2	923847
55-7.1	2350410	**1**	2350410	2350410	2350410	2350410	1	2350410	1	2350410

Fig. 6: Closed, complete or nearly complete metagenome-assembled genomes were generated using sequencing coverage between 7X-115X



Preliminary analysis of the genome content of *B. breve* revealed a variety of oligosaccharide transport proteins, glycosyl hydrolases genes, genes involved in the production of fucose and sialic acid, and the unique sugar metabolic pathway "bifid shunt". These results indicate *B. breve* remarkable capacity to digest and consume human milk oligosaccharide (HMO).

Take a picture for our first publication on this topic

Take a picture for PacBio webinar on Sequel II capabilities presenting our data!

Check out Booth #861 for MARYLAND GENOMICS

FUNDING

- UMB Accelerated Translational Incubator Pilot Grant
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