Supplementary Materials

Breast Milk Microbiota at One Month Postpartum in Women from Arequipa, Peru, Assessed by Full-Length 16S rRNA Gene Nanopore Sequencing

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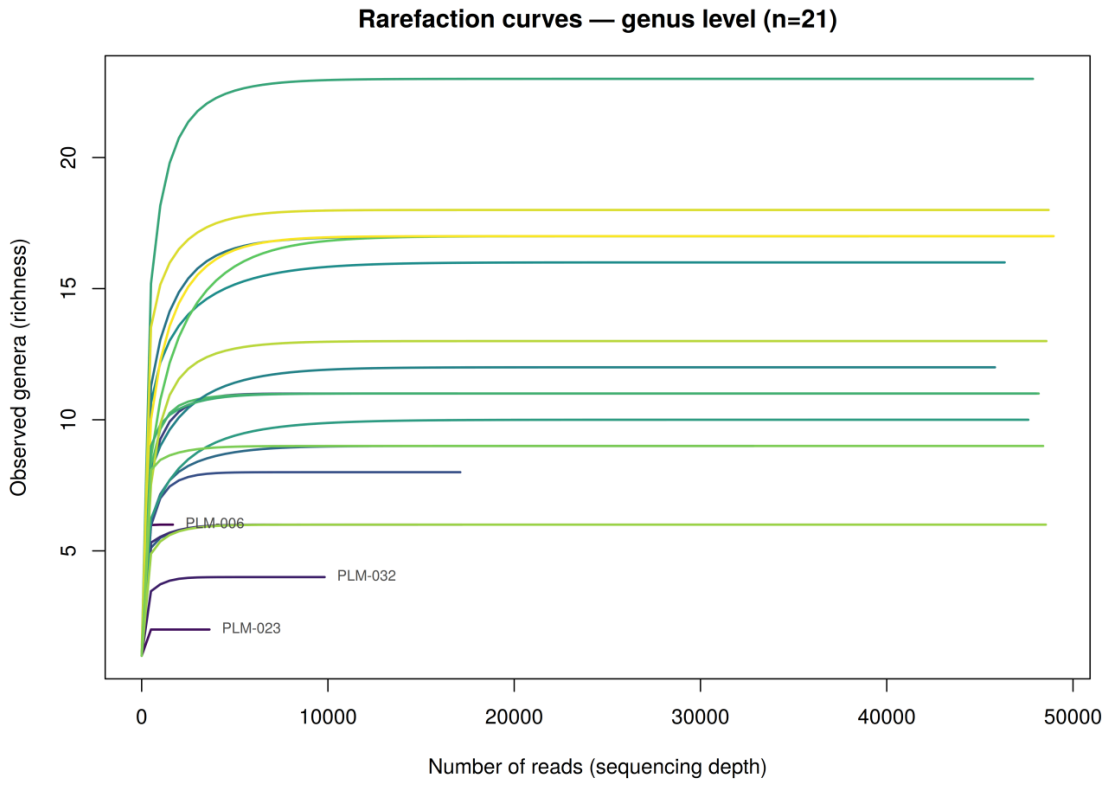


Figure S1. Genus-level rarefaction curves (n = 21). For each sample, the observed genus richness is plotted against sequencing depth (number of reads). All curves plateaued, indicating that the sequencing depth was sufficient to characterize bacterial diversity at the genus level.

Table S1. Sequencing depth, chimera removal and coverage per sample (n = 21).

Sample	Initial reads[1]	Final reads[2]	Chimeras (%)[3]	Observed genera	Good's coverage (%)
PLM-001	122,287	47,847	2.78	23	100
PLM-003	17,165	16,932	1.05	11	100
PLM-006	1716	1676	0.12	6	100
PLM-007	33,558	32,863	1.67	9	100
PLM-008	83,228	48,154	3.60	11	100
PLM-009	48,144	45,808	4.11	12	100
PLM-014	47,774	46,329	2.57	16	100
PLM-015	17,710	17,210	2.77	6	100
PLM-017	160,884	48,388	2.75	17	100
PLM-018	39,983	39,018	1.74	17	100
PLM-021	81,044	48,957	2.01	17	100
PLM-023	3740	3639	2.46	2	100
PLM-024	15,294	14,818	3.01	6	100
PLM-025	17,393	17,107	1.47	8	100
PLM-026	83,197	48,010	3.91	11	100
PLM-027	106,873	48,688	2.55	18	100
PLM-028	522,324	47,598	4.72	10	100
PLM-030	121,355	48,569	2.72	13	100
PLM-032	10,070	9822	2.22	4	100
PLM-034	287,577	48,539	2.82	6	100
PLM-035	266,153	48,395	3.09	9	100

[1] Reads passing length (1300–1800 bp) and quality (Q ≥ 12) filters. [2] Reads retained after subsampling to a maximum of 50,000 reads, chimera removal and taxonomic classification. [3] Percentage of reads identified as chimeric by VSEARCH.

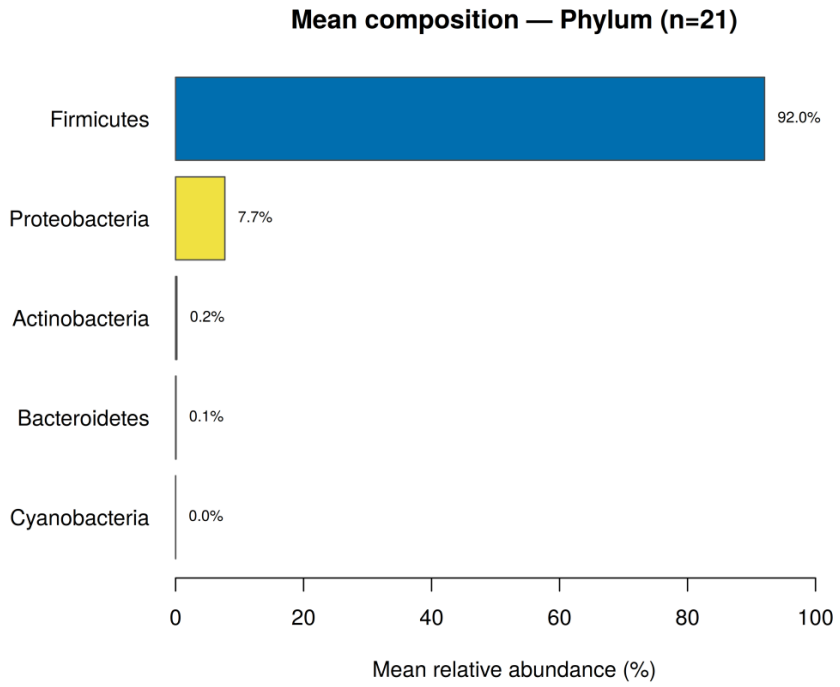


Figure S2. Mean bacterial composition at phylum level (n = 21). Horizontal bars show the mean relative abundance per phylum across all samples. Phylum names are shown according to the former nomenclature (Firmicutes = Bacillota; Proteobacteria = Pseudomonadota; Actinobacteria = Actinomycetota; Bacteroidetes = Bacteroidota).

Table S2. Comparison of alpha-diversity indices across maternal, obstetric, and dietary groups (n = 21).

Variable	Metric	Group 1	Group 2	W	p-value
BMI	Observed richness	Normal (median = 16)	Overweight/obesity (median = 7)	93.5	**0.0071**
BMI	Shannon	Normal (median = 1.11)	Overweight/obesity (median = 0.60)	83.0	0.0513
BMI	Simpson	Normal (median = 0.56)	Overweight/obesity (median = 0.26)	76.0	0.1517
Mode of delivery	Observed richness	Cesarean (median = 9.5)	Vaginal (median = 12)	46.0	0.5917
Mode of delivery	Shannon	Cesarean (median = 0.65)	Vaginal (median = 0.66)	51.0	0.8621
Mode of delivery	Simpson	Cesarean (median = 0.28)	Vaginal (median = 0.40)	47.0	0.6511
Maternal supplement use	Observed richness	No (median = 11)	Yes (median = 9.5)	55.0	0.6797
Maternal supplement use	Shannon	No (median = 0.88)	Yes (median = 0.60)	68.0	0.1718
Maternal supplement use	Simpson	No (median = 0.40)	Yes (median = 0.26)	65.0	0.2545
Processed-food diet	Observed richness	Consumes (median = 11)	Avoids (median = 8.5)	53.5	0.5309
Processed-food diet	Shannon	Consumes (median = 0.71)	Avoids (median = 0.65)	52.0	0.6222
Processed-food diet	Simpson	Consumes (median = 0.33)	Avoids (median = 0.27)	53.0	0.5693
Fermented-food intake	Observed richness	High (median = 12)	Low (median = 9.5)	67.0	0.1889
Fermented-food intake	Shannon	High (median = 0.66)	Low (median = 0.65)	46.0	0.8557
Fermented-food intake	Simpson	High (median = 0.27)	Low (median = 0.30)	39.0	0.4880
Fish intake	Observed richness	Consumes (median = 10.5)	Never (median = 11)	54.0	0.7355
Fish intake	Shannon	Consumes (median = 0.61)	Never (median = 0.73)	37.0	0.4003
Fish intake	Simpson	Consumes (median = 0.26)	Never (median = 0.40)	36.0	0.3601

Group comparisons were performed with the Mann–Whitney U test. W, Mann–Whitney test statistic. Values in bold indicate $p < 0.05$ (uncorrected for multiple comparisons). Shannon and Simpson (1−D) indices were calculated at the genus level.