

# High throughput sequencing to investigate the impact of maternal breast milk on infant gut microbiota establishment during early life

**Project: 430**

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## Project Summary

The objectives of the present study were, on the one hand, to assess bacterial population dynamics in the gut of healthy breast-fed neonates delivered vaginally at term, corresponding maternal gut and breast milk; and on the other hand, to identify isogenic viable strains in these three ecosystems in order to support the hypothesis of bacterial translocation of maternal gut bacteria and subsequent vertical mother-neonate transfer via breast milk.

Therefore, fecal and breast milk samples were collected from seven mother-neonate pairs at different time points during the neonatal period, and analyzed by complementing culture-dependent and -independent, state-of-the-art molecular methods. The culture-dependent approach consisted of culture on selective and non-selective agar media and subsequent characterization of isolated strains using 16S rRNA gene sequencing and DNA fingerprinting methods (RAPD, rep-PCR and PFGE), in order to quantify the major gut-associated bacterial populations and to identify isogenic strains in the different samples within mother-neonate pairs. The culture-independent approach consisted of quantitative PCR and 454-pyrosequencing in order to validate the culture-dependent approach and to gain comprehensive knowledge about the bacterial diversity in these three ecosystems.

To date a first manuscript on neonatal gut microbiota and early establishment of anaerobic populations has been published in the PLoS ONE Journal (doi:10.1371/journal.pone.0044595), and a second manuscript comprehensively describing a large bacterial diversity in breast milk including the detection of obligate, gut-associated taxa, has been submitted to the British Journal of Nutrition (please refer to the abstracts below). A third manuscript on the origin of breast milk bacteria and vertical mother-neonate transfer via breast-feeding is currently being prepared. We are grateful to the SFEFS for contributing with the amount of 5000sfr to the funding of this study.

Manuscript 1. New Insights in Gut Microbiota Establishment in Healthy Breast Fed Neonates  
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Abstract. The establishment of a pioneer gut microbiota is increasingly recognized as a crucial stage in neonatal development influencing health throughout life. While current knowledge is mainly based on either culture or molecular analysis of feces, we opted for a comprehensive approach complementing culture with state-of-the-art molecular methods. The bacterial composition in feces from seven healthy vaginally-delivered, breast-fed neonates was analyzed at days 4-6, 9-14 and 25-30 postnatal, using culture, 16S rRNA gene

sequencing of isolates, quantitative PCR and pyrosequencing. Anaerobes outnumbered facultative anaerobes in all seven neonates within the first days of life, owing to high levels of Bifidobacterium and unexpectedly also Bacteroides, which were inversely correlated. Four neonates harbored maternal Bacteroides levels, comprising typical adult species, throughout the neonatal period, while in three only subdominant levels were detected. In contrast, the major adult-type butyrate-producing anaerobic populations, Roseburia and Faecalibacterium, remained undetectable during the neonatal period. The presence of Bacteroidetes as pioneer bacteria in the majority of neonates studied demonstrates that adult-type strict anaerobes may reach adult-like population densities within the first week of life. Consequently the switch from facultative to strict anaerobes may occur earlier than previously assumed in breast-fed neonates, and the establishment of the major butyrate-producing populations may be limited by other factors than the absence of anaerobic conditions. The impact of breast milk components on the timing of establishment of anaerobic pioneer bacteria, as well as opportunistic pathogens should be further studied in regard to priming of the gut-associated immune system and consequences on later health.

Manuscript 2. Bacterial diversity of breast milk revealed using combined culture-independent and -dependent approaches

Ted Jost, Christophe Lacroix\*, Christian P. Braegger and Christophe Chassard

Abstract. The initial colonization of the neonatal gut is a crucial stage for developing a healthy physiology, profoundly influenced by the feeding mode. Exclusive breast-feeding is associated with a low occurrence of pathological disorders, which may not only be attributed to the broad range of bioactive compounds, unequalled by formulae, but also to commensal bacteria, including potential probiotic strains of Bifidobacterium. However, it remains largely unknown whether breast milk can be a source of other gut-associated anaerobes to the neonatal gut. Therefore, the purpose of our study was to investigate the bacterial diversity in breast milk, with emphasis on identifying gut-associated anaerobes. Breast milk collected from seven mothers at three sampling points (days 3-6, 9-14, 25-30 postpartum) was analysed by combined culture-dependent and state-of-the-art, culture-independent methods (Sanger sequencing and 454-pyrosequencing). In addition to the predominance of facultative anaerobes like Staphylococcus, Streptococcus and Propionibacterium, significant populations of obligate anaerobes, including Bifidobacterium and Veillonella, were detected using pyrosequencing, and confirmed by the isolation of viable strains. Pyrosequencing also revealed the presence of DNA of multiple major gut-associated obligate anaerobes like Bacteroides and for the first time several members of the Clostridia, including butyrate-producers, such as Faecalibacterium and Roseburia, which are important for colonic health. Our study suggests that breast milk may be a major source of bacterial diversity to the neonatal gut with potential roles in the establishment of an early trophic chain involving lactate-producers and -utilizers (i.e. Propionibacterium and Veillonella) and may thus significantly contribute to the colonization and gut maturation process.