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Maternal Probiotic Supplementation During Lactation Shapes Gut Microbiota and Metabolome in Dams and Offspring: A Multi-Omics Mouse Study

Junhong Zhou[1,2,3], Jingda Lu[1,2,3], Cuijiao Feng[1,2,3], Fei Sun[1,2,3], Min Nie[1,2,3], Rui Yang[1,2,3], Lai-Yu Kwok[1,2,3], Qiuwen He[1,2,3], Zhi Zhong[1,2,3]*

[1]*Key Laboratory of Dairy Biotechnology and Engineering, Ministry of Education, Inner Mongolia Agricultural University, Hohhot 010018, China*

[2]*Key Laboratory of Dairy Products Processing, Ministry of Agriculture and Rural Affairs, Inner Mongolia Agricultural University, Hohhot 010018, China*

[3]*Inner Mongolia Key Laboratory of Dairy Biotechnology and Engineering, Inner Mongolia Agricultural University, Hohhot 010018, China*

ABSTRACT: The late pregnancy and lactation periods represent critical windows for maternal and infant health, during which the gut microbiome undergoes dynamic changes that influence immune development, metabolism, and overall well-being. This study investigated the effects of maternal supplementation with *Bifidobacterium animalis* subsp. *lactis* Probio-M8 (Probio-M8) during lactation on both dams and their offspring using a mouse model. Probiotic-treated dams exhibited significantly reduced body weight at weaning and increased serum IL-6 levels ($P < 0.05$), indicating immunomodulatory effects. Although no major shifts in overall microbial diversity were observed, specific bacterial taxa, including *Alistipes indistinctus*, *Agathobacter* sp., and *Bifidobacterium pseudocatenulatum*, were enriched in probiotic-fed dams, while pups showed increased abundance of *Anaerobutyricum hallii* and other beneficial species. Metagenomic and carbohydrate-active enzyme profiling revealed enhanced capacity for complex carbohydrate degradation in probiotic-exposed pups, suggesting improved metabolic adaptation during weaning. Fecal metabolomics further identified significant enrichment of pathways related to tyrosine metabolism and polyunsaturated fatty acid biosynthesis in pups, including α-linolenic acid metabolism, key processes linked to neurological development and immune regulation. Correlation analyses highlighted associations between these metabolic shifts and differentially abundant bacterial taxa, underscoring the interplay between microbiota composition and host physiology. Collectively, our findings demonstrate that maternal probiotic administration during lactation modulates both maternal and offspring gut ecosystems, promoting favorable microbial and metabolic profiles that may support growth, immunity, and developmental programming. These results provide mechanistic insights into how early-life microbial exposure via breastfeeding can be leveraged for nutritional interventions aimed at improving lifelong health outcomes.

Keywords: *Bifidobacterium animalis* subsp. *lactis*; breast milk microbiota; early-life development; functional metagenomics; intestinal barrier function; neurodevelopmental metabolites; weaning

1. Introduction

The gut microbiome, the largest microbial reservoir within the human body, is increasingly recognized for its pivotal role in human health and disease(Ursell, Metcalf, Wegener, & Rob, 2012). During late

***Corresponding author**
imu150zhongzhi@163.com

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pregnancy and lactation, women experience profound physiological changes, including weight gain, hormonal fluctuations, and dynamic shifts in both the gut microbiome and immune responses(Kohlhepp et al., 2018). These transitions are not only crucial for maternal adaptation but also for shaping the early-life microbiota of the infant. Maternal dietary modifications during this period have been shown to influence both the composition of the maternal gut and breast milk microbiomes, which together serve as key determinants of the infant's early-life microbial colonization(Calkins & Devaskar, 2011). Compared to adults, the infant gut microbiome is typically less diverse and stable, and its development is influenced by multiple factors, such as mode of delivery, feeding practices, antibiotic exposure, and environmental interactions. This microbial community gradually matures into an adult-like composition around the age of three(Yatsunenko et al., 2012).

Recognized as the optimal food for infants, breastfeeding provides vital nutrients, immunological factors, and microbial benefits that foster newborn health and development(Damaceno et al., 2017). The maternal microbiome, encompassing microbial communities from various anatomical sites, serves as a primary source of microbial colonization for the infant(Bäckhed et al., 2015; Damaceno et al., 2017). Notably, breast milk is not only a rich source of essential nutrients but also contains a diverse array of commensal bacteria, with estimates suggesting that approximately 800 mL of daily consumption may deliver between 10^5 and 10^7 bacterial cells to the infant(Jeurink et al., 2013). Vertical transmission of microbiota from mother to child is well-documented(Inoue & Ushida, 2003), and breastfeeding represents one of the most direct and effective routes for this microbial transfer, playing a critical role in shaping the infant gut microbiome(Pannaraj et al., 2017).

Emerging evidence highlights the potential of probiotic supplementation to enhance this process. For instance, Zhong et al. (2022) used *Bifidobacterium animalis* subsp. *lactis* Probio-M8 (Probio-M8) as a biological tracer to investigate vertical transmission and strain-level genetic variation of bifidobacteria from mothers to infants, employing both traditional culture techniques and high-throughput sequencing(Zhong et al., 2022). Their findings demonstrated that orally administered maternal probiotics can successfully colonize and adapt genetically within the infant gut, supporting the hypothesis of vertical transmission through breastfeeding and highlighting the long-term implications of early probiotic exposure. Bifidobacteria and other probiotics have been shown to modulate the infant gut microbiome, enhance nutrient absorption, strengthen immune function, and mitigate gastrointestinal symptoms such as diarrhea and constipation(Azad, Sarker, Li, & Yin, 2018). Moreover, reduced colonization of bifidobacteria during early life has been associated with an elevated risk of persistent gut dysbiosis(Dominguez-Bello, Godoy-Vitorino, Knight, & Blaser, 2019), underscoring the importance of early-life probiotic interventions for both short- and long-term health outcomes.

In light of these findings, this study aimed to investigate the effects of administering Probio-M8 to lactating mice on the gut microbial composition and functional profiles of both mothers and their offspring. Using a combination of physiological assessments, metagenomic sequencing, and metabolomic analyses,

this study sought to elucidate how maternal probiotic supplementation influences intergenerational microbial dynamics. Furthermore, this work offers novel insights into the indirect delivery of beneficial nutrients and microbes to infants via breastfeeding, paving the way for the development of next-generation functional foods designed to support early-life gut microbiome assembly and promote long-term health outcomes.

2. Methods

2.1 Ethics statement

All experimental procedures involving mice were approved by the Experimental Animal Welfare and Ethics Committee of Inner Mongolia Agricultural University (Approval No.: NND2023044).

2.2 Experimental design

A total of 45 eight-week-old C57BL/6J mice (30 females and 15 males) were procured from Beijing SPF Bioscience Co., Ltd. (Beijing, China). Upon arrival, the mice were housed in a specific pathogen-free environment at the animal facility of Inner Mongolia Agricultural University. Throughout the study, all mice had *ad libitum* access to standard rodent diet and water, and were maintained under controlled environmental conditions (temperature: 22 ± 2°C; humidity: 50 ± 10%) with a 12-hour light/dark cycle.

Following a one-week acclimatization period, male and female mice were randomly paired at a ratio of 1:2 for one week to promote successful mating. Pregnant dams were randomly assigned to either the control group (n = 13) or the probiotic group (n = 14) and housed individually in preparation for parturition.

The control group received daily oral gavage of 0.1 mL saline solution from postpartum day 0 until weaning on day 21. The probiotic group received a daily oral gavage of 0.1 mL Probio-M8 suspension containing 10^{10} CFU over the same period. The number of pups per dam was recorded at birth. Body weights of dams were monitored weekly throughout lactation, while pup body weights were recorded on postnatal days 14 and 21.

On postnatal day 21, all mother mice and 69 randomly selected pups (Supplementary Table 1) were humanely euthanized by carbon dioxide inhalation following approved ethical protocols. Whole-blood samples were collected in endotoxin-free tubes, allowed to clot at room temperature for 20 to 30 min, and then centrifuged at 4°C for 10 min at 1000 × *g* to separate serum. Serum samples were either used immediately or stored at −80°C to minimize freeze-thaw cycles. Selected organs from the dams were harvested and weighed to calculate organ indices. On the same day, fecal samples were collected from all dams and pups and stored at −80°C until further processing for metagenomic and metabolomic analyses. Prior to euthanasia, intestinal permeability was assessed in all pups, and ileum tissues were collected and fixed in 4% paraformaldehyde for subsequent histological evaluation.

2.3 Evaluation of intestinal permeability

On postnatal day 21, intestinal epithelial permeability in the pups was evaluated using fluorescein isothiocyanate-dextran (FD4) of 4 kDa molecular weight. A stock solution of FD4 was prepared at a concentration of 50 mg/ml and protected from light to prevent quenching. After a 6-h fasting period with

free access to water, the pups were weighed and administered FD4 via intragastric gavage at a dose of 600 mg/kg body weight. Blood samples were collected 4 h post-gavage from the orbital sinus into endotoxin- and pyrogen-free tubes. Serum was separated by centrifugation and stored at −80°C until analysis. The concentration of FD4 in serum was quantified using fluorescent spectrophotometry (MULTISKAN ASCENT, Thermo Fisher Scientific Inc., Waltham, MA, USA) with excitation and emission wavelengths set at 480 nm and 520 nm, respectively. Fluorescence readings were converted to FD4 concentrations using a standard curve generated from known concentrations of FD4.

2.4 Determination of serum cytokine levels

Serum samples were thawed on ice before analysis. Levels of six cytokines, including interleukin (IL)-2, IL-4, IL-6, IL-10, tumor necrosis factor-alpha (TNF-α), and interferon-gamma (IFN-γ), were quantified using Luminex xMAP® technology. A ProcartaPlex™ multiplex assay kit (Thermo Fisher Scientific Inc., Waltham, MA, USA) was used according to the manufacturer's instructions. Fluorescence intensity was measured using a Luminex 200™ instrument system, and cytokine concentrations were determined by fitting the data to standard curves using a five-parameter logistic regression model. This approach ensured accurate quantification across the dynamic range of each cytokine.

2.5 Histological analysis

Ileum tissue samples fixed in 4% paraformaldehyde were embedded in paraffin, sectioned, and stained with hematoxylin and eosin for histological evaluation. Tissue sections were scanned using a Pannoramic slide scanner, which captured high-resolution digital images of the entire tissue section by scanning and stitching together individual fields during imaging. Digital slides were stored as folders containing all histological information and were analyzed using CaseViewer v2.4 software. At least five intact intestinal villi and their corresponding crypts were selected per section for morphometric analysis. Images were captured at 100× magnification, ensuring consistent background illumination across all photographs. Using Image-Pro Plus v6.0 software, the length of each villus and the depth of its associated crypt were measured in millimeter units. The villi-to-crypt ratio was calculated as: Villus-to-crypt ratio = Average villus length / Average crypt depth. These measurements provided quantitative insights into intestinal morphology and structural integrity.

2.6 Shotgun fecal metagenomics and bioinformatics analyses

A total of 96 mouse fecal samples (27 from lactating mothers and 69 from their offspring) were

subjected to shotgun metagenomic sequencing. Metagenomic DNA was extracted from these samples using the QIAamp DNA Stool Mini Kit (QIAGEN, Hilden, Germany), following the manufacturer's protocol. Sequencing libraries were prepared using the NEBNext Ultra DNA library Prep Kit (New England Biolabs, Inc., Ipswich, MA, USA), and paired-end sequencing was performed on the Illumina HiSeq 2500 platform at Novogene Co., Ltd. (Beijing, China).

Quality control of raw sequencing reads was conducted using the KneadData pipeline (http://huttenhower.sph.harvard.edu/kneaddata, accessed on 15 August 2024), which filtered out low-quality reads (sequences shorter than 60 nucleotides) and host-derived contaminants. High-quality reads from each sample were then assembled into contigs using MEGAHIT(Li, Liu, Luo, Sadakane, & Lam, 2015). Contigs larger than 2,000 base pairs were binned into metagenome-assembled genomes (MAGs) using a combination of binning tools: MetaBAT2, VAMB, and DAS Tool(Kang et al., 2019; Nissen et al., 2021; Sieber et al., 2018).

Bins generated by different software were merged to improve recovery and reduce redundancy. The quality of the resulting MAGs, including estimates of genome completeness and contamination, was assessed using CheckM (https://github.com/Ecogenomics/CheckM, accessed on 15 August 2024). Subsequently, all MAGs were clustered to generate species-level genomic bins (SGBs). The abundance of each SGB in individual samples was calculated as reads per kilobase million. Taxonomic annotation of SGBs was performed using the Genome Taxonomy Database (https://gtdb.ecogenomic.org/, accessed on 15 August 2024).

To characterize functional profiles, gut metabolic modules (GMMs) related to polysaccharide metabolism and short-chain fatty acid (SCFA) biosynthesis pathways were identified based on the Kyoto Encyclopedia of Genes and Genomes (KEGG) Orthologues (KOs). The Omixer-RPM tool (parameter: -c 0.66) was used to identify SGBs possessing genes associated with these pathways(Darzi, Falony, Vieira-Silva, & Raes, 2016). Additionally, carbohydrate-active enzyme (CAZyme)-encoding genes within the fecal microbiomes of both mother and offspring mice were detected using dbCAN2(Caspi et al., 2020; Magnúsdóttir et al., 2017; Z. Wang & Zhao, 2018).

2.7 Non-targeted fecal metabolomic analysis

A total of 96 mouse fecal samples (27 from lactating mothers and 69 from their offspring) were subjected to non-targeted metabolomic profiling. For each sample, approximately 25 mg (± 1 mg) of fecal material was transferred into a microcentrifuge tube containing beads and 500 μL of extraction solvent composed of methanol, acetonitrile, and water (2:2:1, v/v), supplemented with tritiated internal standards to monitor extraction efficiency and instrument stability. The mixture was vortexed for 30 s, followed by homogenization at 35 Hz for 4 min. Ultrasonic disruption was performed in a 4°C water bath for 5 min, and this step was repeated three times to ensure complete lysis. To precipitate proteins, samples were incubated at -40°C for 1 h. Following centrifugation at 12,000 rpm (relative centrifugal force = 13,800 × g, radius = 8.6 cm) at 4°C for 15 min, the supernatant was collected and transferred into liquid chromatography-mass spectrometry vials for analysis. Quality control samples were prepared by pooling equal volumes of all sample supernatants to assess system stability and reproducibility throughout the analytical run.

Polar metabolites were analyzed using a Vanquish ultra-high-performance liquid chromatography system (Thermo Fisher Scientific, Waltham, MA, USA), coupled with a Waters ACQUITY UPLC BEH Amide column (2.1 mm × 50 mm, 1.7 μm; Water Corporation, Milford, MA, USA) for chromatographic

separation. The mobile phase consisted of (A) an aqueous solution containing 25 mmol/L ammonium acetate and 25 mmol/L ammonia, and (B) acetonitrile. The sample tray temperature was maintained at 4°C, with an injection volume of 2 μL.

Raw data files were converted to mzXML format using ProteoWizard software for downstream processing. Metabolite identification was performed using the R package BiotreeDB (v3.0), a curated reference database(Zhou et al., 2020). Multivariate statistical analysis was performed using orthogonal partial least squares discriminant analysis, and the variable importance in projection (VIP) score was calculated from the derived model to assess the contribution of each metabolite to group separation. Metabolites with VIP score > 1 and $P < 0.05$ were considered significantly altered. Differentially abundant metabolites in dams and pups were identified using thresholds of $P < 0.05$, VIP score > 1.0, and fold change > 1.2 or < 0.83. Functional interpretation and pathway enrichment analyses were conducted using MetaboAnalyst v6.0, based on Kyoto Encyclopedia of Genes and Genomes pathways.

2.8 Statistical analysis

Statistical analyses were conducted using R software (v.4.0.2). A normalized reads per kilobase million abundance table was generated to account for differences in sequencing depth across samples. Microbial α-diversity (species richness and evenness) was calculated to assess gut microbiota diversity within each group. Beta-diversity was evaluated using non-metric multidimensional scaling and principal coordinates analysis (Bray-Curtis distance), to visualize intergroup differences in microbial community structure. These analyses were performed using the R packages vegan (v2.5-1) and amplicon (v1.19.0). Differential microbial community structures between groups were visualized and statistically assessed using the ggpubr package (v0.6.0). Additional data visualization, including stacked bar plots depicting species-level relative abundances, was generated using the reshape2 (v1.4.4), knitr (v1.42), and ggplot2 (v3.4.2) packages. All graphical outputs were finalized using R software (v4.0.2) and Adobe Illustrator 2020 for enhanced clarity and presentation quality.

3. Results

3.1 Maternal Probio-M8 supplementation reduced body weight in late-lactating dams

The experimental timeline is shown in Fig. 1A. Litter sizes were recorded on postpartum day 0 (Supplementary Table 1), with body weights of dams and pups monitored throughout gestation and lactation. No significant differences in body weight were observed between the probiotic and control groups during pregnancy or early lactation ($P > 0.05$, Wilcoxon test; Fig. 1B). However, by weaning day (postnatal day 21), dams in the probiotic group exhibited significantly lower body weights compared to those in the control group ($P < 0.01$, Wilcoxon test; Fig. 1B). Owing to ethical considerations aimed at minimizing maternal and pup stress, pup body weights were recorded only on postnatal days 14 and 21. No statistically significant differences in pup body weight were detected between the two groups on either day ($P > 0.05$, Wilcoxon test; Fig. 1C).

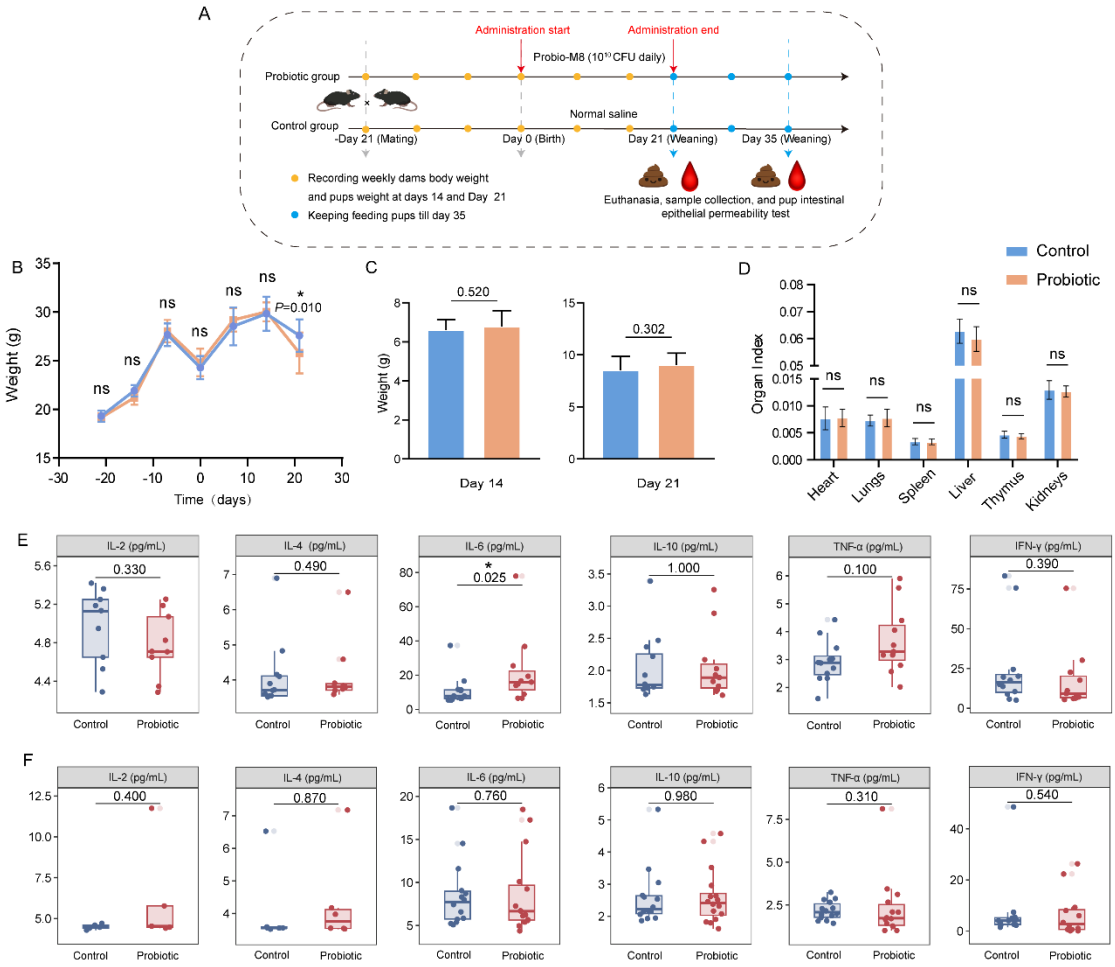

Figure 1. Experimental Design and Physiological Effects of *Bifidobacterium animalis* subsp. *lactis* Probio-M8 Supplementation in Lactating Mice. (A) Timeline of the experimental intervention. Dams in the probiotic group received a daily oral gavage of 0.1 mL of *Bifidobacterium animalis* subsp. *lactis* Probio-M8 suspension containing 10^{10} CFU, while those in the control group received normal saline from postnatal day 0 to day 21 (weaning). (B, C) Body weight measurements of (B) dams throughout the intervention period and (C) pups at postnatal days 14 and 21. (D) Organ index assessment in dams at day 21. (E, F) Serum cytokine levels in (E) dams and (F) pups at day 21. Error bars indicate the standard error of the mean. Statistical comparisons were performed using the Wilcoxon test, with corresponding *P*-values provided (ns represents $P > 0.05$, $^*P < 0.05$). IFN, interferon; IL, interleukin.

Organ indices, determined as the ratio of individual organ weight to total body weight, were assessed in dams following euthanasia to evaluate potential systemic effects of high-dose Probio-M8 supplementation. Six vital organs (heart, lungs, spleen, liver, kidneys, and thymus) were evaluated to assess signs of toxicity or pathological changes. No significant differences in organ indices were observed between the probiotic and control groups ($P > 0.05$, Wilcoxon test; Fig. 1D), suggesting that maternal administration of high-dose Probio-M8 did not induce detectable organ-level toxicity.

3.2 Maternal Probio-M8 supplementation enhanced immune regulation immunity in late-lactating dams

Serum cytokine levels are key indicators of immune status and inflammatory responses. To evaluate the immunomodulatory effects of Probio-M8, six cytokines (IL-2, IL-4, IL-6, IL-10, TNF-α, and IFN-γ) were analyzed in both lactating dams and their offspring on weaning day (postnatal day 21).

In dams, the probiotic group exhibited significantly higher serum IL-6 levels compared to the control group ($P < 0.05$, Wilcoxon test; Fig. 1E), with no significant differences observed for the other cytokines,

suggesting a specific immunomodulatory effect of Probio-M8 on IL-6 production in lactating mothers. In contrast, no significant differences in any of the measured cytokines were detected in the pups ($P > 0.05$, Wilcoxon test; Fig. 1F), indicating that direct systemic immune stimulation via Probio-M8 may be limited in neonates under the current experimental conditions.

These findings suggest that maternal administration of Probio-M8 enhances immune regulation in lactating dams through elevated IL-6 levels. While no direct cytokine-related immune activation was observed in pups, the potential indirect effects of maternal probiotic supplementation on pup immune homeostasis warrant further investigation.

3.3 Maternal Probio-M8 supplementation modulated intestinal permeability in pups

The impact of maternal Probio-M8 supplementation during lactation on pup intestinal health was assessed through histological and functional analyses. Intestinal morphology, particularly villus height and crypt depth, serves as a key indicator of gut development and function. Increased villous height enhances nutrient absorption capacity, while reduced crypt depth reflects greater maturity of intestinal epithelial cells and improved secretory function. The villus-to-crypt ratio is therefore widely used as a marker of intestinal epithelial metabolic activity and overall gut health.

Histological analysis of ileal tissue revealed no significant differences in villus height, crypt depth, or their ratio between pups from probiotic-treated and control dams ($P > 0.05$, Wilcoxon test; Fig. 2A, B). However, functional assessment of intestinal permeability using FD4 demonstrated that pups from Probio-M8-supplemented mothers had significantly higher serum FD4 concentrations compared to controls ($P < 0.05$, Wilcoxon test; Fig. 2C), indicating increased intestinal permeability.

Intestinal permeability reflects the barrier's ability to regulate the passage of substances from the lumen into systemic circulation. While elevated permeability, often referred to as "leaky gut", can allow harmful agents into the bloodstream, potentially triggering inflammation and immune activation, no significant differences were observed in body weight or systemic cytokine levels in the pups at postnatal day 21. This suggests that the observed in permeability may not be pathogenic but could instead reflect a physiological adaptation that facilitates enhanced nutrient uptake. Further studies are needed to clarify the long-term implications of this effect and its potential role in early-life gut development.

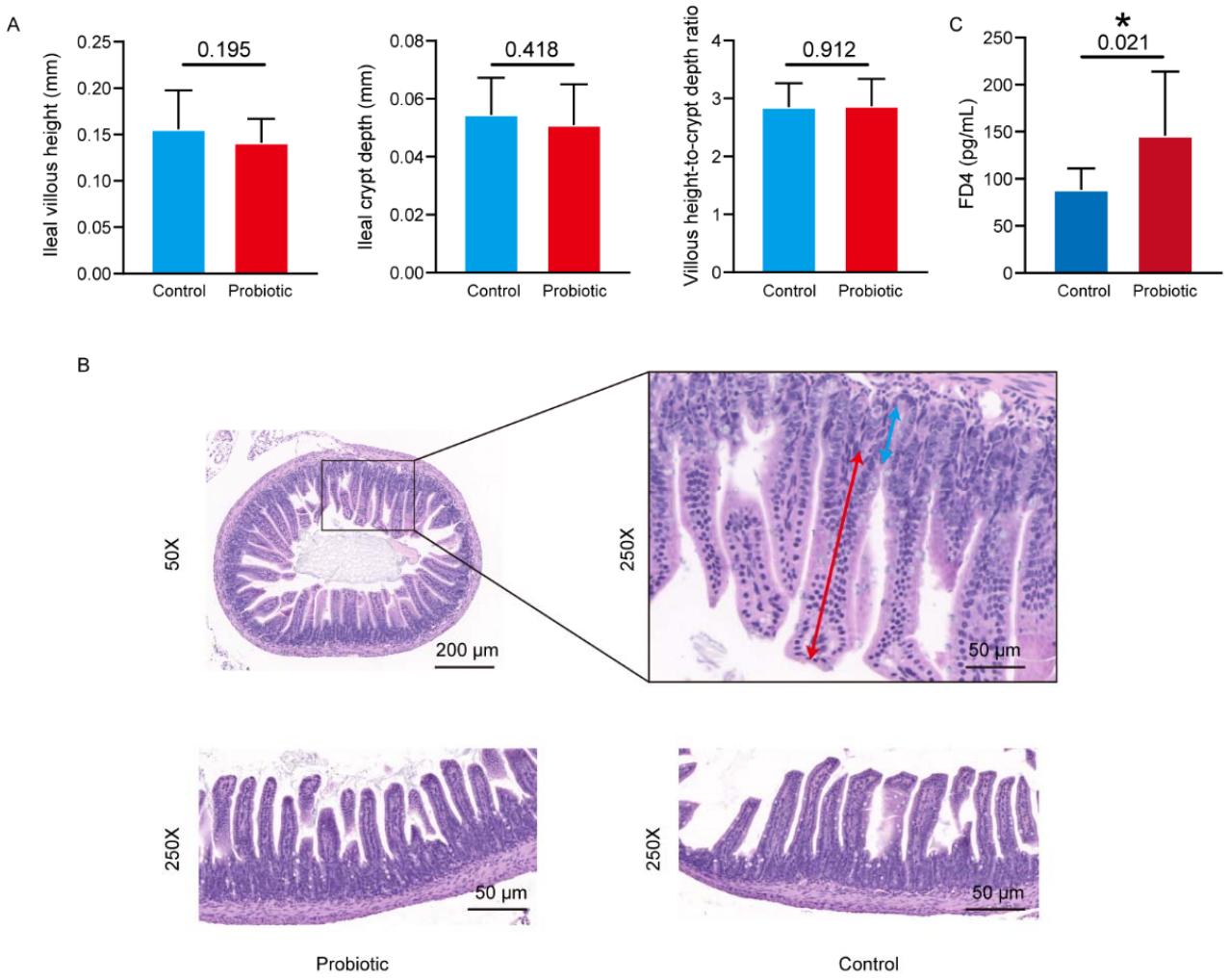

Figure 2. Histological and Functional Assessment of the Ileum in Pups at Day 21. (A) Ileal villous height, crypt depth, and the villous height-to-crypt depth ratio. (B) Representative hematoxylin and eosin-stained ileal tissue sections with the red arrow indicating villus height and the blue arrow denoting crypt depth. (C) Serum concentration of 4 kDa fluorescein isothiocyanate-dextran (FD4) in pups, reflecting intestinal permeability. Error bars indicate the standard error of the mean. Statistical comparisons were performed using the Wilcoxon test, with corresponding *P*-values provided (*$P < 0.05$).

3.4 Effects of maternal Probio-M8 supplementation on the gut microbiota composition of lactating dams and their offspring

The gut microbiome plays a critical role in various physiological processes, including nutrient absorption, immune function modulation, and metabolic regulation. To investigate the impact of maternal Probio-M8 supplementation on the intestinal microbiota of both lactating dams and their pups, shotgun metagenomic sequencing was performed on fecal samples collected at weaning (postnatal day 21; Supplementary Table 2).

At the phylum and genus levels, no major shifts were observed in the overall microbial profiles of dams or pups (Fig. 3A, B). However, significant differences were detected specifically in the relative abundances of two genera, *Evtepia* and *Gemmiger*, in the probiotic group compared to controls among pups ($P < 0.05$, Wilcoxon test; Fig. 3B). These findings suggest that maternal probiotic intervention may selectively influence certain bacterial taxa in offspring.

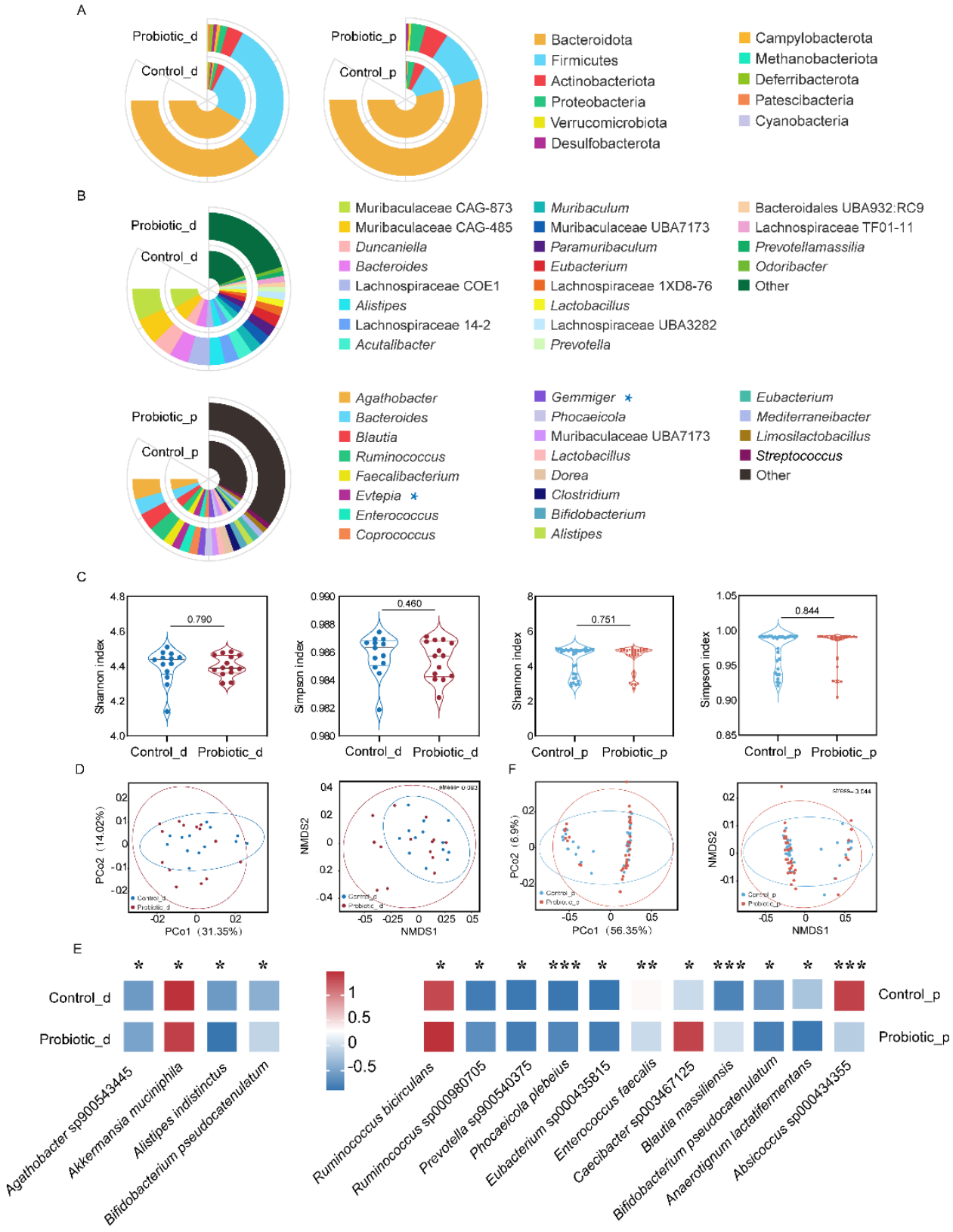

Figure 3. Fecal Microbiomes in Dams and Pups at Day 21. (A, B) Relative abundance of bacterial microbiota composition at the (A) phylum and (B) genus level. Sample codes ending in "d" and "p" denote dams and pups, respectively. Significant differences were observed in the relative abundances of *Evtepia* and *Gemmeriger* in pups from the probiotic group compared to controls, as indicated by a single asterisk (* $P < 0.05$). (C) Alpha diversity (Shannon and Simpson's diversity indices) for both groups. Statistical comparisons were conducted using the Wilcoxon test, with corresponding *P*-values provided. (D) Beta diversity assessed by principal coordinate analysis (PCoA) and non-metric multidimensional scaling (NMDS) for dams and pups. (E) Heatmaps displaying differentially abundant species-level genome bins in dams (left panel) and pups (right panel). The color scale represents relative abundance, ranging from high abundance (red) to low abundance (blue). Significant differences were evaluated using the Wilcoxon test (*$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$).

Alpha diversity indices, including the Shannon and Simpson indices, did not show significant differences between the control and probiotic groups for either dams or pups ($P > 0.05$, Wilcoxon test; Fig. 3C). Similarly, β-diversity analyses using principal coordinates analysis and non-metric multidimensional scaling, based on Bray-Curtis dissimilarities, revealed no distinct clustering between the two groups (Fig. 3D), indicating that Probio-M8 supplementation did not substantially alter the overall diversity or global structure of the gut microbiota in either dams or their offspring. These findings suggest that Probio-M8 supplementation in lactating dams did not significantly affect overall gut microbiota composition.

Despite the absence of broad compositional changes, targeted species-level analysis identified subtle but potentially meaningful shifts in microbial communities. A total of 389 SGBs were identified across all samples, with 123 SGBs detected in dams and 266 in pups. Differential abundance analysis revealed eight and 27 significantly altered SGBs in dams and pups, respectively (Supplementary Tables 3 and 4).

In dams, Probio-M8 supplementation was associated with increased abundance of *Agathobacter sp900543445*, *Alistipes indistinctus*, and *Bifidobacterium pseudocatenulatum*, while *Akkermansia muciniphila* was significantly reduced ($P < 0.05$, Wilcoxon test; Fig. 3E). In pups, maternal probiotic treatment led to elevated levels of *Phocaeicola plebeius*, *Ruminococcus sp000980705*, *Prevotella sp900540375*, *Blautia massiliensis*, and *Blautia stercoris*. Conversely, decreases were observed in *Ruminococcus bicirculans*, *Ruminococcus callidus*, *Enterococcus faecalis*, and *Anaerotignum lactatifermentans* ($P < 0.05$, Wilcoxon test; Fig. 3E).

These results indicate that although maternal Probio-M8 administration does not induce large-scale structural changes in the gut microbiota, it can modulate specific bacterial taxa in both lactating dams and their offspring, potentially contributing to host health through targeted microbial interactions.

3.5 Maternal Probio-M8 supplementation modulated CAZyme profiles

To explore the functional impact of maternal Probio-M8 supplementation on GMMs, the microbial potential for polysaccharide degradation and SCFA biosynthesis in both dams and their offspring was analyzed. A total of 26 GMMs related to these pathways were identified across all samples. Comparative analysis of the cumulative abundance of these GMMs among the 389 SGBs revealed no significant differences between control and probiotic groups in either dams or pups ($P > 0.05$, Wilcoxon test; Supplementary Tables 5 and 6). However, when focusing on differentially abundant SGBs between the two groups, a total of 20 significantly altered GMMs were detected across dams and pups (Tables 1 and 2), indicating that Probio-M8 may influence microbial metabolism through targeted modulation of specific taxa.

Table 1. Differentially Abundant Gut Metabolic Modules (GMMs) in Significant Different SGBs of Dams from Control and Probiotic Groups

GMM ID	Pathway Description	Cumulative Abundance of Genes				*P*-value (Wilcoxon Test)
		Control Group (Mean)	Probiotic Group (Mean)	Control Group (SD)	Probiotic Group (SD)	
GMM005	Acetate degradation	463,925	76,683	879,824	16,610	0.010
GMM008	Propionate synthesis III	3,668,138	2,377,332	1,088,505	1,068,123	0.003

GMM013	Isovaleric acid synthesis II (KADC pathway)	57,776	134,129	43,425	282,653	0.033
GMM022	Tryptophan degradation	4,553,618	3,255,059	299,933	1,238,906	0.004
GMM038	GABA synthesis III	2,611,561	1,899,972	905,615	898,252	0.029
GMM040	Menaquinone synthesis (vitamin K2) I	4,498,698	2,899,650	1,465,410	1,375,285	0.003

Table 2. Differentially Abundant Gut Metabolic Modules (GMMs) in Significant Different SGBs of Pups from Control and Probiotic Groups

GMM ID	Pathway Description	Cumulative Abundance of Genes				P-value (Wilcoxon Test)
		Control Group (Mean)	Probiotic Group (Mean)	Control Group (SD)	Probiotic Group (SD)	
GMM001	Acetate synthesis I	11,840,882	17,564,480	6,953,907	10,327,146	0.022
GMM003	Acetate synthesis III	113,756	259,452	328,995	441,325	0.041
GMM006	Propionate synthesis I	278,031	21,354	861,512	17,842	0.020
GMM007	Propionate synthesis II	496,045	224,135	957,417	631,862	0.014
GMM009	Propionate degradation I	278,031	21,354	861,512	17,842	0.020
GMM010	Butyrate synthesis I	4,142,898	8,702,410	4,631,053	6,071,975	0.002
GMM013	Isovaleric acid synthesis II (KADC pathway)	1,446,990	2,520,129	1,464,727	1,852,496	0.012
GMM021	Tryptophan synthesis	2,168,133	690,074	1,354,216	1,174,146	> 0.001
GMM022	Tryptophan degradation	774,077	245,490	1,415,592	641,519	0.003
GMM030	Quinolinic acid degradation	9,442,362	12,818,367	4,503,135	7,316,653	0.040
GMM033	Melatonin synthesis	278,031	21,354	861,512	17,842	0.020
GMM037	GABA synthesis II	278,031	21,354	861,512	17,842	0.020
GMM038	GABA synthesis III	1,835,686	1,361,191	1,178,366	1,033,593	0.012
GMM039	GABA degradation	278,031	21,354	861,512	17,842	0.020
GMM040	Menaquinone synthesis (vitamin K2) I	3,848,793	2,537,118	1,112,303	1,346,369	> 0.001

To further characterize the enzymatic capacity for complex carbohydrate metabolism, dbCAN2 was used to identify CAZyme-encoding genes within the fecal microbiomes of dams and pups. Across all 389 SGBs, a total of 37,745 CAZyme-encoding genes were detected, 13,913 in dams and 23,832 in pups (Supplementary Tables 7 and 8). The most abundant CAZyme classes were glycoside hydrolases (GHs, 20,262 genes), followed by glycosyltransferases (GTs, 10,850 genes), and carbohydrate esterases (CEs, 3,844 genes; Fig. 4A). Other CAZyme families included carbohydrate-binding modules (CBMs), polysaccharide lyases (PLs), and auxiliary activities (AAs).

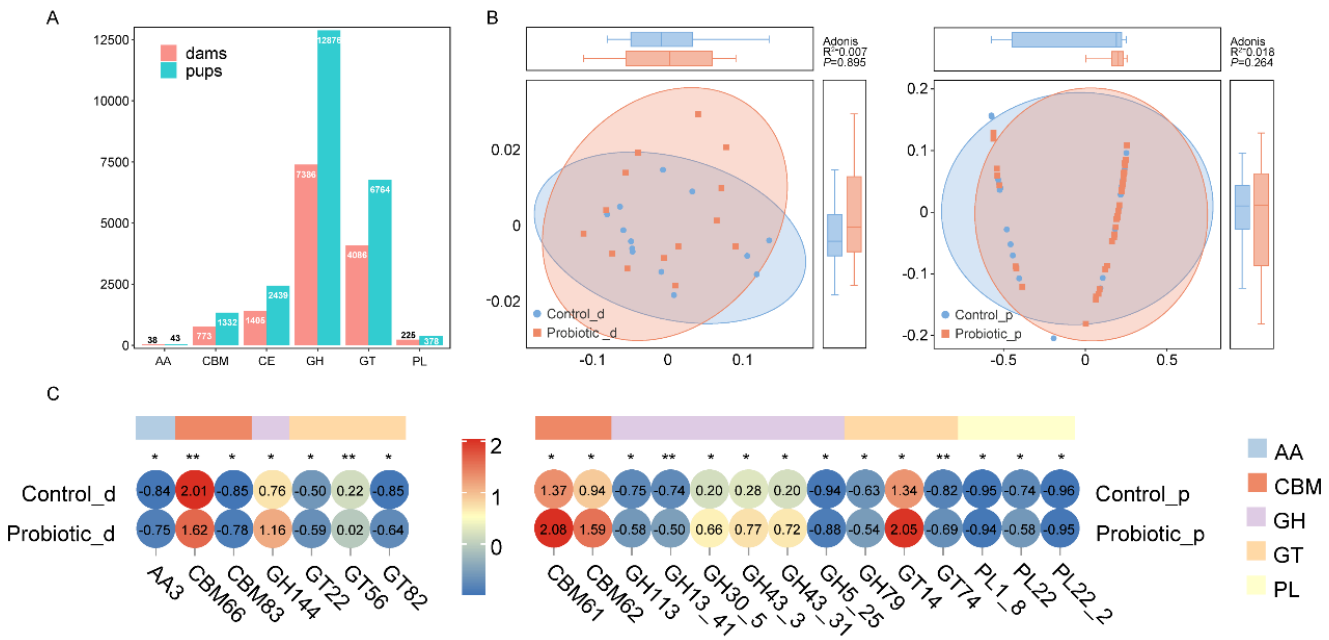

Figure 4. Carbohydrate-Active Enzyme (CAZyme) Profiles in Dams and Pups at Day 21. (A) Distribution of CAZyme families in the fecal metagenomes. (B) Beta diversity based on CAZyme profiles, assessed by principal coordinate analysis and the Adonis test. Sample codes ending in "d" and "p" denote dams and pups, respectively. (C) Heatmaps illustrating significantly different CAZyme subfamilies in dams (left) and pups (right), with normalized abundance values. The color scale represents cumulative abundance, ranging from high abundance (red) to low abundance (blue). AA, auxiliary activities; GH, glycoside hydrolase; GT, glycosyltransferase; CBM, carbohydrates-binding module; PL, polysaccharide lyase. Significant differences were evaluated using the Wilcoxon test (*$P < 0.05$ and **$P < 0.01$).

Although β-diversity analysis of CAZyme profiles using principal coordinates analysis did not reveal significant clustering between groups ($P > 0.05$; Fig. 4B), a higher overall number of CAZyme-encoding genes was observed in pups compared to dams. This increase may reflect the pups' greater reliance oligosaccharides present in breast milk for early-life gut microbiota development .

Further analysis of individual CAZyme subfamilies showed that dams in the probiotic group exhibited enrichment in seven subfamilies: GT22, GT56, GT82, CBM66, CBM83, GH144, and AA3 (Fig. 4C; Supplementary Table 9). In contrast, pups from the probiotic group showed increased abundance in 14 subfamilies, including GH113, GH13_41, GH30_5, GH43_3, GH43_31, GH5_25, GH79, PL1_8, PL22, PL22_2, GT14, GT74, CBM61, and CBM62 (Fig. 4C; Supplementary Table 10).

Substrate prediction for these enriched CAZymes revealed five major substrate categories. Notably, the cumulative gene abundance of enzymes involved in cellulose metabolism was significantly higher in probiotic dams than in controls ($P < 0.05$, Wilcoxon test; Supplementary Table 11), suggesting an enhanced capacity for fiber degradation in lactating mothers. Conversely, pups from the probiotic group showed significantly higher levels of genes associated with pectin and starch metabolism ($P < 0.05$, Wilcoxon test; Supplementary Table 11), potentially reflecting adaptation to dietary carbohydrates following exposure via breast milk.

These findings indicate that maternal administration of Probio-M8 during lactation may promote cellulose degradation in dams and enhance pectin and starch metabolism in offspring, highlighting its role in shaping host-microbe interactions through functional modulation of the gut microbiome.

3.6 Maternal Probio-M8 supplementation enhanced energy metabolism and promoted growth and development in pups

To investigate the metabolic effects of maternal Probio-M8 supplementation, the non-targeted fecal metabolomes of both lactating dams and their offspring were profiled. Principal coordinates analysis of the fecal metabolome revealed tight clustering of quality control samples (Fig. 5A), indicating stable instrument performance and high analytical reproducibility.

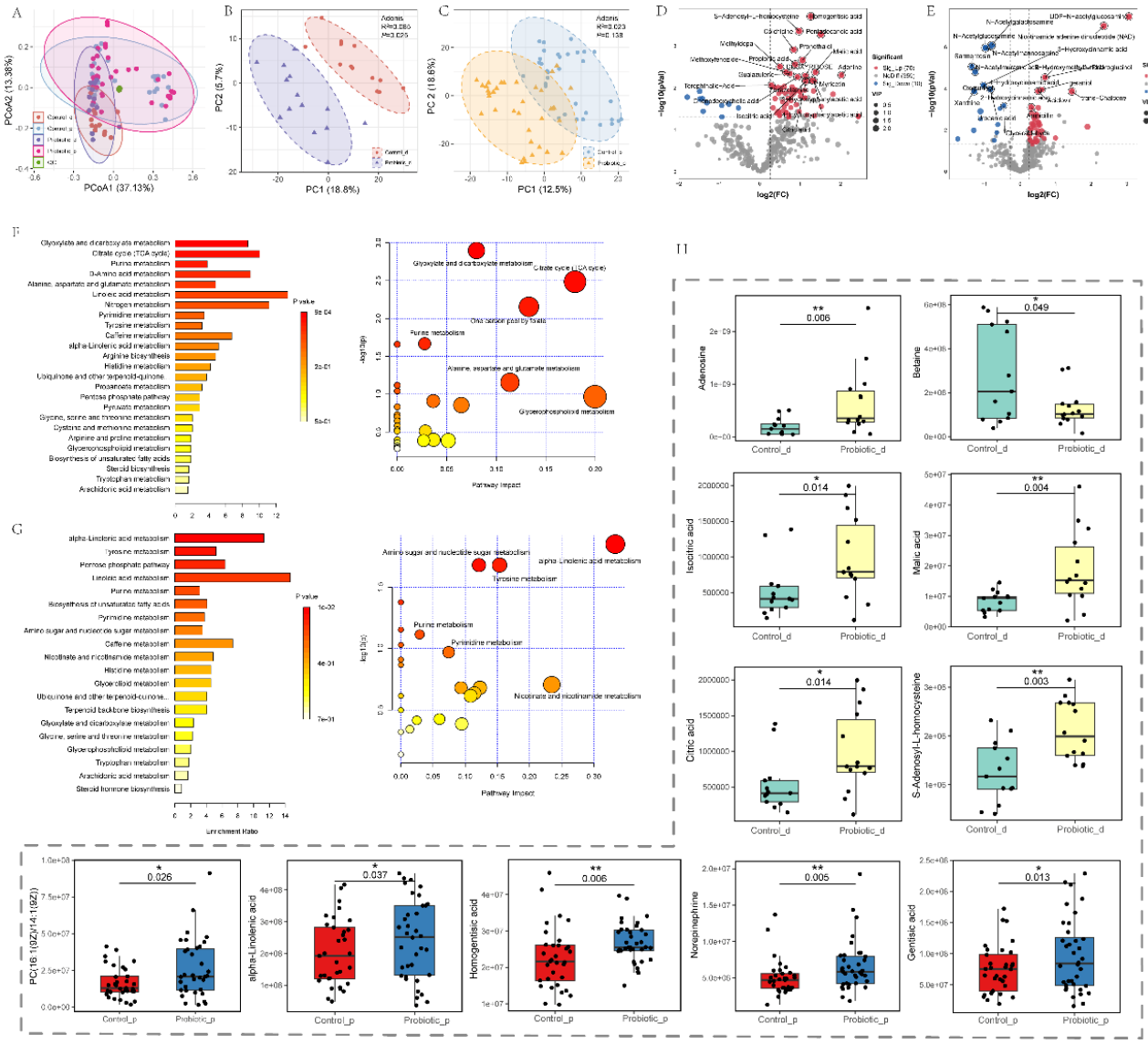

Figure 5. Untargeted Fecal Metabolomic Analysis in Dams and Pups at Day 21. (A) Principal coordinate analysis of all samples, including quality control (QC) samples. Sample codes ending in "d" and "p" denote dams and pups, respectively. (B, C) Partial least squares discriminant analysis for (B) dams and (C) pups, with Adonis test results. (D, E) Volcanic plots depicting differentially abundant metabolites in (D) dams and (E) pups. Significantly increased, decreased, and non-significantly changed metabolites are shown in red, blue, and gray, respectively. "VIP" represents the variable importance in projection score. (F, G) Metabolite enrichment and pathway analysis of differentially abundant metabolites for (F) dams and (G) pups, with color scale representing *P*-values. (H) Significantly different metabolites in dams and pups following intervention, as identified in enrichment analysis. Statistical comparisons were performed using the Wilcoxon test, with corresponding *P*-values provided (*$P < 0.05$ and **$P < 0.01$).

Partial least squares discriminant analysis showed clear separation between control and probiotic groups in dams on weaning day ($R^2 = 0.207$, $P = 0.025$; Fig. 5B). Although a similar trend was observed in pups, it did not reach statistical significance ($R^2 = 0.154$, $P = 0.138$; Fig. 5C).

To identify specific metabolite changes associated with Probio-M8 supplementation, more stringent criteria for differential abundance were applied: $P < 0.05$, VIP score > 1.0, and fold change > 1.2 or < 0.83. A total of 146 differentially abundant metabolites were identified between control and probiotic groups at postnatal day 21, with 80 in dams and 66 in pups (Fig. 5D, E; Supplementary Tables 12 and 13).

Metabolic pathway enrichment analysis was conducted using Metaboanalyst v.6.0 based on Kyoto Encyclopedia of Genes and Genomes annotations. In dams, differentially abundant metabolites were predominantly enriched in pathways related to the tricarboxylic acid (TCA) cycle, glyoxylic and dicarboxylic acid metabolism, and purine metabolism (Fig. 5F). These findings suggest that Probio-M8 may enhance maternal energy metabolism during lactation. In contrast, metabolic alterations in pups from the probiotic group were significantly enriched in α-linolenic acid metabolism, tyrosine metabolism, and the pentose phosphate pathway (Fig. 5G), indicating potential roles in promoting early-life development and redox homeostasis.

Notably, probiotic-supplemented dams exhibited increased levels of key intermediates in energy metabolism, including adenosine, isocitric acid, malic acid, and citric acid, as well as S-adenosyl-L-homocysteine, a central metabolite in methylation reactions. Conversely, betaine, a methyl donor involved in osmoregulation and lipid metabolism, was reduced in the probiotic group (Fig. 5H). In pups, Probio-M8 exposure was associated with elevated levels of phosphatidylcholine, α-linolenic acid, homogentisic acid, norepinephrine, and gentisic acid, metabolites implicated in membrane synthesis, neurodevelopment, and antioxidant defense (Fig. 5H).

Collectively, these results suggest that maternal Probio-M8 supplementation enhances energy metabolism in dams through modulation of gut microbial functions, particularly in purine and TCA cycle-related pathways. In pups, the intervention appears to support developmental processes via increased availability of polyunsaturated fatty acids, amino acid derivatives, and neuromodulatory compounds, potentially facilitating neurodevelopment and cellular growth.

3.7 Probio-M8 modulated gut microbial metabolism

To explore potential functional interactions between gut microbiota and host metabolism, Spearman's correlation analyses were conducted to link differentially abundant microbial species with altered fecal metabolites identified on weaning day (postnatal day 21; Fig. 6).

In dams, several metabolites showed strong positive correlations with *Bifidobacterium pseudocatenulatum*, a species enriched in the probiotic group. These included isocitric acid, citric acid, oleic acid, and trans-octadecadienoic acid (Spearman's Rho > 0.4). In contrast, malic acid, propranolol, and 5-methyluracil exhibited negative correlations with both *Akkermansia muciniphila* and *Alistipes indistinctus* (Spearman's Rho < -0.4), suggesting that these microbial taxa may have distinct metabolic roles in the maternal gut.

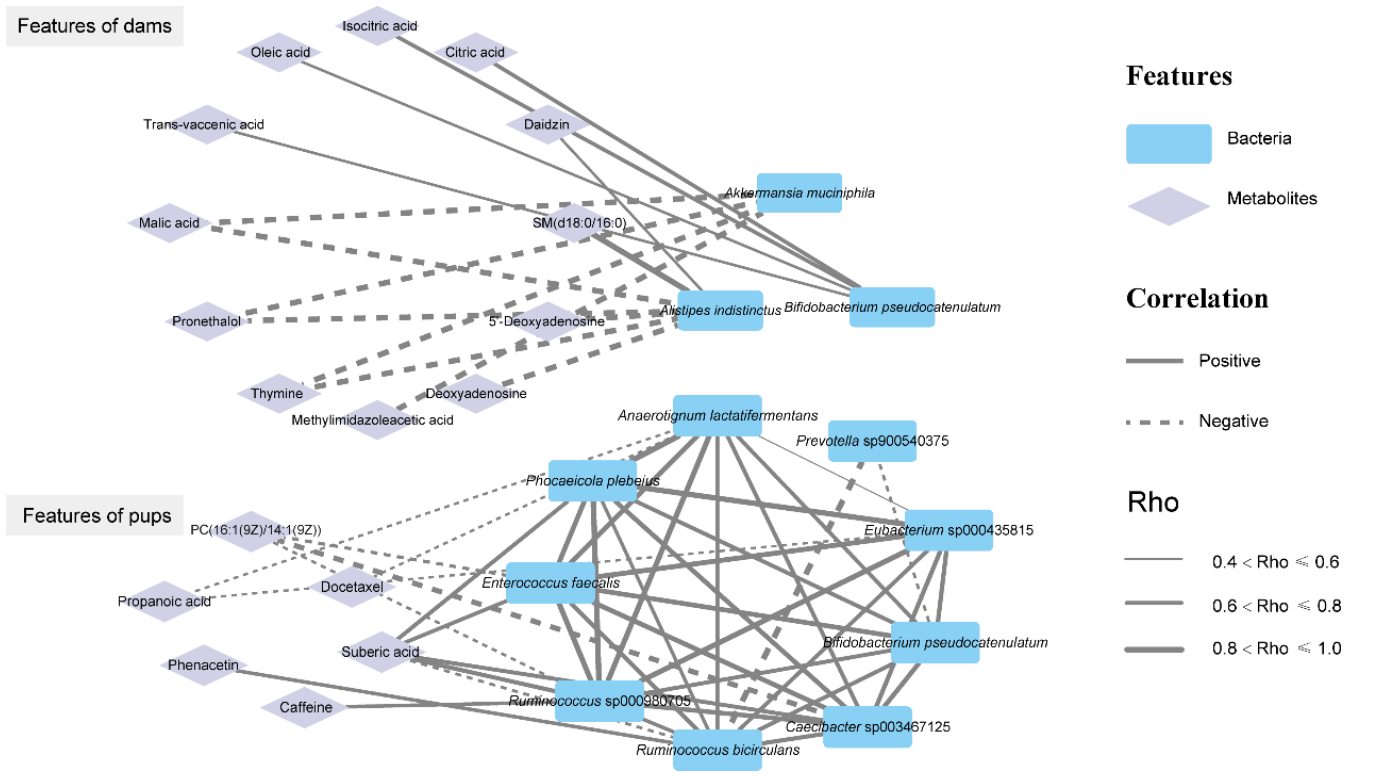

Figure 6. Correlation Networks Between Gut Microbiota and Fecal Metabolites in Dams and Pups. Blue nodes represent differentially abundant bacterial species, while purple nodes represent metabolites. Solid lines indicate positive correlations, dashed lines indicate negative correlations, with line thickness proportional to correlation strength. Spearman's rho ranges: $0.4 < \text{Rho} \leq 0.6$, $0.6 < \text{Rho} \leq 0.8$, and $0.8 < \text{Rho} \leq 1.0$.

In pups, significant correlations were also observed between differentially abundant species and specific metabolites. Notably, caffeine, octanedioic acid, and finasteride displayed positive associations with most probiotic-enriched taxa (Spearman's Rho > 0.4). Conversely, phosphatidylcholine, beta-tyrosine, and docetaxel showed negative correlations with the majority of these species (Spearman's Rho < -0.4), indicating complex microbe-metabolite interactions in the pup gut ecosystem.

These results underscore the complex interplay between gut microbiota and composition metabolism, demonstrating how microbial metabolism actively shapes the intestinal environment. The observed correlations suggest that maternal supplementation with Probio-M8 during lactation influences both the composition of the offspring gut microbiota and associated metabolic pathways. This microbial modulation may underpin some of the health-promoting effects seen in both mothers and offspring, reinforcing the importance of probiotic interventions during critical developmental windows such as lactation.

4. Discussion

The early-life gut microbiota plays a pivotal role in shaping immune, metabolic, and neurological development, making it a key target for maternal interventions during critical developmental windows. This study investigated the impact of maternal supplementation with the breast milk-derived probiotic strain Probio-M8 during lactation in mice. We found that Probio-M8 administration significantly modulated the gut microbiome and metabolome of both dams and offspring, leading to enhanced maternal immune regulation, improved metabolic function in pups, and potential support for neurodevelopmental pathways.

These findings highlight the potential of probiotic supplementation during lactation to influence mother-infant health outcomes through microbial and metabolic mechanisms.

Our findings indicate that maternal probiotic supplementation during lactation shapes the gut microbiota of offspring through entero-mammary pathways, thereby influencing both immune development and metabolic programming. This aligns with previous research demonstrating that maternal probiotics can be transferred to infants through breastfeeding and influence early-life colonization patterns(Azagra-Boronat et al., 2020). Interleukin-6, a key inflammatory cytokine involved in both innate and adaptive immunity, was significantly elevated in dams supplemented with Probio-M8, suggesting an immunomodulatory effect of the intervention. This cytokine promotes acute phase responses and antibody production, enhancing overall immune responsiveness(Tanaka, Narazaki, & Kishimoto, 2014). These findings align with previous studies reporting elevated intestinal IL-6 levels following the administration of other probiotic strains. For instance, supplementation with *Limosilactobacillus fermentum* CECT5716 during pregnancy and lactation was shown to increase IL-6 levels(Azagra-Boronat et al., 2020). Similarly, probiotic use in pregnant and lactating sows has been associated with elevated TNF-α and IL-6 levels in both serum and colostrum, potentially enhancing immune protection for both mothers and offspring(Damaceno et al., 2017). Moreover, *Bifidobacterium animalis* supplementation in lactating mice has been found to stimulate the production of IL-6 and TNF-α, thereby boosting systemic immune responses(Ezendam, de Klerk, Gremmer, & van Loveren, 2008). In addition, our data indicate that on the day of weaning, the probiotic group of dams showed a significant decrease in body weight. Another study showed that mice significantly lost weight after supplementing probiotics, which was achieved by regulating metabolism and immunity rather than reducing food intake(Gao, Zhu, Li, Liu, Li, & Zhang, 2025). This indicates that there were no significant changes in the behavior and food intake of dams after supplementation of Probio-M8. Our data support the hypothesis that maternal Probio-M8 supplementation may confer protection against exogenous infections in both generations by modulating systemic immunity. However, no significant differences were observed in pup cytokine levels, which may reflect the immaturity of the neonatal(Levy, 2007; Siegrist, 2007).

This study also observed increased intestinal permeability in pups from probiotic-supplemented dams, as indicated by higher serum FD4 concentrations. While increased intestinal permeability is often associated with inflammation or injury(Michielan & D'Incà, 2015), our results showed no concomitant elevation in pro-inflammatory cytokine levels or alterations in body weight, suggesting that the observed permeability change may not be pathogenic. Instead, it could reflect physiological adaptations that enhance nutrient absorption. For instance, activation of myosin light chain kinase in intestinal epithelial cells can transiently increase increase epithelial permeability, facilitating water and nutrient uptake without inducing damage(Atisook, Carlson, & Madara, 1990; Meddings & Westergaard, 1989). Moreover, the observed increase in intestinal permeability during neonatal development may represent a physiological adaptation, serving as a facilitator of the essential antigen exposure required for proper immune maturation and the induction of immunologic tolerance(Sanidad & Zeng, 2020). A separate investigation demonstrated that

Zonula Occludens Toxin (ZOT), an endogenous protein produced by Vibrio cholerae, enhances the intestinal absorption of macromolecules, including insulin and immunoglobulin G, through the reversible modulation of epithelial tight junctions(Fasano & Uzzau, 1997). Thus, Probio-M8 may promote beneficial gut adaptations in offspring without triggering harmful immune responses.

A more diverse gut microbiota is widely recognized as essential for maintaining host health and resilience(Lozupone, Stombaugh, Gordon, Jansson, & Knight, 2012; Yatsunenko et al., 2012). Our data show that although overall α- and beta diversity metrics did not differ significantly between groups, several beneficial taxa were enriched in response to maternal probiotic supplementation. In dams, *Agathobacter sp*., *Alistipes indistinctus*, and *Bifidobacterium pseudocatenulatum* were more abundant, while *Akkermansia muciniphila* was reduced. In pups, notable increases were observed in *Anaerobutyricum hallii*, *Alterileibacterium massiliense*, *Bilophila wadsworthia*, and *Alistipes finegoldii*, while *Acetatifactor* sp. and *Blautia* sp. decreases. Several of these taxa have established roles in host health: *Bifidobacterium pseudocatenulatum* has anti-inflammatory properties and enhances bile acid and SCFA production(Chen et al., 2021); *Agathobacter* species are known SCFA producers that improve gut barrier integrity(Jing et al., 2023); and *Alistipes indistinctus* has been linked to improved glucose tolerance in high-fat diet models(Takeuchi et al., 2023). Although we did not detect Probio-M8 directly in metagenomic samples, likely due to its low abundance or insufficient sequencing depth, prior studies indicate that strain-level detection typically requires at least five-fold coverage(Ma et al., 2023; Schloissnig et al., 2013). Another reason may be due to differences in hosts, physiological structures, immune systems, and gut microbiota backgrounds between humans and rodents, which significantly affect the colonization and transmission effects of probiotics(Chung et al., 2012; W. Wang et al., 2025)**.** Thus, Probio-M8 may still be present and exert indirect effects through microbial cross-feeding or host signaling mechanisms.

Using shotgun metagenomics, we annotated SGBs using the Genome Taxonomy Database, enabling high-resolution taxonomic classification. Microbial genome quality was assessed using CheckM, ensuring accurate reconstruction of SGBs for downstream analysis. Our CAZyme profiling revealed that the probiotic group exhibited significant enrichment in 21 distinct GH, GT, and CBM families. These enzyme classes are critical for breaking down complex carbohydrates into SCFAs and other metabolites that support energy acquisition(Zhou et al., 2020). Integrated CAZyme-substrate analyses showed that genes involved in pectin and starch degradation were significantly enriched in the pups from probiotic-fed dams, potentially enhancing their ability to utilize complex carbohydrates during the weaning transition. Although direct measurements of SCFA production were not included, the presence of these enzymatic pathways suggests that microbial fermentation was enhanced.

Metabolomic profiling further supported these functional insights. In dams, probiotic supplementation led to enrichment of pathways including the TCA cycle, glyoxylate and dicarboxylate metabolism, and purine metabolism. These pathways are central to energy homeostasis, nucleotide synthesis, and cellular signaling(Martínez-Reyes & Chandel, 2020). Notably, elevated levels of malic acid, citric acid, and

isocitrate, intermediates that may enhance microbial fermentation and host energy metabolism, were observed. These organic acids also lower gut pH, creating an environment less favorable for pathogens and promoting the proliferation of beneficial commensals(Quinn et al., 2024). Additionally, adenosine, a neuromodulator involved in the cAMP-PKA pathway, was elevated in probiotic dams, potentially contributing to improved intestinal barrier function and neuroprotection(Deng et al., 2025). The enrichment of purine metabolism may also benefit breast milk composition by supplying nucleotides that support infant immune and gastrointestinal development(Jackson, Shaw, Barber, & Golden, 1981; Yu, 1998). Probio-M8 supplementation in dams triggered an IL-6-mediated immune activation, enhancing both the proliferation and apoptosis of immune cells(He et al., 2025). The associated degradation of intracellular nucleotides resulted in elevated fecal purine metabolites. We propose that this energy-intensive immunologic remodeling is the one of key factor underlying the maternal weight loss.

In contrast, pups from probiotic-fed dams showed enrichment in pathways related to α-linolenic acid metabolism, the pentose phosphate pathway, and tyrosine metabolism. Alpha-linolenic acid and its derivatives, such as docosahexaenoic acid, are essential for brain development and visual function(Barringer, 2012; Lauritzen, Brambilla, Mazzocchi, Harsløf, Ciappolino, & Agostoni, 2016). The enrichment of these pathways may reflect enhanced lipid metabolism driven by microbial activity, supporting early neurodevelopment. The pentose phosphate pathway, which generates ribose-5-phosphate for DNA synthesis and NADPH for redox regulation, was also upregulated, suggesting improved cellular proliferation and antioxidant defense(TeSlaa, Ralser, Fan, & Rabinowitz, 2023). Elevated norepinephrine levels in probiotic pups further suggest potential benefits in neural development, as this neurotransmitter plays a key role in neuronal plasticity and cognitive function(Hong, Davies, & Whitfield, 2022).

Despite the comprehensive multi-omics approach used, including metagenomics, CAZyme annotation, and metabolomic pathway analysis, this study has several limitations. First, this study did not measure or control dams' diet and daily activity, which are potential confounders to their weight loss on weaning day. Second, the absence of Probio-M8 in metagenomic datasets may reflect either low abundance or technical limitations in strain-level resolution. Third, our sampling was limited to a single time point (weaning), restricting our ability to capture longitudinal dynamics. Fourth, behavioral or neurological assessments in pups were not conducted, limiting conclusions about systemic effects of altered metabolite profiles. Finally, while correlations between microbial taxa and metabolites were observed, causality cannot be inferred without gnotobiotic validation. Nonetheless, our findings highlight the potential of maternal probiotic supplementation to shape both maternal and infant gut ecosystems, offering novel strategies for early-life nutrition and health optimization. If translatable to humans, such interventions could help mitigate risks associated with dysbiosis, such as allergies, metabolic syndrome, and neurodevelopmental disorders.

Although previous literature has shown that consuming probiotics reduces weight by regulating metabolism rather than decreasing food intake, detailed data is still worth recording to rule out confounders interfering. Future research must build upon these findings by utilizing disease-specific models to rigorously

evaluate the intergenerational protective effects of maternal probiotic supplementation on offspring physiology. It is imperative that longitudinal studies track offspring into adulthood to ascertain the long-term programming of metabolic, immune, and behavioral phenotypes. Additionally, integrating deep metagenomic sequencing with culturomic techniques will be crucial to delineate the precise mechanisms—whether direct or mediated through ecological interactions—by which Probio-M8 exerts its influence.

To our knowledge, this is the first study to comprehensively characterize the multi-omics effects of maternal *Bifidobacterium animalis* subsp. *lactis* Probio-M8 supplementation during lactation, revealing novel insights into its role in shaping both maternal and offspring gut ecosystems. These findings provide a foundation for future research into probiotic-based nutritional strategies aimed at optimizing early-life development and long-term health outcomes.

5. Conclusion

In conclusion, maternal supplementation with *Bifidobacterium animalis* subsp. *lactis* Probio-M8 during lactation exerts multifaceted effects on both dams and offspring. The intervention led to reduced maternal body weight and enhanced immune regulation through elevated IL-6 levels. While global gut microbiota diversity remained stable, distinct shifts in microbial composition were observed, with notable increases in potentially beneficial taxa in pups. Functional analysis revealed an augmented capacity for polysaccharide degradation in offspring, likely supporting dietary adaptation post-weaning.

Metabolomic profiling further demonstrated that probiotic exposure via maternal lactation was associated with enhanced tyrosine and polyunsaturated fatty acid metabolism in pups, pathways closely tied to neurodevelopment and immune homeostasis. These findings suggest that maternal probiotic supplementation influences offspring development not only through direct microbial transfer but also by shaping the functional potential of the gut ecosystem.

Although strain-level detection of Probio-M8 was not achieved in this study, likely due to low abundance or sequencing depth limitations, the observed microbial and metabolic shifts point to indirect yet meaningful effects of maternal probiotic use. These results highlight the potential of targeted probiotic interventions during lactation to support early-life gut development and long-term health outcomes, warranting further investigation in human clinical studies.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The metagenomic data generated in this study have been deposited in the CNCB database (https://ngdc.cncb.ac.cn/gsub/submit/bioproject/PRJCA031676).

References

Atisook, K., Carlson, S., & Madara, J. L. (1990). Effects of phlorizin and sodium on glucose-elicited alterations of cell junctions in intestinal epithelia. *Am J Physiol, 258*(1 Pt 1), C77-85. https://doi.org/10.1152/ajpcell.1990.258.1.C77.

Azad, M. A. K., Sarker, M., Li, T., & Yin, J. (2018). Probiotic Species in the Modulation of Gut Microbiota: An Overview. *Biomed Res Int, 2018*, 9478630. https://doi.org/10.1155/2018/9478630.

Azagra-Boronat, I., Tres, A., Massot-Cladera, M., Franch, À., Castell, M., Guardiola, F., . . . Rodríguez-Lagunas, M. J. (2020). Lactobacillus fermentum CECT5716 Supplementation in Rats during Pregnancy and Lactation Impacts Maternal and Offspring Lipid Profile, Immune System and Microbiota. *Cells, 9*(3). https://doi.org/10.3390/cells9030575.

Bäckhed, F., Roswall, J., Peng, Y., Feng, Q., Jia, H., Kovatcheva-Datchary, P., . . . Wang, J. (2015). Dynamics and Stabilization of the Human Gut Microbiome during the First Year of Life. *Cell Host Microbe, 17*(5), 690-703. https://doi.org/10.1016/j.chom.2015.04.004.

Barringer, T. A. (2012). Do omega-3 Fatty acids have a role in prevention of cardiovascular disease? *Front Physiol, 3*, 395. https://doi.org/10.3389/fphys.2012.00395.

Calkins, K., & Devaskar, S. U. (2011). Fetal origins of adult disease. *Curr Probl Pediatr Adolesc Health Care, 41*(6), 158-176. https://doi.org/10.1016/j.cppeds.2011.01.001.

Caspi, R., Billington, R., Keseler, I. M., Kothari, A., Krummenacker, M., Midford, P. E., . . . Karp, P. D. (2020). The MetaCyc database of metabolic pathways and enzymes - a 2019 update. *Nucleic Acids Res, 48*(D1), D445-d453. https://doi.org/10.1093/nar/gkz862.

Chen, Y., Yang, B., Stanton, C., Ross, R. P., Zhao, J., Zhang, H., & Chen, W. (2021). Bifidobacterium pseudocatenulatum Ameliorates DSS-Induced Colitis by Maintaining Intestinal Mechanical Barrier, Blocking Proinflammatory Cytokines, Inhibiting TLR4/NF-κB Signaling, and Altering Gut Microbiota. *J Agric Food Chem, 69*(5), 1496-1512. https://doi.org/10.1021/acs.jafc.0c06329.

Chung, H., Pamp, S. J., Hill, J. A., Surana, N. K., Edelman, S. M., Troy, E. B., . . . Kasper, D. L. (2012). Gut immune maturation depends on colonization with a host-specific microbiota. *Cell, 149*(7), 1578-1593. https://doi.org/10.1016/j.cell.2012.04.037.

Damaceno, Q. S., Souza, J. P., Nicoli, J. R., Paula, R. L., Assis, G. B., Figueiredo, H. C., . . . Martins, F. S. (2017). Evaluation of Potential Probiotics Isolated from Human Milk and Colostrum. *Probiotics Antimicrob Proteins, 9*(4), 371-379. https://doi.org/10.1007/s12602-017-9270-1.

Darzi, Y., Falony, G., Vieira-Silva, S., & Raes, J. (2016). Towards biome-specific analysis of meta-omics data. *Isme j, 10*(5), 1025-1028. https://doi.org/10.1038/ismej.2015.188.

Deng, F., Yang, D., Qing, L., Chen, Y., Zou, J., Jia, M., . . . Huang, L. (2025). Exploring the interaction between the gut microbiota and cyclic adenosine monophosphate-protein kinase A signaling pathway: a potential therapeutic approach for neurodegenerative diseases. *Neural Regen Res, 20*(11), 3095-3112. https://doi.org/10.4103/nrr.Nrr-d-24-00607.

Dominguez-Bello, M. G., Godoy-Vitorino, F., Knight, R., & Blaser, M. J. (2019). Role of the microbiome in human development. *Gut, 68*(6), 1108-1114. https://doi.org/10.1136/gutjnl-2018-317503.

Ezendam, J., de Klerk, A., Gremmer, E. R., & van Loveren, H. (2008). Effects of Bifidobacterium animalis administered during lactation on allergic and autoimmune responses in rodents. *Clin Exp Immunol, 154*(3), 424-431. https://doi.org/10.1111/j.1365-2249.2008.03788.x.

Fasano, A., & Uzzau, S. (1997). Modulation of intestinal tight junctions by Zonula occludens toxin permits enteral administration of insulin and other macromolecules in an animal model. *J Clin Invest, 99*(6), 1158-1164. https://doi.org/10.1172/jci119271.

Gao, Y., Zhu, A., Li, J., Liu, H., Li, X., & Zhang, H. (2025). Lactiplantibacillus plantarum Attenuates Diet-Induced Obesity and Insulin Resistance Through Gut Microbiota-Driven PPAR/PI3K-Axis Modulation. *Microb Biotechnol, 18*(11), e70227. https://doi.org/10.1111/1751-7915.70227.

He, Q., Sun, Y., Jin, H., Kwok, L. Y., Ma, T., Quan, K., . . . Zhang, H. (2025). Deciphering gut microbial dynamics and multi-omics changes in yogurt-based dietary interventions. *Sci Bull (Beijing)*. https://doi.org/10.1016/j.scib.2025.05.021.

Hong, L., Davies, M., & Whitfield, K. (2022). Noradrenaline use for neonatal circulatory support. *J Paediatr Child Health, 58*(11), 2084-2090. https://doi.org/10.1111/jpc.16226.

Inoue, R., & Ushida, K. (2003). Vertical and horizontal transmission of intestinal commensal bacteria in the rat model. *FEMS Microbiol Ecol, 46*(2), 213-219. https://doi.org/10.1016/s0168-6496(03)00215-0.

Jackson, A. A., Shaw, J. C. L., Barber, A., & Golden, M. H. N. (1981). Nitrogen metabolism in preterm infants fed human donor breast milk: the possible essentiality of glycine. *Pediatric Research, 15*(11), 1454-1461.

Jeurink, P. V., van Bergenhenegouwen, J., Jiménez, E., Knippels, L. M., Fernández, L., Garssen, J., . . . Martín, R. (2013). Human milk: a source of more life than we imagine. *Benef Microbes, 4*(1), 17-30. https://doi.org/10.3920/bm2012.0040.

Jing, Y., Yang, D., Bai, F., Wang, Q., Zhang, C., Yan, Y., . . . Yu, Y. (2023). Spinal cord injury-induced gut dysbiosis influences neurological recovery partly through short-chain fatty acids. *NPJ Biofilms Microbiomes, 9*(1), 99. https://doi.org/10.1038/s41522-023-00466-5.

Kang, D. D., Li, F., Kirton, E., Thomas, A., Egan, R., An, H., & Wang, Z. (2019). MetaBAT 2: an adaptive binning algorithm for robust and efficient genome reconstruction from metagenome assemblies. *PeerJ, 7*, e7359. https://doi.org/10.7717/peerj.7359.

Kohlhepp, L. M., Hollerich, G., Vo, L., Hofmann-Kiefer, K., Rehm, M., Louwen, F., . . . Weber, C. F. (2018). [Physiological changes during pregnancy]. *Anaesthesist, 67*(5), 383-396. https://doi.org/10.1007/s00101-018-0437-2.

Lauritzen, L., Brambilla, P., Mazzocchi, A., Harsløf, L. B., Ciappolino, V., & Agostoni, C. (2016). DHA Effects in Brain Development and Function. *Nutrients, 8*(1). https://doi.org/10.3390/nu8010006.

Levy, O. (2007). Innate immunity of the newborn: basic mechanisms and clinical correlates. *Nat Rev Immunol, 7*(5), 379-390. https://doi.org/10.1038/nri2075.

Li, D., Liu, C. M., Luo, R., Sadakane, K., & Lam, T. W. (2015). MEGAHIT: an ultra-fast single-node solution for large and complex metagenomics assembly via succinct de Bruijn graph. *Bioinformatics, 31*(10), 1674-1676. https://doi.org/10.1093/bioinformatics/btv033.

Lozupone, C. A., Stombaugh, J. I., Gordon, J. I., Jansson, J. K., & Knight, R. (2012). Diversity, stability and resilience of the human gut microbiota. *Nature, 489*(7415), 220-230. https://doi.org/10.1038/nature11550.

Ma, T., Huang, W., Li, Y., Jin, H., Kwok, L. Y., Sun, Z., & Zhang, H. (2023). Probiotics alleviate constipation and inflammation in late gestating and lactating sows. *NPJ Biofilms Microbiomes, 9*(1), 70. https://doi.org/10.1038/s41522-023-00434-z.

Magnúsdóttir, S., Heinken, A., Kutt, L., Ravcheev, D. A., Bauer, E., Noronha, A., . . . Thiele, I. (2017). Generation of genome-scale metabolic reconstructions for 773 members of the human gut microbiota. *Nat Biotechnol, 35*(1), 81-89. https://doi.org/10.1038/nbt.3703.

Martínez-Reyes, I., & Chandel, N. S. (2020). Mitochondrial TCA cycle metabolites control physiology and disease. *Nat Commun, 11*(1), 102. https://doi.org/10.1038/s41467-019-13668-3.

Meddings, J. B., & Westergaard, H. (1989). Intestinal glucose transport using perfused rat jejunum in vivo: model analysis and derivation of corrected kinetic constants. *Clin Sci (Lond), 76*(4), 403-413. https://doi.org/10.1042/cs0760403.

Michielan, A., & D'Incà, R. (2015). Intestinal Permeability in Inflammatory Bowel Disease: Pathogenesis, Clinical Evaluation, and Therapy of Leaky Gut. *Mediators Inflamm, 2015*, 628157. https://doi.org/10.1155/2015/628157.

Nissen, J. N., Johansen, J., Allesøe, R. L., Sønderby, C. K., Armenteros, J. J. A., Grønbech, C. H., . . . Rasmussen, S. (2021). Improved metagenome binning and assembly using deep variational autoencoders. *Nat Biotechnol, 39*(5), 555-560. https://doi.org/10.1038/s41587-020-00777-4.

Pannaraj, P. S., Li, F., Cerini, C., Bender, J. M., Yang, S., Rollie, A., . . . Aldrovandi, G. M. (2017). Association Between Breast Milk Bacterial Communities and Establishment and Development of the Infant Gut Microbiome. *JAMA Pediatr, 171*(7), 647-654. https://doi.org/10.1001/jamapediatrics.2017.0378.

Quinn, A., El Chazli, Y., Escrig, S., Daraspe, J., Neuschwander, N., McNally, A., . . . Engel, P. (2024). Host-derived organic acids enable gut colonization of the honey bee symbiont Snodgrassella alvi. *Nat Microbiol, 9*(2), 477-489. https://doi.org/10.1038/s41564-023-01572-y.

Sanidad, K. Z., & Zeng, M. Y. (2020). Neonatal gut microbiome and immunity. *Curr Opin Microbiol, 56*, 30-37. https://doi.org/10.1016/j.mib.2020.05.011.

Schloissnig, S., Arumugam, M., Sunagawa, S., Mitreva, M., Tap, J., Zhu, A., . . . Bork, P. (2013). Genomic variation landscape of the human gut microbiome. *Nature, 493*(7430), 45-50. https://doi.org/10.1038/nature11711.

Sieber, C. M. K., Probst, A. J., Sharrar, A., Thomas, B. C., Hess, M., Tringe, S. G., & Banfield, J. F. (2018). Recovery of genomes from metagenomes via a dereplication, aggregation and scoring strategy. *Nat Microbiol, 3*(7), 836-843. https://doi.org/10.1038/s41564-018-0171-1.

Siegrist, C. A. (2007). The challenges of vaccine responses in early life: selected examples. *J Comp Pathol, 137 Suppl 1*, S4-9. https://doi.org/10.1016/j.jcpa.2007.04.004.

Takeuchi, T., Kubota, T., Nakanishi, Y., Tsugawa, H., Suda, W., Kwon, A. T., . . . Ohno, H. (2023). Gut microbial carbohydrate metabolism contributes to insulin resistance. *Nature, 621*(7978), 389-395. https://doi.org/10.1038/s41586-023-06466-x.

Tanaka, T., Narazaki, M., & Kishimoto, T. (2014). IL-6 in inflammation, immunity, and disease. *Cold Spring Harb Perspect Biol, 6*(10), a016295. https://doi.org/10.1101/cshperspect.a016295.

TeSlaa, T., Ralser, M., Fan, J., & Rabinowitz, J. D. (2023). The pentose phosphate pathway in health and disease. *Nat Metab, 5*(8), 1275-1289. https://doi.org/10.1038/s42255-023-00863-2.

Ursell, L. K., Metcalf, J. L., Wegener, P. L., & Rob, K. (2012). Defining the human microbiome. *Nutrition Reviews*(suppl_1), S38-S44.

Wang, W., Gu, W., Schweitzer, R., Koren, O., Khatib, S., Tseng, G., & Konnikova, L. (2025). In utero human intestine contains maternally derived bacterial metabolites. *Microbiome, 13*(1), 116. https://doi.org/10.1186/s40168-025-02110-0.

Wang, Z., & Zhao, Y. (2018). Gut microbiota derived metabolites in cardiovascular health and disease. *Protein Cell, 9*(5), 416-431. https://doi.org/10.1007/s13238-018-0549-0.

Yatsunenko, T., Rey, F. E., Manary, M. J., Trehan, I., Dominguez-Bello, M. G., Contreras, M., . . . Gordon, J. I. (2012). Human gut microbiome viewed across age and geography. *Nature, 486*(7402), 222-227. https://doi.org/10.1038/nature11053.

Yu, V. Y. H. (1998). The Role of Dietary Nucleotides in Neonatal and Infant Nutrition. *Singapore Medical Journal, 39*(4), 145-150.

Zhong, Z., Tang, H., Shen, T., Ma, X., Zhao, F., Kwok, L. Y., . . . Zhang, H. (2022). Bifidobacterium animalis subsp. lactis Probio-M8 undergoes host adaptive evolution by glcU mutation and translocates to the infant's gut via oral-/entero-mammary routes through lactation. *Microbiome, 10*(1), 197. https://doi.org/10.1186/s40168-022-01398-6.

Zhou, S., Luo, R., Gong, G., Wang, Y., Gesang, Z., Wang, K., . . . Suolang, S. (2020). Characterization of Metagenome-Assembled Genomes and Carbohydrate-Degrading Genes in the Gut Microbiota of Tibetan Pig. *Front Microbiol, 11*, 595066. https://doi.org/10.3389/fmicb.2020.595066.