

MAMMALIAN MILK MICROBIOMES: ASSOCIATIONS WITH PHYLOGENY AND ADULT DIET

Michael L. Power, PhD¹, Morgan Bragg, BS², Jenna C. Pastel, BA^{1}, and Carly R. Muletz-Wolz, PhD²*

¹*Nutrition Laboratory, Smithsonian National Zoological Park and Conservation Biology Institute, 3001 Connecticut Ave NW, Washington DC 20008, USA.*

²*Center for Conservation Genomics, Smithsonian National Zoological Park and Conservation Biology Institute, 3001 Connecticut Ave NW, Washington DC 20008, USA.*

Abstract

Lactation, the production of milk for offspring, is a fundamental adaptation of the mammalian lineage. Lactation is a unique maternal signaling mechanism by which mothers can affect the development of their offspring. In the ancestral mammalian lineage, milk was the earliest mechanism by which mothers interacted biochemically with their offspring, predating the placenta by more than 100 million years (Power & Schulkin, 2013). Although the nutritional importance of milk is well established, milk provides more than food for infants. It provides immune factors, growth factors, hormones, and other bioactive factors that serve to regulate and guide infant growth and development. Vertically transmitted microorganisms in mother's milk is another potential guiding mechanism.

Milk was previously considered to be a relatively sterile body fluid but is now known to harbor a diverse microbial community. Microbes found in milk were previously thought to represent contamination from skin or the environment or a sign of infection in the mammary gland. However, DNA metabarcoding using next-generation sequencing has shown that milk contains a unique microbiome, distinct from the areolar skin (Pannaraj *et al.*, 2017), which changes with infection (Catozzi *et al.*, 2017) and time (Cabrera-Rubio *et al.* 2012, Muletz-Wolz *et al.*, 2019).

This unique microbiome appears to have a direct effect on infant gut microbial colonization (Martín *et al.*, 2004; Fernández *et al.*, 2013; Perez *et al.*, 2007) and potentially affects infant metabolism. The gut microbiomes of breastfed infants reflect their mother's gut microbiome and differ from those of bottle-fed infants (Bezirtzoglou *et al.*, 2011). Cross fostering mouse pups of lean dams to nurse from obese mothers results in the lean pups becoming vulnerable to obesity and metabolic disease (Oben *et al.*, 2010) possibly by establishing an obesity-associated gut microbiome (Turnbaugh *et al.*, 2006). The milk microbiome may be one of the aspects of milk that influences both neonatal and later adult health through inoculation of the infant gut with maternal gut microbes, presumably aiding in establishing a healthy infant gut microbiome from an early age (Murphy *et al.*, 2017).

This research project seeks to combine two fundamental aspects of mammalian biology, milk and lactation with the microbiome. We hypothesize that mammalian species will harbor milk microbiota that are to some extent unique for each species, though with potential similarities due to phylogeny and adult diet. We propose to test this hypothesis by investigating the milk microbial community structures of 26 species from 11 mammalian orders. Milk samples will represent species from all four superorders of placental mammals (Xenarthra, Afrotheria, Laurasiatheria, and Eurachontoglires), with at least two orders for each superorder. Each superorder has assorted

dietary regimes that are either primarily plant-based (frugivorous/folivorous), primarily animal-based (carnivorous/insectivorous/piscivorous), or omnivorous.

Preliminary evidence from primates suggests that species do have unique aspects to their milk microbiota, with rhesus macaques, humans, and mantled howler monkeys having notably distinct milk microbiomes (Muletz-Wolz *et al.*, 2019). Currently, the project is addressing technical issues regarding extracting DNA from high protein milks. High fat and high sugar content in milk does not appear to negatively affect DNA extraction. We are confident of being able to characterize the milk microbiota of primates, perissodactyls, artiodactyls, and marine mammals. The high protein content of milk from carnivorous and insectivorous species may present a problem, but we are working on techniques to address the issue.

Literature Cited

- Bezirtzoglou E, Tsiotsias A, and Welling, GW (2011) Microbiota profile in feces of breast-and formula-fed newborns by using fluorescence *in situ* hybridization (FISH). *Anaerobe* 17:468-482.
- Cabrera-Rubio R, Collado MC, Laitinen K, Salminen S, Isolauri E, and Mira A (2012) The human milk microbiome changes over lactation and is shaped by maternal weight and mode of delivery. *Am J Clin Nutr* 96(3):544-51.
- Catozzi C, Sanchez Bonastre A, Francino O, Lecchi C, De Carlo E, Vecchio D, Martucciello A, Fraulo P, Bronzo V, Cuscó A, D'Andreano S, and Ceciliani F (2017) The microbiota of water buffalo milk during mastitis. *PLoS One* 12(9): e0184710
- Fernández L, Langa S, Martín V, Maldonado A, Jiménez E, Martín R, and Rodríguez JM (2013) The human milk microbiota: origin and potential roles in health and disease. *Pharmacol Res* 69:1-10.
- Martín R, Langa S, Reviriego C, Jiménez E, Marín ML, Olivares M, and Rodríguez JM (2004) The commensal microflora of human milk: new perspectives for food bacteriotherapy and probiotics. *Trends Food Sci Tech* 15:121-127.
- Muletz-Wolz CR, Kurata NP, Himschoot EA, Wenker ES, Quinn EA, Hinde K, Power ML, and Fleischer RC (2019) Diversity and temporal dynamics of primate milk microbiomes. *Am J Primatol.* 1-14; e22994.
- Murphy K, Curley D, O'Callaghan TF, O'Shea C-A, Dempsey EM, O'Toole PW, Ross, RP, Ryan CA, and Stanton C (2017) The composition of human milk and infant faecal microbiota over the first three months of life: a pilot study. *Sci Rep* 1-10:40597
- Oben JA, Mouralidarane A, Samuelsson A-M, Matthews PJ, Morgan ML, Mckee C, Soeda J, Fernandez-Twinn DS, Martin-Gronert MS, Osanne SE, Sigala B, Novelli M, Poston L, and Taylor PD (2010) Maternal obesity during pregnancy and lactation programs the development of offspring non-alcoholic fatty liver disease in mice. *J Hepatol* 52:913-920.
- Pannaraj PS, Li F, Cerini C, Bender JM, Yang S, Rollie A, Adisetiyo H, Zabih S, Lincez PJ, Bittinger K, Bailey A, Bushman FD, Sleasman JW, and Aldrovandi GM (2017) Association

between breast milk bacterial communities and establishment and development of the infant gut microbiome. *JAMA Pediatr* 171:647-654.

Power ML and Schulkin J (2013) Maternal regulation of offspring development in mammals is an ancient adaptation tied to lactation. *Appl Transl Genom.* 2:55-63

Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, and Gordon JI (2006) An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature* 444:1027–1031.