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Current Opinion in Biotechnology

The human gut microbiota during the initial stages of life: insights from bifidobacteria

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Current scientific literature has identified the infant gut microbiota as a multifaceted organ influencing a range of aspects of host-health and development. Many scientific studies have focused on characterizing the main microbial taxa that constitute the resident bacterial population of the infant gut. This has generated a wealth of information on the bacterial composition of the infant gut microbiota, and on the functional role/s exerted by their key microbial members. In this context, one of the most prevalent, abundant and investigated microbial taxon in the human infant gut is the genus *Bifidobacterium*, due to the purported beneficial activities is bestows upon its host. This review discusses the most recent findings regarding the infant gut microbiota with a particular focus on the molecular mechanisms by which bifidobacteria impact on host health and well-being.

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Current Opinion in Biotechnology 2021, **73**:81–87

This review comes from a themed issue on **Environmental biotechnology**

Edited by **Luigi Vezzulli** and **Marco Ventura**

https://doi.org/10.1016/j.copbio.2021.07.012

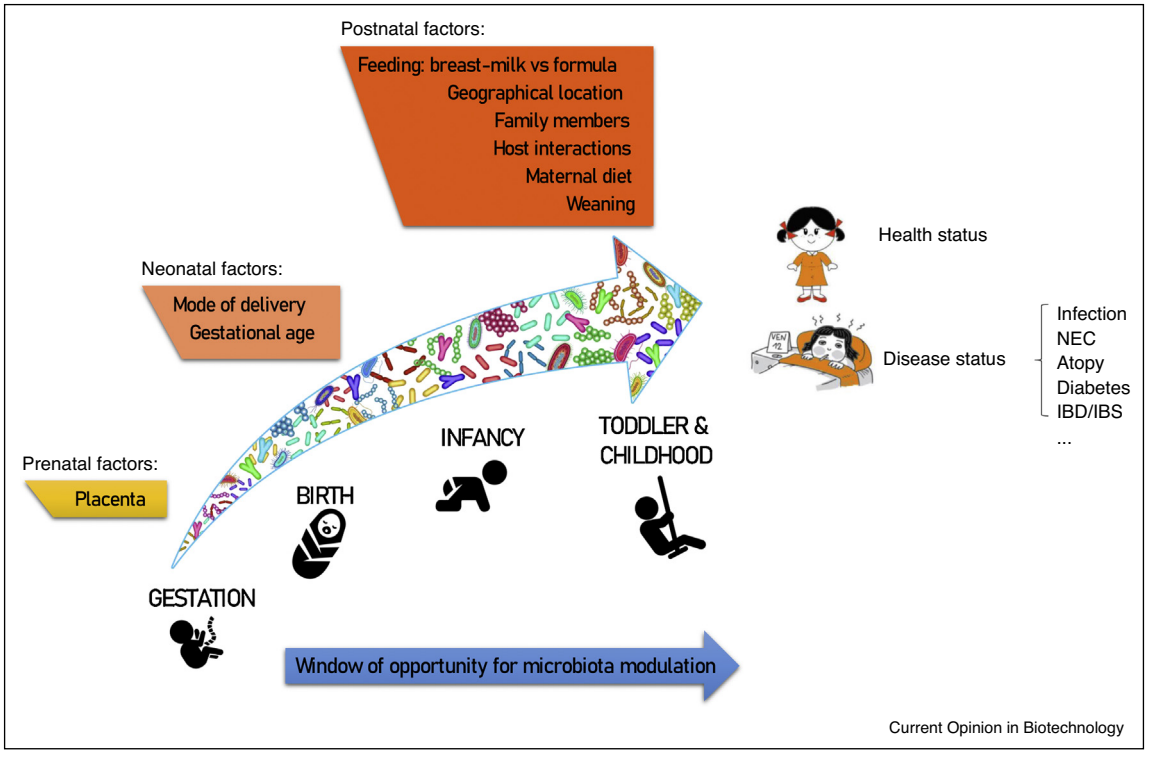
Introduction: the infant gut microbiota

Formerly described as a hidden organ of the human body [1], the complex gut microbial population defined as 'gut microbiota' has enjoyed increasing scientific interest in recent decades due to the variety of beneficial interactions between several of its members and their host [2]. This complex microbe–host interplay on human health is being actively investigated in order to unravel the biological mechanisms responsible for their functional effects on human physiology at any stage of life [3,4•]. One major finding of this research is that the development of early life or infant gut microbiota represents a foundation event for human health [5,6].

The infant gut microbiota is represented by trillions of microbial cells residing in the small and large intestine, with the latter exhibiting the highest microbial density and complexity. Despite some controversy [7], recent investigations support the concept that initial neonatal gut colonization occurs during, and shortly after birth, being influenced by several perinatal factors, in particular delivery mode, feeding type, and gestational age [5,8] (Figure 1). In this context, the origin and transmission routes of the pioneering bacteria have received considerable scientific interest. Vertical transmission from the maternal microbial reservoir is thought to be an important microbial route for infant gut colonization, in addition to microbial taxa acquired from the external environment [9,10]. The infant gut microbiota develops through a dynamic process that starts at birth and proceeds some 2–3 years, at which point it reaches a relatively stable configuration reminiscent of that of a typical adult microbial taxonomic makeup [5,11]. Intriguingly, the microbes present in the intestinal lumen of infants include autochthonous, that is, indigenous, and allochthonous, that is, transient, microorganisms [12]. This has been confirmed by taxonomic investigations of fecal and mucosa-adherent microbiota, revealing diverging, individual-specific microbial compositions [13,14]. Apparently, certain microbial taxa are introduced during food ingestion and may return to the environment by defecation, while others evolved specific properties to persist in the gut [15,16••]. The latter include extracellular structures for gut mucosa adhesion, in particular pili and fimbriae, and the ability to recover carbon/energy from dietary or host-derived glycans [17]. The progressive disentanglement of microbe–host interactions exposed a pattern of long-term co-evolution between human host and its resident gut microbiota that resulted in a mutualist relationship, which is particularly important for host developmental processes during early life [18,19].

Members of the gut microbiota establish various microbe–microbe interactions ranging from co-operative behavior such as syntrophy or cross-feeding, that is, the metabolic collaboration for the complete catabolism of complex nutritional resources, to antagonism and competition for effective niche colonization [20,21•,22]. In a

Figure 1



Microbiota establishment and modification in the early stage of life.
Timing in which it is possible to modulate the microbiota and the various factors that have an impact on the modulation of it. This window can influence the subject's health status in childhood as well as in the future.

healthy gut, interactions between microbial taxa result in a stable homeostasis characterized by high resilience toward exogenous and endogenous perturbations [23]. Nevertheless, the taxonomic composition and functionality of the gut microbiota can be altered to cause gut homeostasis disruption [24]. This condition is often referred to as gut dysbiosis and may involve intestinal colonization by pathogens due to reduced niche competition exerted by resident microbiota or even overgrowth of opportunistic pathogens, which under homeostatic conditions act as harmless commensals [24,25]. While the concept of dysbiosis is still controversial due to the high inter-interindividual variability of the gut microbiota composition that prevents the definition of a 'normal' microbiota, aberrant gut microbiota development and its functional alteration has been linked to a wide range of neonatal disorders and diseases such as Failure To Thrive (FTT) due to gastrointestinal disorders or Necrotizing Entero Colitis (NEC). Moreover, early life dysbiosis may induce long-term effects in childhood or even adulthood that can be responsible for the development of inflammatory bowel disease (IBD), diabetes and obesity [26–30] (Figure 1).

The current knowledge driven by multi-omics investigations on infant microbiota

Culture-independent 'omics' investigations have significantly advanced our understanding of both the ecology and physiology of the infant gut microbiota. As mentioned above, the current view is that the infant gut is sterile during gestation [31]. The mother is perceived to represent the main source of microorganisms that initiate neonatal gut colonization, with delivery being a key initiating event for transmission and colonization [9]. During these early stages, human milk exerts a crucial role in supporting the establishment and persistence of bacteria by providing specific prebiotic nutrients, in particular Human Milk Oligosaccharides (HMOs), an interactive process resulting from microbe–host coevolution [20,32]. The host and its associated chemical-physical-nutritional-environmental conditions guide the assembly of the intestinal microbiota in terms of functionality and biodiversity [16••,33]. Along with bacteria, the mother-newborn microbial transmission route include viruses, in particular bacteriophages [34,35•], which are thought to influence gut microbiota homeostasis by shaping the microbial population through ecological forces, such as 'kill the winner', 'kill the relative',

'community shuffling' and 'invade the relative' strategies [36]. Altogether, these processes establish the early infant gut microbiota which then forms the foundation for the development of gut microbial communities at later stages of life [37••].

A healthy infant gut microbiota elicits a range of positive effects on infant development, including stimulation of the immune system, induction of gut mucosal layer production and its turn-over, protection from (opportunistic) pathogens and participation in metabolic activities, such as breakdown of food components and biosynthesis of bioactive compounds such as short chain fatty acids, vitamins and even neurotransmitters impacting on the central nervous system [5]. Recently, studies involving humans and germ-free animal models underlined the pivotal role played by the gut microbiota in shaping innate and adaptive immunity [38,39]. Moreover, investigation of gut microbial composition and immune system homeostasis has revealed several correlations between colonization by specific microbial taxa and biological activity of various innate immune components, such as neutrophils, lymphocytes and antigen presenting cells, as well as of adaptive immunity players, such as T and B cells [40]. From a clinical perspective, disruption of immune system homeostasis in terms of taxonomic composition and metabolite profiles has been linked to increased risk of pathogenic infections and even development of extraintestinal or gut-localized autoimmune disorders such as atopy [41•], type 1 diabetes [42], rheumatoid arthritis [43] and nervous system's demyelination-related pathologies [44], or Crohn's disease and ulcerative colitis [45], respectively.

The infant gut microbiota also stimulates the enteric mucus layer, that is, the glycoprotein matrix produced by intestinal goblet cells, which provide both lubrification and protection of the gut surface [46]. The role exerted by the host immune system and mucus barrier in controlling and driving gut colonization is supported by the gut microbiota itself through niche competition [47•]. For this reason, an aberrant infant gut microbiota is often associated with (opportunistic) pathogenic infections with major health implications [47•]. This condition is commonly linked to pre-term and/or Cesarean delivery and can be due to lack of vertical transmission of bacteria from the mother, thereby exposing the newborn to colonization by harmful environmental bacteria such as multidrug resistant pathogens [48]. Notably, anomalous infant gut microbiota development due to non-vaginal delivery can be reverted by means of maternal fecal microbiota transplantation [49,50]. The latter approach involves oral or colonoscopic transfer of the microbial population extracted from fecal samples of healthy individuals in order to induce a beneficial shift in the receiver's gut microbiota composition [51].

Microorganisms resident in the infant gut environment extensively participate in catabolic and anabolic activities in close harmony with their host [52]. Among the most relevant activities, the gut microbiota is involved in the breakdown of complex food components [53]. Lactation allows the host to select microbial species which have co-evolved to metabolize complex host-derived glycans, in particular HMOs and mucin, the main glycoprotein found in human secreted mucus, including the gut mucus layer [54]. Moreover, following weaning and introduction of a complex solid diet, the metabolic activities driven by the gut microbiota provides the host access to indigestible food components, thus playing a pivotal role in energy harvesting [54]. Indeed, the gut microbial population produces metabolic end products, which may serve as nutrition for intestinal cells, particularly Short Chain Fatty Acids (SCFAs) [54], yet may also exert a range of activities in distant body sites such as the central nervous system [55], muscles [56], liver [57] and bladder [58,59].

Considering their health implications many studies have scrutinized the main microbial taxa resident in the infant gut, resulting in the identification, through meta-analysis of all publicly available cohorts, of the most prevalent Community State Type (CSTs), that is, common microbial assemblies [60]. Among the key bacterial genera identified in infant gut CSTs, members of the genus *Bifidobacterium* stand out, being considered prototypical health-promoting bacteria [61].

The genus *Bifidobacterium* and its role in the infant gut microbiota

Although the human intestinal microbiota is generally considered to be complex, the microbial assembly during early life is nonetheless relatively simple. In particular, the genus *Bifidobacterium* is typically a dominant microbial taxon especially during the first six months after birth, though the relative abundance of this taxon decreases following weaning [60].

Various physiological, biochemical and in recent years also ecological and genetic aspects have been investigated to understand early life gut colonization events involving bifidobacteria. Certainly, maternal contact represents an important and priming factor that directs bifidobacterial colonization. In fact, bifidobacteria are able to follow a vertical transmission route from a maternal origin [35•]. Bifidobacteria, in fact, are able to colonize and persist in the infant gut until weaning at much higher levels compared to other bacteria for various reasons. One of the main colonization factors in this respect is the neonatal diet of Human Milk (HM), which represents the optimal diet stimulating the development of the most appropriate microbiota for the newborn [62]. This allows the early and high abundance colonization of bifidobacteria with a consequent production of their main fermentation metabolites acetic and lactic acid, which elicit

antagonistic effects toward detrimental microorganisms like *Salmonella* and *Listeria* [63]. Bifidobacteria also possess genetic features that allow the production of various extracellular structures such as pili and exopolysaccharides, and that favor their interactions with the human host and other gut microorganisms [64]. In addition to the maternal origin of bifidobacteria being obtained by the neonate through vertical transmission, another important source of these microorganisms is represented by HM itself, which represents an important vehicle containing bifidogenic substances and microorganisms, including bifidobacteria [65]. In fact, HM is a complete biological body fluid from a nutritional point of view for the newborn consisting of macro-nutrients and micro-nutrients in a mainly aqueous solution in which there are also hormones, immunomodulatory components, growth factors, microRNAs, and HMOs in different concentrations according to the lactation period and genetic factors [66•]. Recently the role of this biofluid has been investigated in depth, revealing various physiological benefits to the newborn (e.g. establishment and nourishment of the gut microbiota, immuno-stimulatory influence, gut brain axis involvement, impact on bacterial and viral infections), yet also eliciting long-lasting health effects [62,67]. HMOs represent, after lactose and lipids, the third most important solid component in HM. Chemically they are unconjugated glycans with a lactose core varying in chain length, glycosidic linkages and monosaccharide composition [which include glucose, galactose, fucose, *N*-acetylneuraminic, fucose, *N*-acetylglucosamine, *N*-acetylneuraminic acid and sialic acid] [68••]. Various HMO-mediated cross-feeding activities have been observed among bifidobacterial species, some of which are able to internalize extended forms of HMOs, while others secrete extracellular Glycosyl Hydrolases (GHs) that catalyze extracellular breakdown of these glycans. These extracellular enzymes allow hydrolysis of HMO structures into mono/di-saccharides, which may then be internalized by other bifidobacteria sharing the same ecological niche, yet unable to degrade intact HMOs [62,68••,69]. *Bifidobacterium breve*, *Bifidobacterium longum* subsp. *longum* and *Bifidobacterium bifidum* are the dominant bifidobacterial species in the infant gut microbiota followed by others such as *Bifidobacterium pseudocatenulatum*, *B. longum* subsp. *infantis*, *Bifidobacterium kashiwanohense* [61,68••,70,71]. Genome analysis of such infant-associated bifidobacterial species has allowed the identification of the genetic arsenal responsible for HMO metabolism [20], represented by genes encoding enzymes involved in HMO internalization and hydrolysis, for example, fucosidase, sialidase, β-hexosaminidase, and β-galactosidase, extracellular solute binding proteins and ABC transporter systems [21•,69]. Careful genomic characterization of bifidobacterial strains isolated from breastfed infants revealed a distinct genomic architecture enriched with strain-specific or species-specific genes involved in HMO metabolism [68••]. This may lead to particular metabolite profiles, especially when considering different cross-feeding interactions that can be established depending on the microorganisms present in the environment [68••,72]. Simpler HMOs and metabolites produced also play important roles in fueling cross-feeding reactions with other gut bacteria, such as with *Eubacterium hallii* [73] and *Anaerostipes caccae* [74]. The syntrophic interactions that can be established between microorganisms are many and varied [21•]. Other known cross-feeding interactions established by bifidobacteria and other human gut commensals involve various nutrients such several plant cell wall oligosaccharides [75], xylan [72], xyloglucan substrates [76], mucin [77,78], arabinogalactan [79], fructooligosaccharide and starch [72,80], inulin [81] and fucose and rhamnose [82]. All these trophic interactions represent a clear example of host–microbe adaptation and co-evolution between various gut microbes and the human host [32,68••,83]. In addition, cross-feeding behavior exerted by many bifidobacterial species highlight the key ecological role of these microorganisms in obtaining and providing substrates from and to other gut bacteria and thus in supporting a metabolically interactive microbiota in the human gut.

Bifidobacteria appear to elicit several beneficial activities in the human body [84•]. In fact, the relative abundance of this microbial group in the infant gut microbiota seems to be negatively correlated with several disease states, thus supporting the notion of bifidobacteria as early microbial biomarkers associated with human wellbeing [85]. In this context, there is compelling scientific evidence supporting the crucial role played by bifidobacteria in the proper functioning of the human immune system [85,86••]. Depletion of bifidobacteria in the infant gut has been implicated in childhood atopy and asthma development as well as in the establishment of many autoimmune diseases [87,88]. Furthermore, the relative abundance of bifidobacteria during early infancy appears to enhance protective efficacy of typical infant vaccines by enhancing immune memory [89].

Increasing scientific evidence has implied a connection between the gut to the brain via the nervous system [90]. One can therefore envisage an exchange of gut-brain messages mediated by the central nervous system that may already commence at birth and conveyed by the intestinal microbiota. It has recently been hypothesized that the intestinal microbiota may somehow assist in the functional organization of neural circuits in the brain during the first years of life through the regulation of synaptic gene expression and modulation of microglial functionality [91]. Notably, bifidobacteria seem to play a role in such neurodevelopment (i.e. by the production of neurotransmitters, such as acetylcholine, GABA, and serotonin, that can influence, directly and indirectly, brain cell physiology), although the exact mechanisms by which gut microbes communicate with the host brain

are yet to be identified [91,92]. Assuming that there is a connection between intestinal microbial composition and functional brain connectivity already at neonatal level, it will be very intriguing to elucidate the long-term consequences on brain function including mental health outcomes.

Conclusions

It is widely accepted that the intestinal microbiota is an organ whose activities contribute to the establishment and maintenance of human health. In the past decade, a lot of effort has been invested to elucidate the molecular mechanisms facilitating gut colonization and persistence, and the beneficial effects of (particular) intestinal microorganisms. Host-driven selection of early colonizers, as well as their temporal order of colonization, are believed to be a crucial step in guiding gut microbiota assembly in terms of biodiversity and functionality. Bifidobacteria are one of the most abundant microbial genera present in the intestine of a full-term, breast-fed, healthy newborn. It is universally accepted that these bacteria play an important role in the maintenance and protection of human health and for this reason they can be used as important microbial gut biomarkers whose decline is linked to the occurrence of a range of human diseases that not only occur during infancy but may also emerge at later stages of life.

Conflict of interest statement

Nothing declared.

Acknowledgements

We thank GenProbio srl for financial support of the Laboratory of Probiogenomics. Part of this research is conducted using the High Performance Computing (HPC) facility of the University of Parma, Italy. D. vS. is member of APC microbiome Ireland which is funded by Science Foundation Ireland (SFI) through the Irish Government's National Development Plan (Grant Numbers SFI/12/RC/2273-P1 and SFI/12/RC/2273-P2).

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