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Probiotics tailored to the infant: a window of opportunity

Christophe Chassard, Tomas de Wouters and Christophe Lacroix

Initial neonatal gut colonization is a crucial stage for developing a healthy physiology, beneficially influenced by breast-feeding. Breast milk has been shown not only to provide nutrients and bioactive immunological compounds, but also commensal bacteria, including gut-associated anaerobic bacteria such as *Bifidobacterium* species. Infant formulas are increasingly supplemented with probiotic bacteria despite uncertainties regarding their efficacy, and lack of mechanistic understanding. Breast milk may be a valuable source of such bacteria which, upon validation of their mechanism of action, might open a window of opportunity for developing probiotic-supplemented infant formula with proven efficacy.

Addresses
Laboratory of Food Biotechnology, Institute of Food, Nutrition and Health, ETH Zürich, Schmelzbergstrasse 7, Zürich CH-8092, Switzerland

Corresponding author: Lacroix, Christophe (christophe.lacroix@hest.ethz.ch)

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Introduction

The human gastrointestinal tract harbors one of the densest bacterial ecosystems found in nature, conferring its host with increased digestive capacity, beneficial metabolites and vitamins, and protection against pathogens. In fact the collective microbial activity in the colon resembles that of an additional human organ, which is why it has been referred to as 'the forgotten organ' or 'virtual organ within an organ' [1,2]. The mutualistic interactions between the enteric microbiota and the host are essential for health [3,4]. Imbalances in host–microbe communication can lead to aberrant responses towards the microbiota, resulting in immune-related disorders, of which the most studied are inflammatory bowel diseases (IBD). Establishment of the neonatal gut microbiota starts from birth. It has been suggested that the first contacts with pioneer bacteria could be deterministic for subsequent gut maturation, metabolic and immunologic programming, and consequently for short-term and long-term health.

The infant microbiome is partly inherited from the mother and the environment during the first 2 years of life. Recent research has indicated that breast milk also contains a broad diversity of microbes present in the maternal gut and are transmitted to the baby [5••,6,7]. A recent hypothesis suggests a novel way of mother–neonate communication, in which maternal gut bacteria reach breast milk via intestinal translocation and blood carriage, describing an internal entero-mammary pathway to influence neonatal gut colonization and maturation of the immune system. This novel pathway of bacterial transfer would support the addition of carefully selected bacteria from mother's breast milk to formula and opens new windows of opportunity for designing probiotics tailored to the infant. Probiotics are defined as live organisms, which when administered in sufficient amounts, can have a beneficial effect on the host's health [8].

This review will discuss the current knowledge of the infant gut colonization considering both phylogenetic and functional diversity in the establishment of a balanced trophic chain. The potential of 'traditional probiotics' in shaping the infant gut microbiota and influencing intestinal functions and host health over the long term will be addressed. Opportunities to develop efficient probiotic strategies based on increased understanding of the roles and functions of the gut microbiota and on mechanisms of probiotic activity that support development and health of the infant will also be discussed.

The enteric microbiota, a complex and adaptive organ

The human gastrointestinal tract (GIT) is colonized by a highly diverse, commensal microbiota, comprising mainly bacteria, which with a total number of $\sim 10^{14}$ cells form one of the densest known ecosystems and outnumber the cells and gene repertoire of their host by an estimated factor of 10 and 150, respectively [9]. Using advanced molecular tools, it has been estimated that each individual harbors at least 100–200 different bacterial species and that the collective human gut microbiota encompasses over 1800 genera, 16 000 phylotypes at the species-level and 35 000 phylotypes at the strain-level [10,11]. More than 99% of the gut microbiota are obligate anaerobes, most belonging to the Clostridia class within the Firmicutes phylum, as well as to the Bacteroidetes phylum. The gut microbiota is involved in the regulation of multiple host physiological pathways, connecting the gastrointestinal tract, liver, immune system, adipose tissue, muscle, and brain [12,13].

Establishment of the gastrointestinal microbiota in the infant

It is generally accepted that the fetal GIT is sterile and that the establishment of the gut microbiota is immediately initiated at birth and characterized by a dynamic succession of bacterial populations until a homeostatic adult-like microbiota is established by the age of 2–3. The diversity of the early microbiota is relatively low, and inter-individual variations in diversity and functionality are higher as compared to adults [14]. The neonatal gut microbiota establishment is non-random, as opposed to chaotic, and follows a smooth temporal gradient with gradually increasing diversity [15,16]. Early culture-dependent research performed in the second half of the 20th century led to the still widely accepted classical colonization dogma, according to which facultative anaerobic bacteria, mainly *Staphylococcus*, *Enterococcus*, *Streptococcus* spp. and members of the *Enterobacteriaceae*, act as pioneer bacteria to reach high densities within the first days of life. By depletion of oxygen these facultative anaerobes create a reduced environment, which enables the successive establishment of obligate anaerobic populations, such as *Bifidobacterium*, and members of the Bacteroidetes and Clostridia [17]. Culture-independent studies confirmed these findings. As such butyrate-producing members of the Clostridia (e.g. *Roseburia* and *Faecalibacterium* spp.) are essential for adult colonic health but are not usually detected in neonates [6,18•]. Using both anaerobic culture-dependent (i.e. culture, isolation and 16S rRNA gene sequencing) and culture-independent methods (i.e. qPCR and pyrosequencing), Jost *et al.* [5••] recently demonstrated that obligate anaerobes, such as members of the Bacteroidetes, may reach adult-like population densities already within the first week of life and thus earlier than previously assumed. These populations have never been extensively studied in neonates. The fact that other strict anaerobes are able to colonize early suggests that their establishment is rather directed by metabolic cross-feeding between members of the microbiota than by oxygen pressure, as generally assumed.

Factors influencing the infant microbiota

The composition and activity of the gut microbiota co-develop with the host starting from birth, and are subjected to a complex interplay that depends on the hosts' genetics, nutrition, and life-style. Natural delivery and feeding mode (i.e. vaginal delivery at term and exclusive breast-feeding) have been associated with beneficial health effects related to gut colonization and development, such as protection against infection, reduced infant morbidity and mortality, and low incidence of immunological disorders [19,20,21••]. Natural delivery and breast feeding provide early contact with the maternal microbiota and are thought to be crucial for gut maturation, metabolic and immunologic programming, host–microbe homeostasis establishment [22]. The major potential sources of pioneer bacteria and factors that may influence neonatal gut microbiota composition include transmission to the fetal gut in utero [23], delivery mode, environmental factors such as geographical location [24••], familial environment, sanitary conditions, mode of feeding, and medical treatment of the mother or the infant [19].

Human breast milk is a complex nutrient shaped by millions of years of evolutionary adaptation. It provides tailored nutritional, protective, immunological and developmental functions to suit all the needs of the developing neonate in an age-adapted manner while sustaining a healthy microbiota establishment [25]. This makes mother's milk the undisputable gold standard for early nutrition that no formula milk can compete with [26]. Breast milk itself has been identified as a continuous source of live commensal maternal bacterial, including strict anaerobes, able to colonize the neonatal gut, and thus to influence early host-microbe interactions and neonatal development [5••,27,28,29]. The way of entry of bacteria into the mammary gland and breast milk remains unclear to date. Bacteria may enter the mammary gland by contamination from outside, and/or from within, via a bacterial entero-mammary pathway [7]. In a recent study, using a combination of culture and culture-independent methods, Jost *et al.* [5••] showed that gut-associated obligate anaerobic genera, such as *Bifidobacterium*, *Bacteroides*, *Parabacteroides*, and members of the Clostridia (*Blautia*, *Clostridium*, *Collinsella* and *Veillonella*) were shared between maternal feces, breast milk and neonatal feces. Furthermore, the same strain of *Bifidobacterium breve* could be isolated in all three ecosystems within one mother–neonate pair. Several butyrate-producing members of the Clostridia (*Coprococcus*, *Faecalibacterium*, *Roseburia* and *Subdoligranulum*) were shared between maternal feces and breast milk but remained undetected in neonate feces. Despite this considerable diversity of breast milk bacteria [6], their impact on neonatal gut microbiota establishment, gut maturation, immunity development and consequences on later health status, remain largely unknown.

Establishment of a healthy trophic equilibrium in the gut

Major sources of carbohydrates for the infant colonic microbiota are human milk oligosaccharides (breast milk), prebiotics (formula), endogenous glycans (mucines) of host origin, undigested monosaccharides and lactose [30]. Complex carbohydrates are mainly degraded by *Bifidobacterium*, *Lactobacillus* and *Bacteroides* (human milk oligosaccharides and mucin degraders) into small sugars. Simple sugars are further metabolized together with lactose by numerous glycolytic microbes, including the predominant lactate producing bacteria such as *Streptococcus*, *Staphylococcus*, *Enterococcus* and enterobacteria (Figure 1). Large amounts of lactate are produced by these species during infant gut fermentation. Lactate can