GUT MICROBES
2017, VOL. 8, NO. 2, 143–171
http://dx.doi.org/10.1080/19490976.2016.1278104

Taylor & Francis
Taylor & Francis Group

REVIEW

The role of early life nutrition in the establishment of gastrointestinal microbial composition and function

Erin C. Davis[a], Mei Wang[b], and Sharon M. Donovan[a,b]

[a]Division of Nutritional Sciences, Department of Food Science and Human Nutrition, University of Illinois, Urbana, IL USA; [b]Department of Food Science and Human Nutrition, University of Illinois, Urbana, IL USA

ABSTRACT

The development of the human infant intestinal microbiota is a sequential process that begins *in utero* and continues during the first 2 to 3 years of life. Microbial composition and diversity are shaped by host genetics and multiple environmental factors, of which diet is a principal contributor. An understanding of this process is of clinical importance as the microbiota acquired in early life influence gastrointestinal, immune and neural development, and reduced microbial diversity or dysbiosis during infancy is associated with disorders in infancy and later childhood. The goal of this article was to review the published literature that used culture-independent methods to describe the development of the gastrointestinal microbiota in breast- and formula-fed human infants as well as the impact of prebiotic and probiotic addition to infant formula, and the addition of solid foods.

ARTICLE HISTORY
Received 7 October 2016
Revised 21 December 2016
Accepted 23 December 2016

KEYWORDS
human milk; infant formula; microbiota; oligosaccharides; probiotic

Introduction

Intestinal microbiota development is a complex process that begins *in utero* and continues for the first 2–3 years of life.[1-3] Host genetics and environmental factors such as gestational age, delivery mode, diet, pre- and probiotics, antibiotics, maternal weight and stress influence the process.[4] The microbiota acquired in early life have long-term implications for host metabolism and gastrointestinal (GI), immune and neurological function.[5,6] Reduced diversity or dysbiosis are linked to childhood and later life disorders, including necrotizing enterocolitis,[7] eczema,[8] asthma,[9] inflammatory bowel diseases,[10] irritable bowel syndrome,[11] obesity,[12] diabetes[13] and autism.[14]

Diet is one of the major determinants of GI microbial diversity. Bacterial composition and alpha-diversity differ between breastfed (BF) and formula-fed (FF) infants,[15-17] and solid food introduction has been associated with rapid and sustained alterations in the fecal microbiota.[1,18] The goal of this review is to summarize our current knowledge on how nutrition may influence GI microbial composition and function in early infancy. The impact of pre- and probiotic supplementation on the colonization of the infant microbiota will also be addressed.

Breast- and formula-feeding

The fecal microbial composition of BF and FF infants has been investigated for more than 40 years. Prior to the early part of the 21st century, microbes were studied primarily using culture-dependent methods, which limited our understanding of microbial diversity and often resulted in conflicting reports.[19] Findings from 16S ribosomal ribonucleic acid (16S rRNA)-based approaches (For a review, see ref. 20) have shown that the infant microbiota is more diverse than previously appreciated. However, the findings are still somewhat varied, likely due to the small number of infants included in some studies (Table 1), geographical differences,[21] differences in analytical methods and sampling time points,[17] changes in infant formula composition over time, and differences in oligosaccharide composition of human milk (HM) and infant formula.[17,20] This review focuses on studies that used 16S rRNA-based methods to investigate the fecal microbial composition in infants over the first yr of life (Table 1).

Initially after birth, the newborn fecal microbiota is composed primarily of facultative anaerobes, such as *Staphylococcus, Streptococcus, Lactobacillus*, and

CONTACT Sharon M. Donovan sdonovan@illinois.edu University of Illinois, Department of Food Science and Human Nutrition, 339 Bevier Hall, 905 S. Goodwin Avenue, Urbana, IL 61801.

Table 1. Differences in fecal microbial composition among infants exposed to different feeding patterns.

Sample Timepoint	Feeding Mode (n)	Method	Results	Reference (Country)
4 mo	BF (63), MF (22), FF (9)	Mixed Sanger sequencing	• Solid foods did not affect microbial composition	[22] (Norway)
3—4 mo	BF (19), MF (5), FF (9)	Illumina sequencing	• No difference in proportion of *Bifidobacterium* • Lower prevalence of *C. difficile* in BF • Higher proportions of Verrucomicrobiaceae (*Akkermansia*) and Peptostreptococcaceae in FF • BF exclusivity was negatively correlated with proportion of Peptostreptococcaceae and prevalence *C. difficile*	[15] (Canada)
3 mo, 12 mo	BF (102), MF (60), FF (36)	Illumina sequencing	• BF had higher proportions of Actinobacteria and Proteobacteria • At 3 mo, BF had higher proportions of *Bifidobacterium* and lower proportions of *Bacteroides* and *Clostridiales* • At 3 mo, BF exclusivity was inversely associated with diversity and proportions of Bacteroidetes and Clostridiales • Higher proportion of Clostridiales at 1 y associated with BF exclusivity at 3 mo • BF exclusivity and duration associated with higher diversity at 1 y. • BF exposed to intrapartum antibiotics (IAP) had higher proportions of *Clostridium* • At 12 mo, FF & MF had lower proportions of Bacteroidaceae and higher proportions of *Clostridiales* if exposed to IAP during emergency CS (compared with FF & MF infants who were not exposed)	[23] (Canada)
1 wk, 4 mo, 12 mo	1 wk: BF (73), MF (24), FF (1)Four mo: BF (67), MF (19), FF (11)	Illumina sequencing	• No differences in MF and BF infants at 1 wk • At 4 mo, BF had higher proportions of *L. johnsonii/L.gasseri, L. paracasei/L.casei* and *B. longum* • At 4 mo, FF had higher proportions of *C. difficile, Granulicatella adjacens, Citrobacter* spp, *Enterobacter cloacae, Bilophila wadsworthia* and *B. adolescentis* • Cessation of breastfeeding followed by increased proportions of *Bacteroides, Bilophila, Roseburia, Clostridium, Anaerostipes*. • *Bifidobacterium, Lactobacillus, Collinsella, Megasphaera, and Veillonella* dominated infants receiving breast milk at 12 mo.	[24] (Sweden)
birth—24 mo	BF-dominant (31),FF-dominant (12)	Illumina sequencing	• α and β-diversity affected by delivery mode, antibiotic use, and diet. • Solid foods associated with a ↑ in Clostridiales • BF- dominant (> 50% of feedings were breast milk from 0–3 mo) had higher abundance of *Lactobacillus, Staphylococcus, Megasphaera*, and Actinobacteria • FF-dominant (> 50% of feedings were formula from 0–3 mo) had greater abundances of Clostridiales and Proteobacteria • Diversity decreased from 12–24 mo in FF-dominant	[25] (US)
3—6 mo	BF (6), FF (6)	Illumina sequencing	• BF had greater proportions of Actinobacteria and Firmicutes, while FF had higher proportions of Proteobacteria • BF dominated by *Streptococcus, Bifidobacterium, Lactobacillus, Clostridium*. • FF dominated by *Klebsiella, Streptococcus, Lactobacillus, Enterococcus, Bifidobacterium*. • BF had greater proportions of *Lactobacillus* and were 2x greater abundance of *Bifidobacterium* • *Klebsiella* was 30x more abundant in FF • Staphylococcaceae and Pasteurellaceae were only present in BF infants. • Bacillaceae, Veillonellaceae, Ruminococcaceae and Comamonadaceae were only found in FF infants.	[26] (China)

d4–6, 9–14, 25–30	BF (7)	Sanger sequencing, pyrosequencing, qPCR	• Actinobacteria was most abundant phyla • *B. breve* was most dominant strain of *Bifidobacterium* • *Lactobacillus* levels were variable and unstable • *Parabacteroides* and *Bacteroides* were most abundant genera from Bacteroidetes • *Escherichia* and *Klebsiella* were most abundant genera from Proteobacteria • *Bacteroides* levels ↑ over sampling period; timing of appearance differed among infants. • Levels of *Bacteroides* and *Bifidobacterium* were negatively correlated • *B. catenulatum, B. adolescentis, Roseburia, Eubacterium, Faecalibacterium*, or *Ruminococcus* not detected	[27] (Switzerland)
birth–2.5 y	BF until d134; MF until 9 mo; Solids started at 4 mo.; FF until 1 y (1)	Pyrosequencing	• Firmicutes dominated until d92; Actinobacteria and Proteobacteria then began to ↑ • Introduction of peas and formula led to a ↑ in Bacteroidetes. • Solid foods caused a shift in phyla abundances, ↑ bacterial load, and ↑ SCFA. • Abundance of Firmicutes and of actinobacterial and proteobacterial genes were negatively correlated. • Composition was consistent after weaning.	[1] (US)
4 wk	BF (10), FF (10)	Pyrosequencing	• All infants dominated by Actinobacteria, Firmicutes, and Proteobacteria • BF had higher proportion of Actinobacteria and lower proportions of Firmicutes and Proteobacteria • Actinobacteria and Firmicutes were almost equally abundant in FF • Proportions of Bifidobacteriaceae, Micrococcaceae, *Bifidobacterium, L. gasseri, B. longum* greater in BF • FF had higher proportions of *Escherichia, Veillonella, Enterococcus*, and Enterobacteria • Low abundance of *Lactobacillus* in FF. • *B. breve, B. bifidum, L. brevis*, and *L. reuteri* only detected in FF	[28] (S. Korea)
6 wk	BF (70), MF (25), FF (6)	Illumina sequencing	• Interaction between delivery mode and diet had no impact on composition. • Greater phylogenetic differences between BF and FF infants than between BF and MF infants • Compositional differences only seen between BF and MF or FF • FF had a greater abundance of Lactococcus compared with BF • MF had largest intra-group compositional differences	[29] (US)
3 mo	BF (6), FF (6)	Pyrosequencing	• BF had greater α-diversity and were less homogenous at phylum-level. • Five of the BF infants dominated by 1 phylum (Actinobacteria, Proteobacteria, or Bacteroidetes). • Actinobacteria and Firmicutes were equally dominant in FF.	[30] (US)
d1, 6 wk, 12 wk	BF (13), FF (13)	Pyrosequencing, qPCR	• In both groups, bifidobacterial counts ↑ from birth to 6 wks • qPCR showed *B. bifidum* and *B. breve* as the dominant species of bifidobacteria in all infants • Pyrosequencing identified *B. breve* and *B. longum* as the dominant bifidobacterial species at different times in all infants • *Bifidobacterium*-dominated 2/3 of BF at 6 wks • *B. pseudocatenulatum* became dominant over time in BF • FF had higher levels of Bacteroidetes and a greater relative abundance of *Peptostreptococcaceae* • Some FF dominated by *Ruminococcus gnavus* at 6 and 12 wks. • Larger ↓ in *Escherichia* counts in BF over the first 6 wks	[31](France, Poland)

(*Continued on next page*)

Table 1. (Continued)

Sample Timepoint	Feeding Mode (n)	Method	Results	Reference (Country)
2 mo (on average)	BF (30), FF (60- 30 CM, 30 GM)	Pyrosequencing	• BF had greater proportions of Bifidobacteriaceae, Bacteroidaceae, and *B. breve* and lower proportions of Lachnospiraceae • BF and goat's milk (GM) formula-fed infants dominated by *Ruminococcus gnavus*. • GM-fed had a Lachnospiraceae composition more similar to BF. • Greater α-diversity in cow's milk (CM) formula group. • Both groups of FF had higher proportions of Erysipelotrichaceaes (*Clostridium ramosum*, *Clostridium innocuum* - most common)	[32] (Australia)
d13—14 mo	Diet was variable (9)	Pyrosequencing	• BF had a core microbiome of *Bifidobacterium* and *Coprobacillus* • MF & FF had a core microbiome of *Veillonella, Clostridiales*, and *Bacteroides* • BF had higher abundances of Actinobacteria and *Bifidobacterium* • Greater proportions of Bacteroidetes, *Eggerthella*, Clostridiales, *Lachnospiraceae, Blautia*, and *Faecalibacterium* in MF & FF • Introduction of solids followed by a ↑ in proportions of Bacteroidetes, *Eggerthella, Blautia, Neisseria*, Peptostreptococcaceae and ↓ in *Staphylococcus and Roseateles* in BF. • Introduction of solids in MF & FF followed by a ↑ in *Bifidobacterium*, Ruminococcaceae, and *Blautia*. • BF had higher proportions of *Lactobacillus* and Ruminococcaceae and MF & FF had greater proportions of Clostridiales, *Blautia, Faecalibacterium, Anaerotruncus, and Eubacterium* after introduction to solids • EBF were more compositionally similar to BF + solids than non-EBF infants.	[33] (US)
1.5—3 mo	BF (7), MF (1), FF (3)	Pyrosequencing	• Bifidobacteriales, Lactobacillales, and Clostridiales were most abundant classes respectively. • 10/11 infants dominated by Bifidobacteriaceae • Large variations in proportions of *Bifidobacterium* species, with *B. longum* being the dominant bifidobacterial species	[34] (Italy, Spain, Ireland)
1 wk, 1 mo, 3 mo,7 mo, 12 mo	Diet was variable; solids introduced between 3–7mo (13)	Pyrosequencing	• Introduction of solid foods followed by large permanent shift toward an adult-like composition. • ↓ in richness (recovered by 1 yr) and ↑ in diversity throughout introduction of solids. • Escherichia ↓ throughout introduction of solid foods. • At 7 mo., *Bifidobacterium* and *Bacteroides* still dominated and *Ruminococcus* and *Akkermansia* began to colonize • Similar composition early in life did not predict similar microbial development throughout the first year.	[35] (Spain)
2—5 mo	BF (8), FF (10)	Pyrosequencing, shotgun sequencing	• 48 OTUs discriminated BF and FF • BF had higher proportion of *Bifidobacterium, Actinomyces, Erwinia, and Haemophilus* • FF had greater levels of Firmicutes and Bacteroidetes	[36] (Venezuela, Malawi, US)
d1—12mo.	BF d1–17 (1), BF d1–130 (1), Diets varied after EBF	PCR-DGGE	• Bifidobacteria were present from d3-d4. • *Ruminococcus, Clostridium*, and *Enterobacter* appeared at different times in infants. • At 3 mo, BF composition was more diverse and more dominated by bifidobacteria • *Ruminococcus* levels ↑ upon supplementation and weaning • Streptococci and enterococci ↓ upon weaning.	[37] (The Netherlands)

birth—d160	Diet was variable (5)	PCR-DGGE	• Bifidobacteria appeared earlier in BF infants than those who received formula for 1 d • Diversity of FF infants ↑ within first few days • Enterobacteriaceae and enterococci present at higher counts and for a longer duration in FF infants	[38] (not stated)
8 mo (on average); samples collected for 8 wk	BF (2), FF (3) Some solid foods consumed after 6 mo	PCR-DGGE	• *B. longum* subsp *infantis (*major species), *B. breve, B. bifidum*, and *B. longum* identified in all infants. • BF had a more stable bacterial diversity and greater total numbers of bifidobacteria • FF had a greater diversity among bifidobacterial species • Low proportion of *B. adolescentis* in FF; none detected in BF • Bifidobacteria counts and species in FF began to resemble BF after starting on prebiotic formula	[39] (Netherlands)
5, 13, 21 wk (on average)	Infants sampled while EBF, weaning (MF), post-weaning (11)	PCR-TGGE	• EBF period dominated by *Bifidobacterium* • Prevalence of *B. breve, B. infantis/longum*, and *Ruminococcus* ↑ across feeding periods. • Prevalence of *E. coli* and *Bifidobacterium* remained consistent throughout dietary changes. • Proportion of bifidobacteria not affected by diet changes • Diversity ↑ upon weaning but ↓ post-weaning • Greater relative abundance of *E. coli* and *Ruminococcus* post-weaning	[40] (Algeria)
~d31	BF (27)	PCR	• *B. breve* and *B. infantis* were most frequently detected bifidobacterial species • *B. longum* and *B. bifidum* detected in at least 20% of samples. • Low prevalence of *B. catenulatum, B. dentium, B. adolescentis*, and *B. angulatum*	[41] (Japan)
1—18 mo	BF (7), FF (7)	DGGE, RISA	• Diversity of bifidobacterial species increased upon weaning • *B. bifidum* and *B. breve* were observed in both groups across timepoints. • BF had a more diverse bifidobacterial population and a higher prevalence of *B. longum* and *B. breve* • FF had a more stable *Bifidobacterium* profile across dietary phases	[42] (UK)
~1, 7 mo.	BF (7), FF (6); Solids introduced between 12–21 wks	DGGE, PCR, Dot blot analysis	• Shifts in bifidobacterial populations pre- and post-weaning differed among infants; no significant difference in bifidobacterial profiles between groups at either timepoint • *B. infantis* was the most prevalent species across both feeding groups. • *B. breve* not detected in FF infants • *Lactobacillus* was prevalent in <50% of infants and shifts pre- and post-weaning were variable among infants • *L. acidophilus* was the most common species of *Lactobacillus* • Composition and diversity did not significantly differ between groups pre- or post-weaning	[43] (Scotland, The Netherlands,)
d30	BF (31), MF (26), FF (11)	PCR, T-RFLP	• No differences in *Bifidobacterium* species between feeding groups. • The 3 most common strains were *B. longum* longum, *B. longum* infantis, and *B. breve* respectively.	[44] (Japan)
1, 2, 4 wk; Two, 6, 12 mo	BF until 4 mo; FF + introduction of solids (1). MF until 6 mo; introduced to solids at 4 mo. (1)	T-RFLP	• BF infant dominated by *Bacteroides* spp, *E. coli, Citrobacter, Veillonella* spp, and members of clostridia cluster at 1 wk • MF infant dominated by *E. coli* and *Bacteroides* at 1 wk • *Bacteroides* spp was dominant at all sample timepoints. • *Bacteroides* spp was dominant at all sample timepoints. • *Bifidobacterium* not detected during BF period but was detected during MF period. • Composition during the weaning period was different than during BF or MF. • Weaning followed by a ↑ in clostridia, ↓ in Enterobacteriaceae, and ↑ in overall diversity.	[8] (Sweden)

(*Continued on next page*)

Table 1. (Continued)

Sample Timepoint	Feeding Mode (n)	Method	Results	Reference (Country)
9 mo	Partially BF - 168 Weaned - 141	qPCR	• BF infants had lower levels of butyrate-producing bacteria • BF duration was positively correlated with *Lactobacillus* and *Bifidobacterium* and negatively correlated with *C. leptum*, *E. hallii, Roseburia* spp, *Bacteriodes/Prevotella* groups, *Bacteroides fragilis, Bacteroides vulgatus*, *Desulfovibrio* spp, and *A. muciniphila*. • BF was positively associated with greater abundance of *Lactobacillus* spp, *Bifidobacterium* spp, *and B. longum* and lower levels of *Delsulfovibrio* spp, *Akkermansia muciniphila*, and Bacteriodetes. • Intake of formula was negatively associated with abundance of *Lactobacillus* spp • Introduction of solids followed by ↓ in *Lactobacillus* spp and Enterobacteriaceae and a ↑ in Bacteroidetes. • Composition heavily influenced by the cessation of breastfeeding.	[45] (Denmark)
birth—12 mo (26 samples)	Diet was variable across infants and sample timepoints (14)	qPCR	• Each infant had a distinct microbial profile. • Infants were dominated by *Flexibacter, Ctyophaga*, and *Bacteroides*. • Infants were dominated by *Flexibacter, Ctyophaga*, and *Bacteroides*. • Bifidobacteria were not present for several months and only in minor amounts • Appearance of *Bacteroides* was largely variable among infants • Introduction to solid foods was followed by a more adult-like microbiota. • Composition at 1 y resembled an adult-like microbiota and was largely different than earlier timepoints.	[2] (US)
4 wks	BF (50), FF (50)	qPCR	• No significant difference in *Bifidobacterium* spp counts between groups • Counts and prevalence of *E. coli* and *C. difficile* were higher in FF infants.	[46] (The Netherlands)
1 mo	BF (200), MF (98), FF (232)	qPCR	• BF born by VD at home had highest counts of *Bifidobacterium* and lowest counts of *C. difficile* and *E. coli*. • FF & MF infants had higher counts and prevalence of *E. coli, C. difficile*, and *B. fragilis* • Lactobacilli counts were greater in FF than BF infants.	[16] (The Netherlands)
Meconium, d2, d7, d30, d90, d180, 1 wk post weaning	Diet was variable across infants and timepoints (108)	qPCR	• BF delivered by VD were most often colonized by *B. bifidum* and *L. gasseri.* • FF & MF more often colonized by *L. reuteri* compared with BF, CS-delivered infants • BF, CS-delivered infants reached *B. bifidum* levels more similar to BF, VD infants sooner than FF or MF, CS-delivered infants. • *Enterococcus, C. coccoides, Atopobium* cluster, *B. vulgatus, C. leptum* group and *B. longum* subsp. *longum* were more prevalent in MF & FF • Counts of *Staphylococcus* were higher in BF over the first 3 mo • BF had lower counts of *Bifidobacterium* at earlier timepoints. (May have been affected by some FF infants who received probiotic-supplemented formula) • BF for the first 3 mo. was associated with greater counts of *Bifidobacterium* and greater prevalence of *L. gasseri, L. casei*, and *B. adolescentis* at 6 mo • *C. perfringens* and *L. casei* were less prevalent in BF, but prevalence was more likely to increase over the first 3 mo. than in FF. • Prevalence of *C. leptum* group increased more over first 3 mos. in MF & FF	[47] (Belgium)

			• Introduction of solids was associated with a higher prevalence of *Atopobium* cluster, *C. coccoides, and B. longum* subsp *longum* • *Enterobacteriaceae* and *Staphylococcus* ↓ after introduction of solids but prevalence of infant-type *Bifidobacterium* did not change	
d21—30	BF (71), MF (77), FF (80), FF + Prebiotic (77)	qPCR	• BF infants had higher levels of lactobacilli and *S. aureus*. • *S. aureus* colonization rate was higher in infants BF and VD • Colonization of *S. aureus* ↑ at 60 d in FF + prebiotic group.	[48] (US)
0—6 mo (for BF/FF)	BF (7), FF (15), Weaned (18)	qPCR; Northern blot	• BF had higher proportions of *Bifidobacterium*; however, results were only significant if not age-matched. • qPCR showed greater proportions of *Bacteroides* and *Enterococcus faecalis* in BF.	[49] (Scotland)
d1—21 (7 samples)	BF (6), FF (6)	FISH; RAPD-PCR	• *Bifidobacterium* and *Escherichia coli* dominated in both groups. • BF characterized by streptococci and lactobacilli, while FF characterized by staphylococci and clostridia • Levels of lactic acid bacteria were higher in BF • FF had higher counts of clostridia and *Bacteroides* • FISH showed bifidobacteria and *Bacteroides* numbers to be approximately equal in FF infants. PCR showed much lower numbers.	[50] (The Netherlands)
d11—22 (BF) d14—36 (FF)	BF (6), FF (6)	FISH	• Both groups were dominated by *Bifidobacterium, Bacteroides*, and *Atopobium*. • *E. coli* colonization was similar across both feeding groups. • FF had higher levels of *Bacteroides* and *Atopobium* • BF had 2x the number of *Bifidobacterium* • *Streptococcus* and *Staphylococcus* were present at low levels (< 1%) in BF • Bifidobacterial populations were more stable in BF	[51] (Greece)
6 wk	BF (312), MF (112), FF (182)	FISH	• BF had lower proportions *C coccoides and Lactobacillus* than FF • Proportions of *Bacteroides* were lower in BF compared with both FF and MF • FF had lower levels of *C perfringens* compared with BF infants. • BF and MF infants had higher levels of bifidobacteria • No differences in levels of *C. difficile*	[52] (Italy, Spain, Scotland, Sweden, Germany)
6 wk, 4 wk post intro to solids	BF (358), MF (85), FF (162)	FISH-FC	• Pre-weaning feeding method impacted composition after introduction of solid foods. • Infants BF (pre-weaning) had greater post-weaning proportions of bifidobacteria and lower levels of *C. coccoides* and *Bacteroides* than FF (pre-weaning). • Post-weaning proportions of *C. coccoides* were greater in infants that were MF compared with BF pre-weaning. • Changes in *C. leptum, C. difficile*, and *C. perfringens* group, before and after solids foods, were slower in BF infants compared with FF and MF. • Introduction of solid foods correlated with ↑ in proportions of *C. leptum, C. coccoides* and ↓ in proportions of bifidobacteria, *C. difficile* + *C. perfringens*, and enterobacteria. • Bifidobacteria were more diverse post-weaning but remained dominant • Proportions of *Bacteroides* remained stable before and after weaning. • Greater post-weaning increase in *C. leptum* in infants who were previously FF. • The decrease in *C. difficile* + *C. perfringens* post-weaning was greater in those who had been FF compared with MF.	[18] (Italy, Spain, Scotland, Sweden, Germany)

(*Continued on next page*)

Table 1. (Continued)

Sample Timepoint	Feeding Mode (n)	Method	Results	Reference (Country)
12 wk	BF (31), FF (27)	FISH-FC	• BF had lower proportions of *Atopobium* cluster, *Lactobacillus, C leptum*, and *Streptococcus* groups • High levels of *Bifidobacterium* were associated with lower levels of Enterobacteriaceae in both groups and lower levels of *Streptococcus* in BF and *C. leptum* in FF infants • Proportions of *C. coccoides* were positively associated with proportions of *Atopobium* cluster in BF and *Streptococcus* and *C. perfringens* + *C. difficile* in FF. • In BF, lower levels of *Bacteroides* were associated with higher levels of *Lactobacillus*. • Higher levels of *Atopobium* cluster were associated with higher levels of *Bacteroides* in FF but lower levels of in BF	[53] (Spain)
6, 12 mo	BF (46), FF (50)	FISH	• At 6 mo, *Bifidobacterium* counts were greater in infants BF for the first 3 mo compared with those BF for less than 3 mos. • Infants still receiving breast milk at 6 mo had higher counts of *Bifidobacterium* and *Lactobacillus/Enterococcus.* • No differences in the counts of *Bifidobacterium, Lactobacillus, and Enterococcus* between groups at 12 mo.	[54] (Finland)
1—18 mo	BF (7), FF (7)	FISH, DGGE	• Similarity within and between the feeding groups was higher 10 weeks post-weaning • *Bifidobacterium* levels were higher in BF before and during the weaning period. • BF exhibited greater intraindividual changes, especially among *Clostridium*, throughout the study. • FF had higher levels of bacteria from *Clostridium* clusters throughout sampling.	[55] (UK)

Note. Abbreviations: BF, breastfed; FF, formula-fed; MF, mixed-fed (receiving both human milk and infant formula); EBF, exclusively breastfed; d, day; wk, week; mo, month; yr, year; VD, vaginal delivery; CS, cesarean section delivery; qPCR, real-time polymerase chain reaction; PCR, polymerase chain reaction; GM, goat's milk; CM, cow's milk; DGGE, denaturing gradient gel electrophoresis; TGGE, temperature gradient gel electrophoresis; RISA, *ribosomal intergenic spacer analysis*; T-RFLP, terminal restriction fragment length polymorphism; FISH, *fluorescence in situ hybridization;* RAPD, random amplified polymorphic DNA; FC, flow cytometry

Enterobacteriaceae,[1,27,56,57] which are thought to consume oxygen and prime the GI tract for colonization with obligate anaerobes such as *Bifidobacterium, Bacteroides*, and *Clostridium*. From the first wk of life, different colonization patterns are present in BF and FF infants.[26,49] The fecal microbiota of BF infants is more stable over time,[26,39] and characterized by a lower alpha-diversity compared to FF infants.[15,23,31,33] Exclusive breastfeeding is inversely related to alpha-diversity in 3 mo-old infants.[23] Conversely, exclusive breastfeeding and breastfeeding duration,[23] as well as predominant breastfeeding for the first 3 mos,[25] are positively related to alpha-diversity at 12 mos.

Many studies have reported that BF infants are colonized with higher proportions of microbes from the phylum Actinobacteria and less Firmicutes than FF infants.[23,25,26,28,33] Other studies report that BF infants have similar levels of Actinobacteria and Firmicutes[28,30] but lower proportions of Bacteroidetes[31,36] and Proteobacteria[26,28] compared to FF infants.

At the genus level, *Bifidobacterium* has long been described to predominate in BF versus FF infants[19]; however, results are varied. Several studies found bifidobacteria to be significantly greater in BF infants[18,23,26,28,33,36,37,39,47,51]; Fan and colleagues[26] reported roughly double the relative abundance of bifidobacteria in BF than FF infants. Bifidobacteria have been shown to account for 70% of the sequences of exclusively breastfed (EBF) infants[40] and appear earlier in the feces of EBF than in FF infants[38] In addition, infants who were exclusively breastfed in early life maintained greater colonization with bifidobacteria later in infancy.[54] Although this evidence is substantial, other findings suggest that *Bifidobacterium* exists in similar proportions[18,34] and numbers[44,46] in BF and FF infants and dominate the microbiota across both groups.[34,50,51] Conversely, Palmer and colleagues[2] reported very low levels of bifidobacteria in all infants with varied dietary patterns; however, these findings are likely due to the 8F universal primer that was used in the study having a three base pair mismatch against *B. longum*, and that *Bifidobacterium* genus in general does not have 100% sequence identity to the 8F primer sequence.[58] Infants harboring similar relative abundances of *Bifidobacterium* still exhibit some variance in diversity,[34,39,42] and stability[51] of populations at the species level. For example, *B. longum* and *B. breve* are the dominant species in BF infants,[27,41-43] whereas FF infants also harbor adult-associated bifidobacterial species, such as *B. adolescentis*.[24,39,42]

While many studies have focused on bifidobacterial colonization, breastfeeding supports other differences in microbiota composition. Within the phyla Bacteroidetes and Firmicutes, BF infants contain lower levels of *Bacteroides*,[33,50-53] *Atopobium*,[51,53] Clostridiales, *Lachnospiraceae*, and *Faecalibacterium* than FF.[33] Fan and colleagues[26] found that *Bifidobacterium, Streptococcus*, and *Lactobacillus* were dominant members in both feeding groups sampled between 3 and 6 mos; other dominant genera included *Clostridium* in BF infants and *Klebsiella* and *Enterococcus* in FF infants. Bergstrom and coworkers[45] reported a positive association between breastfeeding duration and levels of *Lactobacillus spp.*; however, effects of feeding on *Lactobacillus* abundance are inconsistent across other studies. Increased proportions of *Lactobacillus*,[25,26,48,54] as well as greater abundances of *L. gasseri*[28,47] in BF compared to FF infants have been reported, whereas other studies found opposite results[16,52,53] or reported intergroup variability and instability over time.[27,43]

A greater prevalence[15] and higher proportion[24] and total counts[16,46] of *C. difficile* in FF compared to BF infants, as well as, significantly more *Peptostreptococcaceae*,[31] *Akkermansia*,[15] *Veillonella*, and *Enterococcus*[28] have been described. *Escherichia* abundance was also higher in FF than BF infants[28] and decreased at a slower rate over time.[31] Furthermore, other results have indicated *E. coli* to be dominant across both groups[50] or present in similar total numbers,[51] while Penders et al.[16] found this species significantly increased in FF infants at 4 wks of age. *Bacteroides* abundance has been shown to increase over time in BF infants; however, the timing of initial colonization is variable among infants.[27] Jost et al.[27] also found that EBF infants who had higher proportions of and were colonized earlier with *Bacteroides* also harbored higher proportions of pathogenic bacteria, such as *Klebsiella* and *Clostridium*.

Few studies have investigated the fecal microbial composition of mixed-fed (MF) infants – those receiving both HM and infant formula.[29,52] The dietary patterns of MF infants are highly variable within and across studies, and are often not well described, which presents an obstacle when trying to draw conclusions relative to the effect of the proportion of HM and

formula consumed on microbial composition. At 6 wks of age, MF infants exhibited the largest within group compositional differences and harbored a microbiota significantly different than BF but not FF infants.[29] As further shown in Table 1, MF infants tend to group with FF infants and differ from BF infants.[16,18,47] Conversely, Fallani and associates[52] detected, in a much larger sample of infants, significantly higher proportions of bifidobacteria in MF compared to FF infants of the same age. Azad and colleagues[23] also found breastfeeding exclusivity, at 3 mos, to be associated with fecal microbiota composition at 3 mos and 1 yr of age, with MF infants exhibiting increased abundances of Bacteroidetes, Lachnospiraceae, Ruminococcaceae and Verrucomicrobia and decreased abundances of Enterobacteriaceae and Bifidobacteriaceae at 3 mos compared to EBF infants. Differences in Bifidobacteriaceae and Bacteroidetes abundance still remained at 1 yr of age; Veillonellaceae abundance was also increased in EBF infants. Mixed feeding is a common dietary pattern in early life, and these wide-ranging results emphasize the need for further exploration of the fecal microbiota of infants receiving both HM and formula. Additional studies investigating the long-term impacts of early feeding regimes on GI profiles beyond infancy are also necessary, as existing evidence has demonstrated that infants who were fed with HM for at least 50% of feedings during the first 3 mos of life maintained a different fecal microbial composition between 12 and 24 mos than those who had received less than 50% of feedings from HM.[54] In future studies, it will be important to carefully document the relative proportions of HM and formula the MF infant is consuming in addition to when the formula was introduced to the infant. For example, the microbiota of an infant who was mixed-fed from birth would likely differ from an infant who was exclusively breastfed from birth until 3 mos of age at which time formula was introduced.

Diet also interacts with other environmental factors and life events during infancy to affect GI colonization. Breastfeeding may compensate for factors shown to negatively impact the infant's GI microbiota. For example, infants delivered by Cesarean section (CS) had different fecal microbiota depending on if they had been breast- or formula-fed.[47] The BF infants achieved numbers of *B. bifidum* comparable to those of vaginally-delivered (VD) infants earlier than FF or MF infants. Findings have also shown that infant feeding likely moderates the effects of intrapartum antibiotic prophylaxis (IAP) exposure on microbial colonization (Table 1).[23] Regardless of feeding mode, IAP-exposed infants, delivered by emergency CS, presented with dybioses at 3 mos. Compared to infants who were not exposed to IAP, these infants had a lower abundance of Bacteroidetes and increased abundance of Firmicutes. These differences, between IAP-exposed and non-exposed, remained at 1 yr, but only in infants who were not exclusively breastfed at 3 mos. Results, at 1 yr, were significant when compared with VD infants not exposed to IAP and of either feeding type at 3 mos. These findings further support the notion that breastfeeding may help to reconcile imbalances in an infant's microbial composition resulting from adverse life events.

To date, few have described functional changes accompanying microbial variations between BF and FF infants. Some investigators have utilized metagenomics, an analysis of the microbial genetic material, in order to identify possible roles in various metabolic pathways and therefore functions of the microbes in a community.[20] Bäckhed and coworkers[24] used Kyoto Encyclopedia of Genes and Genomes (KEGG) orthology (KO) modules to identify functional differences, according to feeding pattern, in the stool microbiome of 4 mo-old infants. The FF infant microbiome was enriched in functions characteristic of a more mature microbiota (e.g. more similar to that of an adult) such as bile acid synthesis, methanogenesis, and the phosphotransferase system, while the fecal microbiome of BF infants was enriched in KO modules associated with synthesis of B vitamins and oxidative phosphorylation. In addition, virulence factors of the microbiota of BF and FF infants affect the expression of immunity and defense genes in the host GI epithelium,[30] potentially contributing to programming of the intestinal immunological tone. More recently, Bokulich and colleagues[25] showed maturational differences, measured by age-dependent stages characterized by specific taxa that serve as markers for the rate of microbiota development, in BF and FF infant microbiota. These assessments are of interest to researchers as delayed development is often linked to physiological perturbations. Over the first 6 mos of life, FF infants matured at a faster rate, compared to BF infants, as indicated by a lower abundance of several gene pathways involved in metabolism; however, between the ages of 12–24 mos, these FF infants exhibited decreased

microbiota maturation. These findings illustrate the potential impact of microbial composition to developmentally program host function and should direct the efforts of future research studies.

While it is clear that HM plays a substantial role in establishing the fecal microbiota, details regarding the interactions between HM components and infant microbes that contribute to compositional and functional differences among infants remain to be determined. Complex oligosaccharides and bacteria present in HM may contribute, but do not explain all compositional differences.

Human milk microbiota

The microbes in HM are hypothesized to be one of the contributors to the observed differences in the fecal microbiota of BF vs. FF infants. In the last two decades, HM, which was once thought to be sterile, has been shown to be a source of potentially probiotic bacteria for the infant.[59] Human milk contains bacterial genera also present in the infant fecal microbiota, including *Staphylococcus, Streptococcus, Lactobacillus*, and *Bifidobacterium*.[60-63] Hunt and coworkers[64] described a "core" HM microbiota comprised of nine OTUs that were present in all three samples collected from each mother and accounted for roughly half of the relative abundance of total bacteria in all HM samples. The remaining 50% consisted of several different genera, highlighting that each mother has a unique HM microbiota. These findings were further supported by a study showing that the HM microbiota composition was associated with mode of delivery, stage of lactation, and maternal health status.[65] Both Hunt et al.[64] and Cabrera-Rubio et al.[65] only detected few sequences of *Bifidobacterium* upon analysis of milk samples. However, other studies have identified this genus in over 90% of samples taken from mothers[61] and have found associations between levels of bifidobacteria in maternal milk and infant feces,[66] which may partly explain the increased levels of *Bifidobacterium* found in the feces of BF infants. Although taxonomic differences could be attributed to primers used for analysis, as mentioned previously, incomplete cell lysis during DNA isolation, due to the thick bacterial cell wall, has been recognized to influence the ability to detect bifidobacteria in samples. This may be another factor which led to varying results seen among milk, as well as fecal microbiota, and has prompted many researchers to include a bead-beating step to adequately lyse cells.[58] Further studies have also detected identical strains of *Lactobacillus, Bifidobacterium, and Staphylococcus* in both HM and infant feces, reaffirming HM as a potential source of probiotic and commensal bacteria involved in colonization of the immature infant GI tract.[61,67]

Culture-independent methods have detected more than 700 different bacterial species in milk, including obligate anaerobes and butyrate-producers.[59,65] However, the origin of the HM microbiota remains unclear. Previously, the bacteria present in HM were thought to be a result of contamination from the infant's oral cavity, the nipple and surrounding breast skin, as a result of backflow of milk into the mammary ducts during feeding.[64] While it is still hypothesized that breast skin may contribute a portion of the bacteria found in HM, notable differences found in bacterial phylotypes between HM and the breast skin of the same mother indicate additional sources for milk bacteria.[64] The presence of bacterial DNA in HM samples before breastfeeding initiation and the subsequent lack of typical neonate oral bacteria in HM after infants began to feed from the breast support a similar conclusion.[62] Identification of identical strains of bacteria in maternal feces, HM, and infant feces from the same mother-infant pair have led researchers to the concept of an "enteromammary pathway" through which bacteria translocate from the maternal GI tract to the mammary gland via dendritic cells.[59,68]

Jost et al.[59] proposed several possible functions of the HM microbes. Certain strains of bacteria previously isolated from HM produce bacteriocins and others prevent growth of various GI-associated pathogens, which could contribute to the decreased prevalence of illness and other health problems observed in BF vs. FF infants.[59] Bacterial genera identified in milk using molecular, but not culture-dependent, methods suggest that bacterial DNA in HM may correspond to dead bacteria. While there could be other reasons explaining this lack of identification with culture-dependent methods, including sample collection or culture techniques, this may be a result of the interaction of these bacteria with antimicrobial components in milk or of an out competition by other bacterial species.[59]

Further research on the HM microbiota is needed to reveal how they interact with other components found in HM and how these constituents work

together to facilitate healthy maturation of the infant GI microbiota.[69,70] Continued investigation of how the HM microbiota differs between mothers as well as the effects of transient exposure to certain bacteria, without colonization, will provide further insight into the complex and long-lasting functional differences witnessed between growing infants.[70]

Human milk oligosaccharides

Differences in the colonization patterns and microbial composition in BF vs. FF infants are proposed to be guided by complex sugars present in HM of each mother, known as human milk oligosaccharides (HMO). These sugars are the third largest component of HM and have been identified in over 200 distinct forms.[71] Abundance of HMOs decreases throughout lactation and maternal genetic differences determine both the composition and orientations of the 5 monosaccharides known to comprise these glycans – L-fucose, D-glucose, D-galactose, N-acetylglucosamine, and N-acetylneuraminic acid.[71,72] Being resistant to enzymatic hydrolysis, the HMOs pass intact through the infant stomach and upper GI tract to the distal small intestine and colon. HMOs exert prebiotic effects and inhibit the binding of pathogenic bacteria, thereby modulating the infant immune system and shaping the composition of the resident GI microbes.[72,73]

Of particular interest to researchers is the ability of *Bifidobacterium* to metabolize HMOs. Certain HMO show bifidogenic effects *in vitro*,[72,74] selectively stimulating the growth of certain commensal bacteria often identified in BF infants, such as *B. infantis, B. bifidum, B. breve* and *B. longum*.[34,42] Additionally, HMOs also serve as a carbon source for various species of *Bacteroides* common to the infant GI tract, but do not function as a substrate for other members of the Firmicutes phylum or *Bifidobacterium* species primarily present in adults, such as *B. adolescentis* and *B. animalis;* these microbes lack the enzymatic capacity to break down and consume HMOs.[59,72,75] A recent study published by Wang et al.[17] revealed both positive and negative associations between multiple HMOs and the relative abundance of bacterial genera, suggesting that HMO profiles can predict infant fecal microbial composition. Certain species, namely *B. infantis*, exhibit the ability to grow in the presence of a wide range of HMOs, while other species of *Bifidobacterium* and *Bacteroides* metabolize fewer structural types.[17,72] Moreover, Wang et al.[17] hypothesize that the inverse relationship detected between *Bifidobacterium* and *Bacteroides*, in their study as well as by other earlier investigators,[27,53] may be due to the ability of different species to thrive in the presence of particular HMOs. Therefore, differences in selectivity for certain HMOs likely contribute to variance among species of *Bifidobacterium* and *Bacteroides* isolated from feces of BF infants. Lewis et al.[76] showed that bifidobacterial colonization was delayed in infants consuming HM from non-secretor mothers that is devoid of a particular type of HMO, 2′-fucosyllactose (2′FL), further supporting this theory regarding a possible origin for variations in *Bifidobacterium* observed among BF infants. This further leads to the question of how these HMOs interact with commensal bacteria residing in HM, and in turn, how this complex network of HM components functions in the näive infant GI tract. Lastly, HMOs have been implicated in upregulating the expression of genes related to carbohydrate utilization,[77] effects which may continue to be important later in infancy.[26]

Weaning, complementary feeding, and introduction of solids

The impact of the introduction of solids and weaning has been understudied in regards to their effects on infant microbiota composition and function. The transition toward a more adult-like microbiota is observed during the introduction of solids foods[1,2,35]; however, more recent studies suggest that this shift toward a more mature microbiota is more likely attributed to the cessation of breastfeeding, rather than the introduction of complementary foods.[24,45] Additional findings demonstrate that pre-weaning feeding mode remains associated with the post-weaning microbial communities.[18,33,55] For example, continued breastfeeding during the introduction of solid foods seems to support consistent levels of *Lactobacillus* spp. and *Bifidobacterium* spp.[24,40,45,47,55] (Table 1).

Thompson et al.[33] found that EBF infants and infants receiving HM and solid foods (EBF + S) clustered closer together than EBF and non-EBF infants. The addition of solid foods also appeared to have a more dramatic impact on the fecal microbiota of non-EBF infants. While there were marked differences between the EBF and EBF + S groups, the separation

between clusters of non-EBF and non-EBF + S groups was more distinct. The lack of any significant microbiota changes accompanying the introduction of complementary foods reported in BF infants by some groups[22,43] further supports the impact of HM withdrawal. Others have found that infants still receiving HM at 9 mos harbored lower levels of butyrate-producing bacteria, *C. leptum, C. coccoides*, and *Roseburia*, as well as Bacteroidetes[45] which have been previously noted to increase when infants begin to consume solid foods.[1,20] Infants still partially breastfed at 9 mos, compared to those already weaned, also had an increased relative abundance of *Bifidobacterium* spp. and *Lactobacillus* spp.[45] Furthermore, in the cohort studied by Bäckhed et al.,[24] it was not until infants stopped breastfeeding that their microbiota showed the functional capacity to degrade polysaccharides, which is characteristic of a shift toward a more adult-like microbiota. Taken together, these findings have led to the idea that it is the withdrawal of HM that triggers the compositional maturation of the infant GI tract, marked by an increase in members of the Firmicutes and Bacteroidetes phyla, with some studies noting specific increases in *Clostridium, Roseburia, Bacteroides, Bilophila, and Anaerostipes* in feces.[1,24,33,45,47] Valles et al.,[35] who proposed that colonization can be separated into 2 phases, before and after the introduction of solid foods, still found *Bifidobacterium* and *Bacteroides* to be dominant in samples taken before and after all infants had begun to eat solid foods, which could be due to the fact that 70% of infants were still being breastfed when the second sample was taken.

The introduction of solid foods has been associated with increases in Bacteroidetes and Firmicutes phyla. As outlined in Table 1, several changes at the genus level have been reported, with some common findings including increases in *Atopobium, Clostridium, Akkermansia, Bacteroides*, and *Ruminococcus* and decreases in *Escherichia* and *Staphylococcus*.[33,35] Thompson et al.[33] also found that all infants, despite pre-weaning feeding type, had a microbiota dominated by Actinobacteria, Firmicutes, and Bacteroidetes, respectively, after the introduction of solids. However, as previously described, whether or not the infants had been previously breastfed or mixed- or formula-fed did appear to have an impact at the family and genus levels, both during complementary feeding and weaning periods. In EBF infants, introduction of solids was associated with increases in *Eggerthella, Blautia, Neisseria, Peptostreptococcaceae* and Bacteroidetes and led to higher post-weaning abundances of *Lactobacillus* and *Ruminococcaceae*.[33] Both FF and MF infants experienced significant increases in Actinobacteria, specifically *Bifidobacterium, Ruminococcaceae*, and *Blautia* at the start of complementary feeding and showed greater abundances of *Faecalibacterium, Eubacterium*, and *Blautia* post-weaning compared to infants who had been previously exclusively breastfed.[33] Fallani et al.[18] found similar results, showing that feeding mode at 6 wks was associated with long-term differences in microbial composition. Those who were breastfed at 6 wks had higher proportions of fecal *Bifidobacterium* and lower proportions of *Bacteroides* and *C. coccoides* at 4 wks post-weaning compared to infants receiving any formula. Those previously formula-fed also had a greater increase in *C. leptum* post-weaning than BF infants.[18] *Bifidobacterium* populations remain relatively consistent throughout the introduction of solids[18,35,40,42]; however, some infants experience an increase in diversity at the species level throughout complementary feeding and weaning.[18,40,42]

Beyond compositional shifts, perhaps the most important outcomes of these dietary modifications are the functional changes that accompany complementary feeding and weaning as infants are exposed to several metabolic substrates for the first time, which promote microbial growth that was not supported by HM and/or formula.[33] Bäckhed et al.[24] noted an increase in genes involved in carbohydrate and pyruvate metabolism at 12 mos, supporting new ways of obtaining energy from the increasing percentage of solid foods. Additionally, an increase in short chain fatty acid (SCFA)-producing species was observed in accordance with an elevated intake of starch and plant fibers,[1,35] and an increased expression of genes involved in amino acid metabolism and vitamin biosynthesis followed complementary feeding and weaning.[1,33] Analysis of 16s sequencing data using PICRUSt (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States) to infer metabolic effects associated with the fecal microbial community upon the introduction of solid foods, further revealed that these changes also differ among infants receiving HM or formula prior to solid foods.[33] An increase in a variety of genes associated with metabolism pathways, for example, biosynthesis of secondary metabolites and energy metabolism, was observed in

the microbiota of EBF infants. In contrast, the introduction of solids coincided with enriched expression of cellular process pathways, such as genetic information processing and immune system diseases, in the microbiota of MF and FF infants. Overall, infants who were not exclusively breastfed experienced significantly more functional changes after complementary feeding began.[33] These researchers hypothesized that the consistently changing composition of HM could explain why functional differences were less dramatic in BF infants, and that some of the differentially over-represented genes in non-EBF infants may help to explain the protective effect of breastfeeding against certain diseases.[33]

Prebiotics and probiotics

As noted above, human milk contains microbes and a diverse complement of oligosaccharides. Infant formulas are sterilized, killing any live microbes. In addition, bovine milk, the base of most infant formula, contains only trace amounts of less complex oligosaccharides.[71] Thus, in an attempt to narrow the compositional gap between human milk and infant formula, prebiotics and probiotics have been supplemented to formula and functional outcomes have been assessed.

Prebiotics

The prebiotic concept was introduced by Glenn Gibson and Marcel Roberfroid, who defined a prebiotic as 'a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon and thus improves host health'.[78] Since then, the definition has been refined several times.[79-82] Most recently, Bindels and colleagues[83] proposed a new definition for prebiotics as 'a nondigestible compound that, through its metabolization by microorganisms in the gut, modulates composition and/or activity of the gut microbiota, thus conferring a beneficial physiological effect on the host'. Most previous definitions required the prebiotic to be selective for stimulation of growth and/or activities of health-promoting bacteria, while the new definition removed the selectivity criterion and places more emphasis on the causal link between the microbial metabolism of the compound, the resulting modulation of the GI microbe and the beneficial physiology effects.[83]

Several *in vitro* studies have shown that HMOs serve as the primary substrate for the growth of *Bifidobacterium* and other bacteria.[84,85] Furthermore, we[17] and others[86,87] have demonstrated correlations between several bacterial genera present in BF infant feces, with the HMO in their mother's milk[17] or present in the infant feces.[86,87] Until recently, HMOs have not been commercially available in large quantities; therefore, other prebiotics that share some of the functional attributes of HMOs are currently supplemented to infant formula.

The most studied prebiotic addition to infant formula is a 9:1 mixture of short-chain galactooligosaccharides (scGOS) and long-chain fructooligosaccharides (lcFOS). Investigators have identified significant clinical impacts of these prebiotics on the immune and metabolic development of infants. Infants who consume formula supplemented with this mixture have a lower fecal pH and exhibit a fecal SCFA profile and stool characteristics comparable to that of BF infants. Findings have also shown that infants exposed to scGOS and lcFOS to require fewer doses of antibiotics and have a decreased incidence of infection likely due to an increased colonization resistance to enteric pathogens.[88-90] The other prebiotics added to infant formula individually or in combination include, GOS, FOS, oligofructose and inulin, polydextrose (PDX), lactulose (LOS) and acidic oligosaccharides (AOS) (Table 2). Prebiotics are resistant to gastric acidity and enzymatic hydrolysis in the upper gastrointestinal tract and enter the colon intact, where they are metabolized by colonic microbiota.[78] Short-chain prebiotics, such as FOS and GOS, are mainly fermented in the ascending colon, while longer-chain prebiotics, like PDX and inulin, are fermented along the entire colon.[114]

Clinical studies show that prebiotics modulate the composition of the infant fecal microbiota (Table 2, [88,90]). Most studies showed that supplementation of prebiotics to infant formula increased the numbers of beneficial bacteria, mainly *Bifidobacterium* and sometimes *Lactobacillus* (Table 2, [88,90]). Some studies also demonstrated a decrease in the levels of opportunistic pathogens, such as *Escherichia coli, Enterococcus* and clostridia with prebiotic supplementation.[92,97,103] As 'selectivity' was previously considered as a key characteristic of prebiotics, many studies focused on documenting changes in the abundance of specific groups/species of bacteria, such as *Bifidobacterium* and *Lactobacillus*, using traditional plate counting or by

Table 2. Impact of prebiotic supplementation on the composition of infant microbiota.

Prebiotic (dose) Source	Nutrition base	Subject (feeding duration)	Study group (n)	Major effect of the prebiotic	References (Country)
GOS (2.4 g/L) Frisolac Advanced, Friesland Nutrition, Netherlands	Infant formula	Healthy term infant < 28 d (3 mo)	GOS (20) Control (18) HM reference (15) GOS + HM (29)	• ↑ counts of bifidobacteria and lactobacilli • ↑ acetic acid level and stool frequency • ↓ fecal pH	[91] (China)
GOS (4 g/L) Vivinal GOS; Friesland Foods Domo, Amersfoort, The Netherlands	Infant formula	Healthy term infants at 15 d (up to 70 d of age)	GOS (83) Control (80) HM reference (199)	• ↓ *Clostridium* count	[92] (Italy)
GOS (4.4 g/L) Source not reported	Infant formula	Healthy term infants < 8 wk (until 4 mo of age), microbiota were analyzed at 4 mo of age	GOS (40) Control (29)	• ↑ *Bifidobacterium* count • ↑ proportion of acetate • ↑ stool frequency and softness • ↓ fecal pH, proportions of propionate and butyrate	[93] (Spain)
GOS (2 g/L) Source not reported	Infant formula	Healthy term infants	GOS (43) Control (17) HM reference (20)	• ↑ counts of bifidobacteria and lactobacilli	[94] (Not reported)
GOS (3 g/L) OM55N; Yakult Pharmaceutical Industry, Tokyo, Japan	Infant formula	Healthy term infants at 31–54 d (2 wk)	GOS (9) Control (13)	• ↑ *Bifidobacterium* abundance • ↓ Shannon index	[95] (Japan)
FOS (1.5 or 3 g/L) Raftilose®P95; Orafti, Tienen, Belgium	Infant formula	Healthy term infants at 2–6 wk (1wk)	FOS 1.5 and FOS 3 as crossover (58) HM reference (14)	• The counts of *Bifidobacterium, Lactobacillus* and clostridia were similar among the 2 FOS groups • FOS 3 had softer stool than FOS 1.5.	[96] (US)
FOS (4 g/L) Source not reported	Infant formula	Preterm infants at 0–14 d (14 d), microbiota were analyzed at study d 7	FOS (36) Maltodextrins (20)	• ↑ numbers of bifidobacteria and bacteroides • ↑ stool frequency • ↓ counts of *E. coli* and enterococci	[97] (Greece)
scFOS (4 g/L) Acilight 950P; Beghin Meiji, Marckolsheim, France	Infant formula Maltodextrins (15)	Healthy term infants at 0–7 d (4 mo)	FOS (18)	• Greater increase in *Bifidobacterium* counts after 2 mo feeding	[98] (France)
OF (3 g/L) OraftiR P95; BENEO-Orafti	Infant formula	Health term infants at 5–14 d (8 wk)	OF (20) Control (19) HM reference (23)	• Greater increase in *Bifidobacterium* • Softer stool	[99] (US)
FOS (2 or 3 g/ L) Source not reported	Infant formula	Healthy term infants at 0–6 d (4 wk)	FOS 2 (14) FOS 3 (20) Control (14) HM reference (17)	• No effects on population of bifidobacteria, lactobacilli, *C. difficile, E. coli* or *Bacteroides*.	[100] (US)

(*Continued on next page*)

Table 2. (Continued)

Prebiotic (dose) Source	Nutrition base	Subject (feeding duration)	Study group (n)	Major effect of the prebiotic	References (Country)
FOS (4.5 g/L) (OF and inulin 70:30 ratio) Raftilose P95 and Raftiline in 70:30 ratio, Orafti	Growing –up Formula	Infants with antibiotic treatment at 1–2 y (3 wk)	FOS (57) Control (56)	• ↑counts of bifidobacteria after 1 wk • The other bacteria were unaffected.	[101] (Chile)
FOS (0.75 g/25 g cereal) Source not reported	Cereal	Infants at 4–11 mos (28 d)	FOS (27) Maltodextrin (29)	• ↑ stool frequency and softness	[102] (France)
OF (2 g/ d) Beneo P95; Orafti	Cereal	Healthy children at 6–24 months (21 d)	OF (10) Maltodextrin (12)	• ↓ *Clostridium* levels • Trend for increase of *Bifidobacterium*	[103] (US)
OF/Inulin (8 g/L, 1:1 ratio) Synergy 1; BENEO-Orafti	Infant formula	Healthy term newborn (until 4 months of age)	OF/inulin(63) Control (68) HM as reference (57)	• ↑ *Bifidobacterium* counts • Softer stools and a higher deposition frequency	[104] (Spain)
OF/inulin (4 or 8 g/L, 1:1 ratio) Synergy 1; BENEO-Orafti	Infant formula	Healthy term infants ≤ 5 d (28d)	OF/inulin 4 (21) OF/inulin 8 (20) Control (21) scGOS+lcFOS (19) HM as reference (29)	• Greater increase in *Bifidobacterium* counts with 8 g/L of OF/inulin • Softer stool	[105] (Belgium)
Inulin, 1.5 g/d Frutafit IQ, Roosendaal, Netherlands	Infant formula	Healthy infants at 5–24 wk (3wk)	Inulin and Control as crossover (14)	• ↑ count s of *Bifidobacterium* and *Lactobacillus*	[106] (Korea)
Inulin (0.75, 1.00 or 1.25 g/d) Frutafit IQ	Follow-up formula	Healthy infants at 5–12 mo (14d)	Inulin 0.75 (10) Inulin 1 (9) Inulin 1.25 (9) Control (8)	• ↓ clostridia counts and fecal pH • ↑ bifidobacteria numbers with 1.25 g/d inulin	[107] (Malaysia)
LOS (5 or 10 g/L) Produced by alkaline isomerization of lactose	Infant formula	Healthy infants at 2–10 wk (3 wk)	LOS 5, LOS 10 and Control as crossover (6)	• ↑ *Bifidobacterium* counts • ↓ fecal pH	[108] (Not Reported)
PDX/GOS (4 g/L, 1:1 ratio) PDX/GOS/LOS (8 g/L. 50:33:17 ratio) Source not reported	Infant formula	Healthy term infants at d14 (until 120 d of age)	PDX/GOS (58) PDX/GOS/LOS(48) Control (58)	• Softer stool	[109] (US)
PDX/ GOS (4 g/L, 1:1 ratio) Litesse Two PDX; Danisco, Beaminster, UK) and Vivinal GOS;Friesland Foods Domo, Zwolle, The Netherlands	Infant formula	Healthy term infants at 21–30 d (60 d)	GOS/PDX (78) Control (81) HM as reference (71)	• ↑ *Bifidobacterium* counts • Softer stool	[110] (US)

PDX/GOS (4 g/L; 1:1 ratio) PDX/GOS/LOS (4 or 8 g/L, 3:2:1 ratio) Litesse Two PDX; Danisco, Copenhagen, Denmark; and Vivinal GOS; Friesland Foods Domo, Zwolle, The Netherlands and Morinage Milk Industry, Tokyo, Japan	Infant formula	Healthy term infants at 13–92 d (28 d)	PDX/GOS (23) PDX/GOS/LOS 4 (23) PDX/GOS/LOS 8 (21) Control (21) HM as reference (29)	• No impact on the bacterial composition were observed	[111] (US)
AOS(2 g/L) AOS/GOS/FOS (2 g/L, 25:67.5:7.5 ratio) AOS from Richter Pharma, Wels, Austria; the source for GOS/FOS are unknown	Infant formula	Healthy term newborns (6 wk)	AOS (14) AOS/GOS/FOS (13) Maltodextrin (14)	• ↓ stool pH; softer stool • AOS/GOS/FOS also ↑ *Bifidobacterium* and *Lactobacillus* counts and stool frequency • No effect on the numbers of Clostridia, *E.coli, Proteus, Klebsiella, Pseudomonas, Enterobacter, Citrobacter*, Enterococci	[112] (Italy)
AOS/scGOS/lcFOS/ (1.5 g/kg/d, 20:72:8 ratio) Danone Research, Friedrichsdorf, Germany	Enteral supplementation	Preterm infants < 2d (until 30 d of age)	scGOS/lcFOS/AOS (55) maltodextrin (58)	• ↑ total bacteria count at d14 • ↓ fecal pH	[113] (Netherlands)

Note. AOS, acidic oligosaccharides; *E. coli, Escherichia coli*; FOS, fructooligosaccharide, GOS, galactooligosaccharide; OF, Oligofructose; LOS, lactulose, OF; PDX, polydextrose

molecular methods, such as real-time quantitative PCR and fluorescence *in situ* hybridization.[91-94,96,99] Fewer studies have assessed changes in overall fecal microbial communities by next-generation sequencing or microarray approaches.[95] With the new prebiotic definition,[83] in the future, research should shift from a focus on selective microbial group/species toward ecological and functional characteristics of the GI microbiota and on defining the mechanisms whereby any health benefits of prebiotics are achieved.

Short-chain fatty acids (SCFAs), the fermentation products of anaerobic bacteria, are commonly used to reflect the metabolic activity of the intestinal microbiota. While the role of SCFAs in the development of the infant GI tract are still being investigated, they are known to be absorbed as a source of metabolizable energy while also exerting anti-inflammatory effects on the colonic epithelium and contributing to defense against gut-associated pathogens. Additionally, SCFAs are involved in enteroendocrine hormone regulation and may be involved in de novo lipid synthesis.[90] The fecal SCFA profile represents events that occur in the colon and is closely related to the composition of intestinal microbiota.[93] For example, a positive correlation has been reported between the abundance of *Bifidobacterium* and fecal acetate level; while high propionate and butyrate content in the infant feces may imply the presence of a more complex microbiota, as propionate and butyrate, among others, are produced by the members of *Bacteroides* and *Clostridium*.[93] Several studies have investigated the effect of prebiotics on infant fecal SCFA content (Table 2). In a randomized, double blind, placebo-controlled study, infants who were fed formula with added scGOS/lcFOS (8 g/L) for 6 wks had an increase in proportion of acetate and a decrease in proportion of propionate compared to infants fed control formula, which represented a fermentation profile similar to that of BF infants.[115] Similarly, a higher proportion of acetate and lower proportions of propionate and butyrate were observed in infants fed formula containing GOS (4.4 g/L) for 2 mos compared with infants fed control formula.[93] Thus, supplementation of certain prebiotics to infant formula could modulate the fecal SCFA content to a level as human milk does.

The SCFAs are the principal luminal anions in humans and an increase in their concentrations reduces luminal pH, resulting in more acidic stools, which in turn leads to a mild laxative effect with increased stool frequency and softness.[116,117] BF infants generally have lower fecal pH and softer and more frequent stools compared to FF infants[91,92,118]; therefore, several clinical studies have evaluated the influence of prebiotics on stool characteristics of infants (Table 2). Infants fed formula supplemented with GOS (2.4 or 4.4 g/L) had lower fecal pH, as seen in BF infants, in comparison with infants fed non-supplemented formula.[91,93] Similar patterns were also observed when inulin, LOS, AOS, scGOS/lcFOS or scFOS/lcFOS/AOS was administrated to FF infants.[107,108,112,113] The effect of prebiotics on stool frequency and consistency has been evaluated in a systematic review.[117] Four randomized controlled trials reported that the stool frequency of infants fed prebiotics was similar to that of BF infants and was higher than that of infants fed control formula.[117] In addition, six studies assessed the stool consistency after prebiotic supplementation, and all reported that the stools were softer in infants consuming prebiotics.[117] Taken together, the addition of prebiotics to infant formula could decrease stool pH and increase stool frequency and softness, making the stool characteristics of FF infants resemble those of BF infants.

Probiotics

Probiotics are "live microorganisms which, when consumed in adequate amounts, confer a health benefit on the host."[119] Most studies conducted with probiotics in pediatrics were carried out with strains of *Bifidobacterium* and *Lactobacillus* isolated from the human GI tract or dairy products (Table 3). A few studies used bacteria isolated from HM.[138,139] Several reasons have been used to support probiotic use in infant formula. Human milk, as previously described, contains a variety of potential probiotic bacteria, such as *Bifidobacterium, Lactobacillus* and *Streptococcus*, and these bacteria serve as a source of continuous inoculum to the BF infant GI tract, which may partly contribute to differences in the fecal microbial composition between BF and FF infants.[62,64] Furthermore, administration of specific probiotic bacteria has been shown to improve infant health, including shortening the duration of rotavirus diarrhea,[140] preventing of antibiotic-associated diarrhea,[141] reducing the incidence of eczema in high-risk children[142] and decreasing the risk of necrotizing enterocolitis in very low birth weight infants.[143] Thus, supplementation of probiotics to infant formula has been promoted as an approach to improve infant

Table 3. Impact of probiotic supplementation on the composition of infant microbiota.

Probiotic strain (dose)	Nutrition base	Subject (feeding duration)*	Study groups (n)	Major effect of the probiotic	References (Country)
L. johnsonii La1 (10^8CFU/g)	Infant formula	Healthy term infants at 4 mo (13 wk)	Probiotic (48) FOS (44) Control (61) BF as reference (46)	• ↑ fecal *Lactobacillus* count	[120] (Chile)
LGG (4.5 –8.5 × 10^7 CFU/g)	Hydrolyzed casein formula	Infant with cow's milk allergy at 1–12 mo (6 mo)	Probiotic (12) Control (7) Healthy infants (20)	• ↑ proportions of *Roseburia* and *Anaerofustis* • ↑ fecal butyrate level	[121] (Italy)
L. rhamnosus LPR + *B. longum* BL999 or *B. longum* BL999, (10^9 CFU/strain/d)	Hydrolyzed formula	Infants with familial atopic background >1 mo (4 mo), fecal microbiota were analyzed at 6 mo of age	LPR + BL999 (24) BL999 (25) Control (32) HM as reference (8)	• No effect on the proportions of *Bifidobacterium*, *Bacteroides-Prevotella*, *C. histolyticum*, *Lactobacilluse-Enterococcus* or *A. muciniphila* • ↓ colonization rate of *B. bifidum* in BL999	[122] (Germany)
L. acidophilus NCFM or *B. lactis* Bi-07 (10^{10}CFU/d)	In a capsule	Children with atopic dermatitis at 7–24 mo (8 wk)	NCFM (17) Bi-07 (17) Control (16)	• No effect on the composition and diversity of the main bacterial populations • ↑ numbers of *Lactobacillus* in NCFM	[123] (Denmark)
LGG + *B. longum* BB536 (2 × 10^7 and 10^7CFU/g, respectively)	Infant formula	Newborn infant at high risk of allergy (6 mo)	Probiotic (17) Control (20)	• The proportions of the major bacterial groups were similar • Bacterial colonization pattern was unaffected by probiotics • ↑ detection rates of *B. longum* and *L. rhamnosus* • ↑ lactic acid bacteria counts	[124] (Singapore)
L. fermentum CECT5716 (10^7CFU/g)	Infant formula	Healthy infants at 1 mos (5 mo), fecal samples were analyzed at 3 y of age	Probiotic (61) Control (60)	• No effect on the fecal counts of lactobacilli, bifidobacteria, clostridia and *Bacteroidaceae*	[125] (Spain)
B. lactis LKM512 (6 × 10^9 CFU/d)	Oral feeding	Infants required surgery ≤ 3 d (until 15 d of age)	LKM+ (4) LKM- (4) Control (no surgery, 5)	• ↑ *Streptococcaceae* in LKM+ • ↓ *Bifidobacteriaceae* in LKM+	[126] (Japan)
L. reuteri DSM 17938 (1.2 ×10^9 CFU/L)	Infant formula	Healthy VD or CD term infants ≤ 3d (6 mo)	CSLr (11) VDLr (9) CSCt(10) VDCt (10)	<u>VD infants</u> • No effect on overall microbiota composition • ↑ Proportions of *Lactobacillus* at 2wk and 4 mo. • ↑ Abundance of *Coprococcus* at 4 mo <u>CS infants</u> • ↑ Proportion of Actinobacteria toward VD infants at 2 wk • ↓ unclassified *Enterobacteriaceae* toward VD infants at 2 wk • ↑ Proportions of *Lactobacillus* at 2 wk and 4 mo	[127] (Greece)
B. longum BB536 (10^7 CFU/g)	Infant formula	Healthy term infants at 0–7 d (12 mo)	Probiotic (135) Control (129)	• ↑ bifidobacteria level at 2 and 4 mo	[128] (China)

(*Continued on next page*)

Table 3. (Continued)

Probiotic strain (dose)	Nutrition base	Subject (feeding duration)*	Study groups (n)	Major effect of the probiotic	References (Country)
L. acidophilus LAVRI-A1 (3×10^9CFU/d)	Powder in water	Health term newborn at high risk of allergy (6 mo)	Probiotic (89) Maltodextrin (89)	• ↑ rate of *Lactobacillus* colonization	[129] (Australia)
L. reuteri DSM 17938 (10^8 CFU/d)	Oil drop	BF colicky infants at 10–60 d (21d)	Probiotic (15) Control (14)	• No effect on the overall composition of the microbiota	[130] (Italy)
B. breve M-16V (5×10^8 CFU/d) or *B. breve* M-16V + *B. infantis* M-63 + *B. longum* BB536 (5×10^8CFU/strain/d)	Powder in water	Low birth weight infants ≤ 7 d (6 wk)	M-16V (15) M-16V+M-63+BB536 (13) Control (16)	• ↑ detection rate and numbers of *Bifidobacterium* • ↓ detection rate of *C. perfringens* • ↓ proportion of *Enterobacteriaceae* in M-16V+M-63+BB536	[131] (Japan)
LGG (6×10^9 CFU/d)	Preterm infant formula	Preterm infants at 0–3 d (42 d)	Probiotic (21) Maltodextrins (26)	• ↑ colonization rates of *Lactobacillus*, *Enterobactericeae*, *Enterococcus* and staphylococci	[132] (Poland)
B. lactis Bb12($1.6–4.8 \times 10^9$ CFU/d)	Infant formula	Preterm infants at birth (21d)	Probiotic (37) Control (32)	• ↑ numbers of *Bifidobacterium* • ↓ counts of *Enterobacteriaceae* and *Clostridium* spp	[133] (Germany)
B. longum BB536 + LGG (4×10^8 CFU/d)	Powder in wate	Preterm infants when enteral feeding started (until discharge)	Probiotic (45) Maltodextrin (49)	• No alteration in the composition of intestinal microbiota, except for increase in the colonization rates of *Bifidobacterium* and *Lactobacillus*	[134] (France)
LGG (1.8×10^{10} CFU/d)	In a capsule	Mothers of infants at high risk of allergy at 36 wk gestation (until delivery), infant microbiota were followed until 180 d of age	Probiotic (59) Maltodextrin (57)	• ↑ prevalance of *B. longum* group at 90 d • Concentration of *Bifidobacterium* was similar at any time	[135] (Australia)
B. bifidum W23 + *B. lactis* W52+ *L. lactis* W58 (10^9 CFU/d/strain)	Powder	Mothers of infants at high risk of allergy (6 wk before delivery); infants at birth (1y), infant microbiota were followed up until 6 y	Probiotic (15–26) Maltodextrin (9–12)	• Bacterial composition and a diversity were similar during first 6 y, except relative abundances and Shannon indices of Bacteroidetes and Proteobacteria were lower at 2 wk	[136] (Netherlands)
L. rhamnosus LPR + *B. longum* BL999 or *L. paracasei* ST11+ *B. longum* BL999 (10^9 CFU/strain/d)	Powder in Water	Mothers of infants (2 mo before and 2 mo after delivery during breast feeding), infant microbiota were analyzed at 6 mo of age	LPR + BL999 (28) ST11 + BL999 (29) Control (22)	• ↑ percentage of *Lactobacillus-Enterococcus* and ↓*Bifidobacterium* counts in LPR + BL999 at 6 mos • ↓ colonization rate of *B. infantis* in LPR + BL999 • ↓ colonization rate of *B. longum* in STII + BL999	[122] (Finland)
LGG (1.8×10^{10} CFU/d)	Mothers: capsule; BF infants:powder in water	Mother of infants with high risk of atopy (2–4 wks before delivery); BF mothers or FF infants after delivery (6 mos), infant microbiota were analyzed at 6 and 24 mo of age	Probiotic (46) Control(47)	At 6 mos · No effect on the counts of *Bifidobacterium*, *Bacteroides*, *Lactobacillus/Enterococcus* and total bacteria • ↑ clostridia At 24 mo • ↓ *Lactobacillus/Enterococcus* • ↓ clostridia • No effect on *Bifidobacterium*, *Bacteroides* and total bacteria counts	[137] (Finland)

Note. AD, atopic dermatitis; BF, breastfed ; CS, cesarean section; CFU, colony-forming units; Ct, Control; FF, formula-fed; LGG, *L. rhamnosus* GG; L. *lactis, Lactococcus lactis*; VD, vaginal delivered. The information before the parentheses indicates the time point, or age of the infant, at which the probiotic treatment began. The information presented in the parentheses is the duration for which the probiotic was administered to the infant or mother. Unless otherwise stated, fecal samples analyzed, were those collected after the completion of the probiotic administration.

health and to mimic the probiotic property of human milk. Although the mechanisms by which the probiotic strains exert their positive impact on infant health are unclear, it could be related to their direct interaction with the host or indirect through their modulation of the GI microbiota.

Clinical evidence has shown that administration of probiotics to pregnant women and nursing mothers can influence the establishment and composition of infant fecal microbiota (Table 3). Supplementation of *L. rhamnosus* GG to mothers of infants at high risk of allergy during late pregnancy has been shown to increase the prevalence of *B. longum* group in their infants at 3 mos of age.[135] A Finnish study investigated the effect of probiotic administration (*L. rhamnosus* LPR + *B. longum* BL999) to mothers for the 2 mos prior to and 2 mos after delivery. At 6 mos of age, BF infants whose mothers who received the probiotic treatment had higher percentages of *Lactobacillus-Enterococcus* and lower levels of *Bifidobacterium* compared to infants whose mothers received a placebo control.[122]

Probiotics have also been administrated directly to infants; however, their effects on intestinal microbiota are contradictory (Table 3). For example, Mohan and coworkers[133] evaluated supplementation for modifying the GI microbiota of preterm infants. They found that the numbers of *Bifidobacterium* were higher and the counts of *Enterobacteriaceae* and *Clostridium* spp. were lower in *B. lactis* Bb12 group than in the placebo group after 21 days of feeding. In a randomized double-blind placebo-controlled intervention, administration of *L. acidophilus* NCFM or *B. lactis* Bi-07 to infants with atopic dermatitis for 8 wks did not affect the composition and diversity of the main bacterial populations in feces.[123] Similarly, no effect on the overall microbiota composition was observed when *L. reuteri* DSM 17938 was administrated to BF colicky infants.[130] These inconsistent results may relate to differences in probiotic strain used, daily doses administered, timing and duration of administration, and the methods applied for microbiota analysis. In addition, environmental factors that influence the development of infant microbiota might also affect the outcomes. A recent study monitored the effect of probiotic intervention on the microbiota colonization of infants with different delivery mode. In this study, infants delivered by CS or VD received (within 72h of birth) either control formula or the same formula containing *L. reuteri* DSM 17938 for 6 mos and the fecal microbiota was analyzed at 2 wks- and 4 mos of age. They reported administration of *L. reuteri* DSM 17938 did not modify the overall microbial composition of VD infants; however, it increased proportion of Actinobacteria and decreased the relative abundance of unclassified *Enterobacteriaceae* of CD infants, making the microbiota of the CS infants more similar to the composition of VD infants.[127] The results suggest when a study is designed to describe the impact of the probiotic intervention on the infant microbiota, not only daily doses and timing and duration of administration need to be considered; factors that affect the microbial colonization in early life, such as delivery mode, infant diet and age, should also be controlled.

Conclusions and future directions

The infant GI microbiota undergoes rapid and profound changes during the first year of life. During this process, diet plays a predominant role over other environmental factors in shaping the microbial composition. While this review has outlined significant advances in our understanding of the role of early nutrition in the development of infant GI microbiota, large gaps in our understanding of the specific factors regulating this process still exist. For example, information about the effects of mixed feeding on the composition and function of infant microbiota is scarce. Our knowledge of how feeding mode, weaning, and solid foods, as well as the timing of dietary shifts, influence the functional capacities of infant GI microbiota and host programming is also limited. As reviewed by Chu and colleagues in this supplement,[144] maternal nutrition during pregnancy and lactation has the potential to effect the composition and function of the microbiota of the offspring. Although compelling, much of the evidence is derived from animal models, thus, additional studies in humans and potential intervention trials are needed to assess whether maternal nutritional status and diet lead to changes in infant microbial colonization.

Looking forward, longitudinal studies are necessary to investigate how nutrition (breast-, mixed-, and formula-feeding, solid foods) interacts with other early life events (route of delivery, antibiotics, daycare, pets, etc) to impact microbial composition and function and subsequent effects on health outcomes during and beyond infancy. Given that infant microbiota

composition differs based on geography, sampling from a large cohort of infants located in various sites around the globe in which detailed records of dietary intake and other environmental factors are collected is needed to provide a more complete understanding of the development of the microbiota in infants who are breast- vs. formula-fed.

Studies are also needed to further elucidate how human milk components other than HMOs, such as cytokines, macronutrients, and antimicrobial peptides, may work individually or synergistically to support specific GI microbial profiles as well as the potential for pre- and probiotics in human milk or added to formula to preferentially modulate the infant GI tract to a desired composition. Future intervention studies should aim for consistent timing, duration, and dose amounts of any supplemental ingredients (pre or probiotics), which is instrumental in isolating the effects of different feeding regimes on GI microbial colonization during early life.

Disclosure of potential conflicts of interest

The authors report no conflicts of interest

Funding

The authors report no sources of funding to support this work

References

[1] Koenig JE, Spor A, Scalfone N, Fricker AD, Stombaugh J, Knight R, Angenent LT, Ley RE. Succession of microbial consortia in the developing infant gut microbiome. Proc Natl Acad Sci U S A 2011; 108(Suppl 1):4578-85; PMID:20668239; http://dx.doi.org/10.1073/pnas.1000081107

[2] Palmer C, Bik EM, DiGiulio DB, Relman DA, Brown PO. Development of the human infant intestinal microbiota. PLoS Biol 2007; 5:e177; PMID:17594176; http://dx.doi.org/10.1371/journal.pbio.0050177

[3] Wopereis H, Oozeer R, Knipping K, Belzer C, Knol J. The first thousand days - intestinal microbiology of early life: establishing a symbiosis. Pediatr Allergy Immunol 2014; 25(5):428-38; PMID:24899389; http://dx.doi.org/10.1111/pai.12232

[4] Wang M, Monaco MH, Donovan SM. Impact of early gut microbiota on immune and metabolic development and function. Semin Fetal Neonatal Med 2016; 21(6):380-7 [Epub ahead of print]

[5] Abrams GD, Bauer H, Sprinz H. Influence of the normal flora on mucosal morphology and cellular renewal in the ileum. A comparison of germ-free and conventional mice. Lab Invest 1964; 12:355-364

[6] Round JL, Mazmanian SK. The gut microbiota shapes intestinal immune responses during health and disease. Nat Rev Immunol 2009; 9(5):313-23; PMID:19343057; http://dx.doi.org/10.1038/nri2515

[7] Mai V, Young CM, Ukhanova M, Wang X, Sun Y, Casella G, Theriaque D, Li N, Sharma R, Hudak M, Neu J. Fecal microbiota in premature infants prior to necrotizing enterocolitis. PLoS One 2011; 6(6):e20647; PMID:21674011; http://dx.doi.org/10.1371/journal.pone.0020647

[8] Wang M, Ahrné S, Antonsson M, Molin G. T-RFLP combined with principal component analysis and 16S rRNA gene sequencing: an effective strategy for comparison of fecal microbiota in infants of different ages. J Microbiol Methods 2004; 59(1):53-69; PMID:15325753; http://dx.doi.org/10.1016/j.mimet.2004.06.002

[9] Vael C, Vanheirstraeten L, Desager KN, Goossens H. Denaturing gradient gel electrophoresis of neonatal intestinal microbiota in relation to the development of asthma. BMC Microbiol 2011; 11:68; http://dx.doi.org/10.1186/1471-2180-11-68

[10] Aomatsu T, Imaeda H, Fujimoto T, Takahashi K, Yoden A, Tamai H, Fujiyama Y, Andoh A. Terminal restriction fragment length polymorphism analysis of the gut microbiota profiles of pediatric patients with inflammatory bowel disease. Digestion 2012; 86(2):129-135; PMID:22846404; http://dx.doi.org/10.1159/000339777

[11] Saulnier DM, Riehle K, Mistretta TA, Diaz MA, Mandal D, Raza S, Weidler EM, Qin X, Coarfa C, Milosavljevic A, et al. Gastrointestinal microbiome signatures of pediatric patients with irritable bowel syndrome. Gastroenterology 2011; 141(5):1782-91; PMID:21741921; http://dx.doi.org/10.1053/j.gastro.2011.06.072

[12] Karlsson CL, Onnerfält J, Xu J, Molin G, Ahrné S, Thorngren-Jerneck K. The microbiota of the gut in preschool children with normal and excessive body weight. Obesity 2012; 20(11):2257-61; PMID:22546742; http://dx.doi.org/10.1038/oby.2012.110

[13] Qin J, Li Y, Cai Z, Li S, Zhu J, Zhang F, Liang S, Zhang W, Guan Y, Shen D, et al. A metagenome-wide association study of gut microbiota in type 2 diabetes. Nature 2012; 490(7418):55-60; PMID:23023125; http://dx.doi.org/10.1038/nature11450

[14] Kang DW, Park JG, Ilhan ZE, Wallstrom G, Labaer J, Adams JB, Krajmalnik-Brown R. Reduced incidence of *Prevotella* and other fermenters in intestinal microflora of autistic children. PLoS One 2013; 8(7):e68322; PMID:23844187; http://dx.doi.org/10.1371/journal.pone.0068322

[15] Azad MB, Konya T, Maughan H, Guttman DS, Field CJ, Chari RS, Sears MR, Becker AB, Scott JA, Kozyrskyj AL. Gut microbiota of healthy Canadian infants: profiles by mode of delivery and infant diet at 4 months. CMAJ 2013; 185(5):385-394; PMID:23401405; http://dx.doi.org/10.1503/cmaj.121189

[16] Penders J, Thijs C, Vink C, Stelma FF, Snijders B, Kummeling I, van den Brandt PA, Stobberingh EE. Factors

influencing the composition of the intestinal microbiota in early infancy. Pediatrics 2006; 118(2):511-21; PMID:16882802; http://dx.doi.org/10.1542/peds.2005-2824

[17] Wang M, Li M, Wu S, Lebrilla CB, Chapkin RS, Ivanov I, Donovan SM. Fecal microbiota composition of breast-fed infants is correlated with human milk oligosaccharides consumed. J Pediatr Gastroenterol Nutr 2015; 60(6):825-33; PMID:25651488; http://dx.doi.org/10.1097/MPG.0000000000000752

[18] Fallani M, Amarri S, Uusijarvi A, Adam R, Khanna S, Aguilera M, Gil A, Vieites JM, Norin E, Young D, et al. Determinants of the human infant intestinal microbiota after the introduction of first complementary foods in infant samples from five European centres. Microbiology 2011; 157(Pt 5):1385-92; PMID:21330436; http://dx.doi.org/10.1099/mic.0.042143-0

[19] Adlerberth I, Wold AE. Establishment of the gut microbiota in Western infants. Acta Paediatr 2009; 98:229-38; PMID:19143664; http://dx.doi.org/10.1111/j.1651-2227.2008.01060.x

[20] Fouhy F, Ross RP, Fitzgerald GF, Stanton C, Cotter PD. Composition of the early intestinal microbiota: knowledge, knowledge gaps and the use of high-throughput sequencing to address these gaps. Gut Microbes 2012; 3 (3):203-20. doi:10.4161/gmic.20169; PMID:22572829; http://dx.doi.org/10.4161/gmic.20169

[21] Kemppainen KM, Ardissone AN, Davis-Richardson AG, Fagen JR, Gano KA, León-Novelo LG, Vehik K, Casella G, Simell O, Ziegler AG, Rewers MJ, et al. Early childhood gut microbiomes show strong geographic differences among subjects at high risk for type 1 diabetes. Diabetes Care 2015; 38(2):329-32; PMID:25519450; http://dx.doi.org/10.2337/dc14-0850

[22] Avershina E, Storrø O, Øien T, Johnsen R, Pope P, Rudi K. Major faecal microbiota shifts in composition and diversity with age in a geographically restricted cohort of mothers and their children. FEMS Microbiol Ecol 2014; 87(1):280-90; PMID:24112053; http://dx.doi.org/10.1111/1574-6941.12223

[23] Azad MB, Konya T, Persaud RR, Guttman DS, Chari RS, Field CJ, Sears MR, Mandhane PJ, Turvey SE, Subbarao P, Becker AB, Scott JA, Kozyrskyj AL. Impact of maternal intrapartum antibiotics, method of birth and breastfeeding on gut microbiota during the first year of life: a prospective cohort study. BJOG 2016; 123(6):983-93; PMID:26412384; http://dx.doi.org/10.1111/1471-0528.13601

[24] Bäckhed F, Roswall J, Peng Y, Feng Q, Jia H, Kovatcheva-Datchary P, Li Y, Xia Y, Xie H, Zhong H, et al. Dynamics and stabilization of the human gut microbiome during the first year of life. Cell Host Microbe 2015; 17(6):852; PMID:26308884; http://dx.doi.org/10.1016/j.chom.2015.05.012

[25] Bokulich NA, Chung J, Battaglia T, Henderson N, Jay M, Li H, D Lieber A, Wu F, Perez-Perez GI, Chen Y, Schweizer W, Zheng X, Contreras M, Dominguez-Bello MG, Blaser MJ. Antibiotics, birth mode, and diet shape microbiome maturation during early life. Sci Transl Med 2016; 8(343):343ra82; PMID:27306664; http://dx.doi.org/10.1126/scitranslmed.aad7121

[26] Fan W, Huo G, Li X, Yang L, Duan C, Wang T, Chen J. Diversity of the intestinal microbiota in different patterns of feeding infants by Illumina high-throughput sequencing. World J Microbiol Biotechnol 2013; 29 (12):2365-2372; PMID:23793940; http://dx.doi.org/10.1007/s11274-013-1404-3

[27] Jost T, Lacroix C, Braegger CP, Chassard C. New insights in gut microbiota establishment in healthy breast fed neonates. PLoS One 2012; 7(8):e44595; PMID:22957008; http://dx.doi.org/10.1371/journal.pone.0044595

[28] Lee SA, Lim JY, Kim BS, Cho SJ, Kim NY, Kim OB, Kim Y. Comparison of the gut microbiota profile in breast-fed and formula-fed Korean infants using pyrosequencing. Nutr Res Pract 2015; 9(3):242-8; http://dx.doi.org/10.4162/nrp.2015.9.3.242

[29] Madan JC, Hoen AG, Lundgren SN, Farzan SF, Cottingham KL, Morrison HG, Sogin ML, Li H, Moore JH, Karagas MR. Association of cesarean delivery and formula supplementation with the intestinal microbiome of 6-week-old infants. JAMA Pediatr 2016; 170(3):212-9; PMID:26752321; http://dx.doi.org/10.1001/jamapediatrics.2015.3732

[30] Schwartz S, Friedberg I, Ivanov IV, Davidson LA, Goldsby JS, Dahl DB, Herman D, Wang M, Donovan SM, Chapkin RS. A metagenomic study of diet-dependent interaction between gut microbiota and host in infants reveals differences in immune response. Genome Biol 2012; 13(4):r32; PMID:22546241; http://dx.doi.org/10.1186/gb-2012-13-4-r32

[31] Simeoni U, Berger B, Junick J, Blaut M, Pecquet S, Rezzonico E, Grathwohl D, Sprenger N, Brüssow H, Szajewska H. Gut microbiota analysis reveals a marked shift to bifidobacteria by a starter infant formula containing a synbiotic of bovine milk-derived oligosaccharides and Bifidobacterium animalis subsp. lactis CNCM I-3446. Environ Microbiol 2016; 18(7):2185-95

[32] Tannock GW, Lawley B, Munro K, Gowri Pathmanathan S, Zhou SJ, Makrides M, Gibson RA, Sullivan T, Prosser CG, Lowry D, et al. Comparison of the compositions of the stool microbiotas of infants fed goat milk formula, cow milk-based formula, or breast milk. Appl Environ Microbiol 2013; 79(9):3040-8; PMID:23455335; http://dx.doi.org/10.1128/AEM.03910-12

[33] Thompson AL, Monteagudo-Mera A, Cadenas MB, Lampl ML, Azcarate-Peril MA. Milk- and solid-feeding practices and daycare attendance are associated with differences in bacterial diversity, predominant communities, and metabolic and immune function of the infant gut microbiome. Front Cell Infect Microbiol 2015; 5:3; PMID:25705611; http://dx.doi.org/10.3389/fcimb.2015.00003

[34] Turroni F, Peano C, Pass DA, Foroni E, Severgnini M, Claesson MJ, Kerr C, Hourihane J, Murray D, Fuligni F, et al. Diversity of bifidobacteria within the infant gut

microbiota. PLoS One 2012; 7(5):e36957; PMID:22606315; http://dx.doi.org/10.1371/journal.pone.0036957

[35] Vallès Y, Artacho A, Pascual-García A, Ferrús ML, Gosalbes MJ, Abellán JJ, Francino MP. Microbial succession in the gut: directional trends of taxonomic and functional change in a birth cohort of Spanish infants. PLoS Genet 2014; 10(6):e1004406; PMID:24901968; http://dx.doi.org/10.1371/journal.pgen.1004406

[36] Yatsunenko T, Rey FE, Manary MJ, Trehan I, Dominguez-Bello MG, Contreras M, Magris M, Hidalgo G, Baldassano RN, Anokhin AP, et al. Human gut microbiome viewed across age and geography. Nature 2012; 486(7402):222-7; PMID:22699611

[37] Favier CF, Vaughan EE, De Vos WM, Akkermans AD. Molecular monitoring of succession of bacterial communities in human neonates. Appl Environ Microbiol 2002; 68(1):219-26; PMID:11772630; http://dx.doi.org/10.1128/AEM.68.1.219-226.2002

[38] Favier CF, de Vos WM, Akkermans ADL. Development of bacterial and bifidobacterial communities in feces of newborn babies. Anaerobe 2003; 9(5):219-29; PMID:16887708; http://dx.doi.org/10.1016/j.anaerobe.2003.07.001

[39] Klaassens ES, Boesten RJ, Haarman M, Knol J, Schuren FH, Vaughan EE, de Vos WM. Mixed-species genomic microarray analysis of fecal samples reveals differential transcriptional responses of bifidobacteria in breast- and formula-fed infants. Appl Environ Microbiol 2009; 75(9):2668-76; PMID:19286790; http://dx.doi.org/10.1128/AEM.02492-08

[40] Magne F, Hachelaf W, Suau A, Boudraa G, Mangin I, Touhami M, Bouziane-Nedjadi K, Pochart P. A longitudinal study of infant faecal microbiota during weaning. FEMS Microbiol Ecol 2006; 58(3):563-71; PMID:17117997; http://dx.doi.org/10.1111/j.1574-6941.2006.00182.x

[41] Matsuki T, Watanabe K, Tanaka R, Fukuda M, Oyaizu H. Distribution of bifidobacterial species in human intestinal microflora examined with 16S rRNA-gene-targeted species-specific primers. Appl Environ Microbiol 1999; 65(10):4506-12

[42] Roger LC, Costabile A, Holland DT, Hoyles L, McCartney AL. Examination of faecal Bifidobacterium populations in breast- and formula-fed infants during the first 18 months of life. Microbiology 2010; 156:3329-41; PMID:20864478; http://dx.doi.org/10.1099/mic.0.043224-0

[43] Satokari RM, Vaughan EE, Favier CF, Doré J, Edwards C, de Vos WM. Diversity of Bifidobacterium and Lactobacillus spp. in breast-fed and formula-fed infants as assessed by 16s rDNA sequence differences. Microb Ecol Health Dis 2002; 14(2):97-105; http://dx.doi.org/10.1080/08910600260081748

[44] Sakata S, Tonooka T, Ishiezeki S, Takada M, Sakamoto M, Fukuyama M, Benno Y. Culture-independent analysis of fecal microbiota in infants, with special reference to *Bifidobacterium* species. FEMS Microbiol Lett 2005; 243(2):417-23; PMID:15686844; http://dx.doi.org/10.1016/j.femsle.2005.01.002

[45] Bergstrom A, Skov TH, Bahl MI, Roager HM, Christensen LB, Ejlerskov KT, Mølgaard C, Michaelsen KF, Licht RT. Establishment of intestinal microbiota during early life: a longitudinal, explorative study of a large cohort of Danish infants. Appl Environ Microbiol 2014; 80(9):2889-900; PMID:24584251; http://dx.doi.org/10.1128/AEM.00342-14

[46] Penders J, Vink C, Driessen C, London N, Thijs C, Stobberingh EE. Quantification of *Bifidobacterium* spp., *Escherichia coli* and *Clostridium difficile* in faecal samples of breast-fed and formula-fed infants by real-time PCR. FEMS Microbiol Lett 2005; 243(1):141-7; PMID:15668012; http://dx.doi.org/10.1016/j.femsle.2004.11.052

[47] Martin R, Makino H, Cetinyurek YA, Ben-Amor K, Roelofs M, Ishikawa E, Kubota H, Swinkels S, Sakai T, Oishi K, et al. Early-life events, including mode of delivery and type of feeding, siblings and gender, shape the developing gut microbiota. PLoS One 2016; 11(6):e0158498; PMID:27362264; http://dx.doi.org/10.1371/journal.pone.0158498

[48] Salminen S, Endo A, Isolauri E, Scalabrin D. Early gut colonization with lactobacilli and staphylococcus in infants: the hygiene hypothesis extended. J Pediatr Gastroenterol Nutr 2016; 62(1):80-6; PMID:26230902; http://dx.doi.org/10.1097/MPG.0000000000000925

[49] Hopkins MJ, Macfarlane GT, Furrie E, Fite A, Macfarlane S. Characterisation of intestinal bacteria in infant stools using real-time PCR and northern hybridisation analyses. FEMS Microbiol Ecol 2005; 54(1):77-85; PMID:16329974; http://dx.doi.org/10.1016/j.femsec.2005.03.001

[50] Harmsen HJ, Wildeboer-Veloo AC, Raangs GC, Wagendorp AA, Klijn N, Bindels JG, Welling GW. Analysis of intestinal flora development in breast-fed and formula-fed infants by using molecular identification and detection methods. J Pediatr Gastroenterol Nutr 2000; 30(1):61-7; PMID:10630441; http://dx.doi.org/10.1097/00005176-200001000-00019

[51] Bezirtzoglou E, Tsiotsias A, Welling GW. Microbiota profile in feces of breast- and formula-fed newborns by using fluorescence in situ hybridization (FISH). Anaerobe 2011; 17(6):478-82; PMID:21497661; http://dx.doi.org/10.1016/j.anaerobe.2011.03.009

[52] Fallani M, Young D, Scott J, Norin E, Amarri S, Adam R, Aguilera M, Khanna S, Gil A, Edwards CA, et al. Intestinal microbiota of 6-week-old infants across Europe: geographic influence beyond delivery mode, breast-feeding, and antibiotics. J Pediatr Gastroenterol Nutr 2010; 51(1):77-84; PMID:20479681; http://dx.doi.org/10.1097/MPG.0b013e3181d1b11e

[53] Gomez-Llorente C, Plaza-Diaz J, Aguilera M, Muñoz-Quezada S, Bermudez-Brito M, Peso-Echarri P, Martinez-Silla R, Vasallo-Morillas MI, Campaña-Martin L, Vives-Piñera I. Three main factors define changes in fecal microbiota associated with feeding modality in infants. J Pediatr Gastroenterol Nutr 2013; 57(4):461-6;

PMID:23752082; http://dx.doi.org/10.1097/MPG.0b013e31829d519a

[54] Rinne M, Kalliomaki M, Arvilommi H, Salminen S, Isolauri E. Effect of probiotics and breastfeeding on the bifidobacterium and lactobacillus/enterococcus microbiota and humoral immune responses. J Pediatr 2005; 147(2):186-91; http://dx.doi.org/10.1016/j.jpeds.2005.03.053

[55] Roger LC, McCartney AL. Longitudinal investigation of the faecal microbiota of healthy full-term infants using fluorescence in situ hybridization and denaturing gradient gel electrophoresis. Microbiology 2010; 156(Pt 11):3317-28

[56] Rodriguez J.M., Murphy K., Stanton C., Ross R.P., Kober O.I., Juge N., et al. The composition of the gut microbiota throughout life, with an emphasis on early life. Microb. Ecol. Health Dis 2015; 26:26050; PMID:25651996

[57] Collado MC, Cernada M, Baüerl C, Vento M, Pérez-Martínez G. Microbial ecology and host-microbiota interactions during early life stages. Gut Microbes 2012; 3(4):352-65; PMID:22743759; http://dx.doi.org/10.4161/gmic.21215

[58] Sim K, Cox MJ, Wopereis H, Martin R, Knol J, Li M-S, Cookson WOCM, Moffatt MF, Krol JS. Improved detection of bifidobacteria with optimised 16S rRNA-gene based pyrosequencing. PLoS ONE 2012; 7(3): e32543; PMID:22470420; http://dx.doi.org/10.1371/journal.pone.0032543

[59] Jost T, Lacroix C, Braegger C, Chassard C. Impact of human milk bacteria and oligosaccharides on neonatal gut microbiota establishment and gut health. Nutr Rev 2015; 73(7):426-37; PMID:26081453; http://dx.doi.org/10.1093/nutrit/nuu016

[60] Jost T, Lacroix C, Braegger C, Chassard C. Assessment of bacterial diversity in breast milk using culture-dependent and culture-independent approaches. Br J Nutr 2013; 110(7):1253-62; PMID:23507238; http://dx.doi.org/10.1017/S0007114513000597

[61] Jost T, Lacroix C, Braegger CP, Rochat F, Chassard C. Vertical mother–neonate transfer of maternal gut bacteria via breastfeeding. Environ Microbiol 2014; 16 (9):2891-904; PMID:24033881; http://dx.doi.org/10.1111/1462-2920.12238

[62] Fernández L, Langa S, Martin V, Maldonado A, Jimenez E, Martin R, Rodriguez JM. The human milk microbiota: origin and potential roles in health and disease. Pharmacol Res 2013; 69(1):1-10; http://dx.doi.org/10.1016/j.phrs.2012.09.001

[63] Martín V, Maldonado-Barragán A, Moles L, Rodriguez-Baños M, Campo RD, Fernández L, Rodriguez JM, Jimenez E. Sharing of bacterial strains between breast milk and infant feces. J Hum Lact 2012; 28(1):36-44; PMID:22267318; http://dx.doi.org/10.1177/0890334411424729

[64] Hunt KM, Foster JA, Forney LJ, Schütte UM, Beck DL, Abdo Z, et al. Characterization of the diversity and temporal stability of bacterial communities in human milk. PLoS One 2011; 6(6):e21313; PMID:21695057; http://dx.doi.org/10.1371/journal.pone.0021313

[65] Cabrera-Rubio R, Collado MC, Laitinen K, Salminen S, Isolauri E, Mira A. The human milk microbiome changes over lactation and is shaped by maternal weight and mode of delivery. Am J Clin Nutr 2012; 96(3):544-51; PMID:22836031; http://dx.doi.org/10.3945/ajcn.112.037382

[66] Grönlund MM, Gueimonde M, Laitinen K, Kociubinski G, Grönroos T, Salminen S, Isolauri E. Maternal breast-milk and intestinal bifidobacteria guide the compositional development of the Bifidobacterium microbiota in infants at risk of allergic disease. Clin Exp Allergy 2007; 37(12):1764-72; PMID:17941914; http://dx.doi.org/10.1111/j.1365-2222.2007.02849.x

[67] Martín R, Heilig HG, Zoetendal EG, Jiménez E, Fernández L, Smidt H, Rodriguez JM. Cultivation-independent assessment of the bacterial diversity of breast milk among healthy women. Res Microbiol 2007; 158(1):31-7; PMID:17224259; http://dx.doi.org/10.1016/j.resmic.2006.11.004

[68] Rodriguez JM. The origin of human milk bacteria: is there a bacterial entero-mammary pathway during late pregnancy and lactation? Adv Nutr 2014; 5(6):779-84; PMID:25398740; http://dx.doi.org/10.3945/an.114.007229

[69] Bode L, McGuire M, Rodriguez JM, Geddes DT, Hassiotou F, Hartmann PE, McGuire MK. It's alive: microbes and cells in human milk and their potential benefits to mother and infant. Adv Nutr 2014; 5(5):571-3; PMID:25469400; http://dx.doi.org/10.3945/an.114.006643

[70] Urbaniak C, Angelini M, Gloor G, Reid G. Human milk microbiota profiles in relation to birthing method, gestation, and infant gender. Microbiome 2016; 4(1); PMID:26739322; http://dx.doi.org/10.1186/s40168-015-0145-y

[71] Bode L. Recent advances on structure, metabolism, and function of human milk oligosaccharides. J Nutr 2006; 136(8):2127-2130; PMID:16857829

[72] Garrido D, Dallas DC, Mills DA. Consumption of human milk glycoconjugates by infant-associated bifidobacteria: mechanisms and implications. Microbiology 2013; 159(Pt 4):649-64; PMID:23460033; http://dx.doi.org/10.1099/mic.0.064113-0

[73] Donovan SM, Wang M, Li M, Friedberg I, Schwartz SL, Chapkin RS. Host-microbe interactions in the neonatal intestine: role of human milk oligosaccharides. Adv Nutr 2012; 3(3):450S-455S; PMID:22585924; http://dx.doi.org/10.3945/an.112.001859

[74] Asakuma S, Hatakeyama E, Urashima T, Yoshida E, Katayama T, Yamamoto K, Kumagai H, Ashida H, Hirose J, Kitaoka M. Physiology of consumption of human milk oligosaccharides by infant gut-associated bifidobacteria. J Biol Chem 2011; 286(4):34583-92; PMID:21832085; http://dx.doi.org/10.1074/jbc.M111.248138

[75] Sela DA, Mills DA. Nursing our microbiota: molecular linkages between bifidobacteria and milk oligosaccharides. Trends Microbiol 2010; 18(7):298-307; http://dx.doi.org/10.1016/j.tim.2010.03.008

[76] Lewis ZT, Totten SM, Smilowitz JT, Popovic M, Parker E, Lemay DG, Van Tassell ML, Miller MJ, Jin YS, German JB, et al. Maternal fucosyltransferase 2 status affects the gut bifidobacterial communities of breastfed infants. Microbiome 2015; 3(13); PMID:25922665

[77] Gonzalez R, Klaassens ES, Malinen E, de Vos WM, Vaughan EE. Differential transcriptional response of *Bifidobacterium longum* to human milk, formula milk, and galactooligosaccharide. Appl Environ Microbiol 2008; 74(15):4686-94; PMID:18539808; http://dx.doi.org/10.1128/AEM.00122-08

[78] Gibson GR, Roberfroid MB. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. J Nutr 1995; 125(6):1401-12; PMID:7782892

[79] Gibson GR, Probert HM, Loo JV, Rastall RA, Roberfroid MB. Dietary modulation of the human colonic microbiota: updating the concept of prebiotics. Nutr Res Rev 2004; 17(2):259-75; PMID:19079930; http://dx.doi.org/10.1079/NRR200479

[80] Reid G, Sanders ME, Gaskins HR, Gibson GR, Mercenier A, Rastall R, Roberfroid M, Rowland I, Cherbut C, Klaenhammer TR. New scientific paradigms for probiotics and prebiotics. J Clin Gastroenterol 2003; 37 (2):105-18; PMID:12869879; http://dx.doi.org/10.1097/00004836-200308000-00004

[81] Roberfroid M. Prebiotics: the concept revisited. J Nutr 2007; 137(3 Suppl 2):830S-7S; PMID:17311983

[82] Roberfroid M, Gibson GR, Hoyles L, McCartney AL, Rastall R, Rowland I, Wolvers D, Watzl B, Szajewska H, Stahl B, et al. Prebiotic effects: metabolic and health benefits. Br J Nutr 2010; 104(Suppl 2):S1-63; PMID:20920376; http://dx.doi.org/10.1017/S0007114510003363

[83] Bindels LB, Delzenne NM, Cani PD, Walter J. Towards a more comprehensive concept for prebiotics. Nat Rev Gastroenterol Hepatol 2015; 12(5):303-10; PMID:25824997; http://dx.doi.org/10.1038/nrgastro.2015.47

[84] Ward RE, Niñonuevo M, Mills DA, Lebrilla CB, German JB. *In vitro* fermentation of breast milk oligosaccharides by *Bifidobacterium infantis* and *Lactobacillus gasseri*. Appl Environ Microbiol 2006; 72(6):4497-9; PMID:16751577; http://dx.doi.org/10.1128/AEM.02515-05

[85] Marcobal A, Barboza M, Froehlich JW, Block DE, German JB, Lebrilla CB, Mills DA. Consumption of human milk oligosaccharides by gut-related microbes. J Agric Food Chem 2010; 58(9):5334-40; PMID:20394371; http://dx.doi.org/10.1021/jf9044205

[86] Davis JC, Totten SM, Huang JO, Nagshbandi S, Kirmiz N, Garrido DA, Lewis ZT, Wu LD, Smilowitz JT, German JB, et al. Identification of oligosaccharides in feces of breast-fed infants and Their Correlation with the Gut Microbial Community. Mol Cell Proteomics 2016; 15 (9):2987-3002; PMID:27435585; http://dx.doi.org/10.1074/mcp.M116.060665

[87] De Leoz ML, Kalanetra KM, Bokulich NA, Strum JS, Underwood MA, German JB, Mills DA, Lebrilla CB. Human milk glycomics and gut microbial genomics in infant feces show a correlation between human milk oligosaccharides and gut microbiota: a proof-of-concept study. J Proteome Res 2015; 14(1):491-502; PMID:25300177; http://dx.doi.org/10.1021/pr500759e

[88] Boehm G, Moro G. Structural and functional aspects of prebiotics used in infant nutrition. J Nutr 2008; 138 (9):1818S-1828S; PMID:18716193

[89] Fanaro S, Boehm G, Garssen J, Knol J, Mosca F, Stahl B, Vigi V. Galacto-oligosaccharides and long-chain fructo-oligosaccharides as prebiotics in infant formulas: a review. Acta Paediatr 2005; 94(Suppl 449):22-6; http://dx.doi.org/10.1080/08035320510043538

[90] Oozeer R, van Limpt K, Ludwig T, Ben Amor K, Martin R, Wind RD, Boehm G, Knol J. Intestinal microbiology in early life: specific prebiotics can have similar functionalities as human-milk oligosaccharides. Am J Clin Nutr 2013; 98(2):561S-71S; PMID:23824728; http://dx.doi.org/10.3945/ajcn.112.038893

[91] Ben XM, Li J, Feng ZT, Shi SY, Lu YD, Chen R, Zhou XY. Low level of galacto-oligosaccharide in infant formula stimulates growth of intestinal bifidobacteria and lactobacilli. World J Gastroenterol 2008; 14(42):6564-8; http://dx.doi.org/10.3748/wjg.14.6564

[92] Giovannini M, Verduci E, Gregori D, Ballali S, Soldi S, Ghisleni D, Riva E; PLAGOS Trial Study Group. Prebiotic effect of an infant formula supplemented with galacto-oligosaccharides: randomized multicenter trial. J Am Coll Nutr 2014; 33(5):385-93; http://dx.doi.org/10.1080/07315724.2013.878232

[93] Sierra C, Bernal MJ, Blasco J, Martínez R, Dalmau J, Ortuño I, Espín B, Vasallo MI, Gil D, Vidal ML, Infante D, Leis R, Maldonado J, Moreno JM, Román E. Prebiotic effect during the first year of life in healthy infants fed formula containing GOS as the only prebiotic: a multicentre, randomised, double-blind and placebo-controlled trial. Eur J Nutr 2015; 54(1):89-99; PMID:24671237; http://dx.doi.org/10.1007/s00394-014-0689-9

[94] Yahiro M, Nishikawa I, Murakami Y, Yoshida H, Ahiko K. Studies on application of galactosyl lactose for infant formula. II. Changes of fecal characteristics on infant fed galactosyl lactose. Reports of Research Laboratory. Snow Brand Milk Products 1992; 78:27-32

[95] Matsuki T, Tajima S, Hara T, Yahagi K, Ogawa E, Kodama H. Infant formula with galacto-oligosaccharides (OM55N) stimulates the growth of indigenous bifidobacteria in healthy term infants. Benef Microbes 2016; 7(4):1-10; http://dx.doi.org/10.3920/BM2015.0168

[96] Euler AR, Mitchell DK, Kline R, Pickering LK. Prebiotic effect of fructo-oligosaccharide supplemented term infant formula at two concentrations compared with unsupplemented formula and human milk. J Pediatr Gastroenterol Nutr 2005; 40(2):157-64; PMID:15699689; http://dx.doi.org/10.1097/00005176-200502000-00014

[97] Kapiki A, Costalos C, Oikonomidou C, Triantafyllidou A, Loukatou E, Pertrohilou V. The effect of a fructo-oligosaccharide supplemented formula on gut flora of preterm infants. Early Hum Dev 2007; 83(5):335-9;

PMID:16978805; http://dx.doi.org/10.1016/j.earlhumdev.2006.07.003

[98] Paineau D, Respondek F, Menet V, Sauvage R, Bornet F, Wagner A. Effects of short-chain fructooligosaccharides on faecal bifidobacteria and specific immune response in formula-fed term infants: a randomized, double-blind, placebo-controlled trial. J Nutr Sci Vitaminol (Tokyo) 2014; 60(3):167-75; http://dx.doi.org/10.3177/jnsv.60.167

[99] Wernimont S, Northington R, Kullen MJ, Yao M, Bettler J. Effect of an α-lactalbumin-enriched infant formula supplemented with oligofructose on fecal microbiota, stool characteristics, and hydration status: a randomized, double-blind, controlled trial. Clin Pediatr (Phila) 2015; 54(4):359-70; PMID:25297064; http://dx.doi.org/10.1177/0009922814553433

[100] Xia Q, Williams T, Hustead D, Price P, Morrison M, Yu Z. Quantitative analysis of intestinal bacterial populations from term infants fed formula supplemented with fructo-oligosaccharides. J Pediatr Gastroenterol Nutr 2012; 55(3):314-20; PMID:22395187; http://dx.doi.org/10.1097/MPG.0b013e3182523254

[101] Brunser O, Gotteland M, Cruchet S, Figueroa G, Garrido D, Steenhout P. Effect of a milk formula with prebiotics on the intestinal microbiota of infants after an antibiotic treatment. Pediatr Res 2006; 59(3):451-6

[102] Waligora-Dupriet AJ, Campeotto F, Nicolis I, Bonet A, Soulaines P, Dupont C, Butel MJ. Effect of oligofructose supplementation on gut microflora and well-being in young children attending a day care centre. Int J Food Microbiol 2007; 113(1):108-13; PMID:16996154; http://dx.doi.org/10.1016/j.ijfoodmicro.2006.07.009

[103] Moore N, Chao C, Yang LP, Storm H, Oliva-Hemker M, Saavedra JM. Effects of fructo-oligosaccharide-supplemented infant cereal: a double-blind, randomized trial. Br J Nutr 2003; 90(3):581-7; PMID:13129464; http://dx.doi.org/10.1079/BJN2003950

[104] Closa-Monasterolo R, Gispert-Llaurado M, Luque V, Ferre N, Rubio-Torrents C, Zaragoza-Jordana M, Escribano J. Safety and efficacy of inulin and oligofructose supplementation in infant formula: results from a randomized clinical trial. Clin Nutr 2013; 32(6):918-27; PMID:23498848; http://dx.doi.org/10.1016/j.clnu.2013.02.009

[105] Veereman-Wauters G, Staelens S, Van de Broek H, Plaskie K, Wesling F, Roger LC, McCartney AL, Assam P. Physiological and bifidogenic effects of prebiotic supplements in infant formulae. J Pediatr Gastroenterol Nutr 2011; 52(6):763-71; PMID:21593649; http://dx.doi.org/10.1097/MPG.0b013e3182139f39

[106] Kim SH, Lee DH, Meyer D. Supplementation of baby formula with native inulin has a prebiotic effect in formula-fed babies. Asia Pac J Clin Nutr 2007; 16(1):172-7; PMID:17215195

[107] K W Yap W, Mohamed S, Husni Jamal M, Diederick M, A Manap Y. Changes in infants faecal characteristics and microbiota by inulin supplementation. J Clin Biochem Nutr 2008; 43(3):159-66; PMID:19015750; http://dx.doi.org/10.3164/jcbn.2008055

[108] Nagendra R, Vishwanatha S, Rao SV, Ravish SR. Effect of feeding infant formula containing lactulose on intestinal flora in the infant. Indian J Pediatr 1992; 59(6):763-6; PMID:1340869; http://dx.doi.org/10.1007/BF02859419

[109] Ziegler E, Vanderhoof JA, Petschow B, Mitmesser SH, Stolz SI, Harris CL, Berseth CL. Term infants fed formula supplemented with selected blends of prebiotics grow normally and have soft stools similar to those reported for breast-fed infants. J Pediatr Gastroenterol Nutr 2007; 44(3):359-64; PMID:17325558; http://dx.doi.org/10.1097/MPG.0b013e31802fca8c

[110] Scalabrin DM, Mitmesser SH, Welling GW, Harris CL, Marunycz JD, Walker DC, Bos NA, Tölkkö S, Salminen S, Vanderhoof JA. New prebiotic blend of polydextrose and galacto-oligosaccharides has a bifidogenic effect in young infants. J Pediatr Gastroenterol Nutr 2012; 54(3):343-52; PMID:21946836; http://dx.doi.org/10.1097/MPG.0b013e318237ed95

[111] Nakamura N, Gaskins HR, Collier CT, Nava GM, Rai D, Petschow B, Russell WM, Harris C, Mackie RI, Wampler JL, Walker DC. Molecular ecological analysis of fecal bacterial populations from term infants fed formula supplemented with selected blends of prebiotics. Appl Environ Microbiol 2009; 75(4):1121-8; PMID:19088307; http://dx.doi.org/10.1128/AEM.02359-07

[112] Fanaro S, Jelinek J, Stahl B, Boehm G, Kock R, Vigi V. Acidic oligosaccharides from pectin hydrolysate as new component for infant formulae: effect on intestinal flora, stool characteristics, and pH. J Pediatr Gastroenterol Nutr 2005; 41(2):186-90; http://dx.doi.org/10.1097/01.mpg.0000172747.64103.d7

[113] Westerbeek EA, Slump RA, Lafeber HN, Knol J, Georgi G, Fetter WP, van Elburg RM. The effect of enteral supplementation of specific neutral and acidic oligosaccharides on the faecal microbiota and intestinal microenvironment in preterm infants. Eur J Clin Microbiol Infect Dis 2013; 32(2):269-76; PMID:22961006; http://dx.doi.org/10.1007/s10096-012-1739-y

[114] Mäkeläinen HS, Mäkivuokko HA, Salminen SJ, Rautonen NE, Ouwehand AC. The effects of polydextrose and xylitol on microbial community and activity in a 4-stage colon simulator. J Food Sci 2007; 72(5):M153-9; PMID:17995737; http://dx.doi.org/10.1111/j.1750-3841.2007.00350.x

[115] Knol J, Scholtens P, Kafka C, Steenbakkers J, Gro S, Helm K, Klarczyk M, Schöpfer H, Böckler HM, Wells J. Colon microflora in infants fed formula with galacto- and fructo-oligosaccharides: more like breast-fed infants. J Pediatr Gastroenterol Nutr 2005; 40(1):36-42; PMID:15625424; http://dx.doi.org/10.1097/00005176-200501000-00007

[116] Topping DL, Clifton PM. Short-chain fatty acids and human colonic function: roles of resistant starch and

nonstarch polysaccharides. Physiol Rev 2001; 81:1031-64; PMID:11427691

[117] Rao S, Srinivasjois R, Patole S. Prebiotic supplementation in full-term neonates: a systematic review of randomized controlled trials. Arch Pediatr Adolesc Med 2009; 163(8):755-64; PMID:19652109; http://dx.doi.org/10.1001/archpediatrics.2009.94

[118] Ogawa K, Ben RA, Pons S, de Paolo MI, Bustos Fernández L. Volatile fatty acids, lactic acid, and pH in the stools of breast-fed and bottle-fed infants. J Pediatr Gastroenterol Nutr 1992; 15:248-52; PMID:1432461; http://dx.doi.org/10.1097/00005176-199210000-00004

[119] Food and Agriculture Organization of the United Nations and World Health Organization. Health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria. 2001. Available at: http://isappscience.org/wp-content/uploads/2015/12/FAO-WHO-2001-Probiotics-Report.pdf. Accessed August 20, 2016

[120] Brunser O, Figueroa G, Gotteland M, Haschke-Becher E, Magliola C, Rochat F, Cruchet S, Palframan R, Gibson G, Chauffard F, et al. Effects of probiotic or prebiotic supplemented milk formulas on fecal microbiota composition of infants. Asia Pac J Clin Nutr 2006; 15 (5):368-76; PMID:16837430

[121] Berni Canani R, Sangwan N, Stefka AT, Nocerino R, Paparo L, Aitoro R, Calignano A, Khan AA, Gilbert JA, Nagler CR. Lactobacillus rhamnosus GG-supplemented formula expands butyrate-producing bacterial strains in food allergic infants. ISME J 2016; 10(3):742-50; PMID:26394008; http://dx.doi.org/10.1038/ismej.2015.151

[122] Grześkowiak Ł, Grönlund MM, Beckmann C, Salminen S, von Berg A, Isolauri E. The impact of perinatal probiotic intervention on gut microbiota: double-blind placebo-controlled trials in Finland and Germany. Anaerobe 2012; 18(1):7-13; PMID:21979491; http://dx.doi.org/10.1016/j.anaerobe.2011.09.006

[123] Larsen N, Vogensen FK, Gøbel R, Michaelsen KF, Abu Al-Soud W, Sørensen SJ, Hansen LH, Jakobsen M. Predominant genera of fecal microbiota in children with atopic dermatitis are not altered by intake of probiotic bacteria Lactobacillus acidophilus NCFM and Bifidobacterium animalis subsp. lactis Bi-07. FEMS Microbiol Ecol 2011; 75(3):482-96; http://dx.doi.org/10.1111/j.1574-6941.2010.01024.x

[124] Mah KW, Chin VI, Wong WS, Lay C, Tannock GW, Shek LP, Aw MM, Chua KY, Wong HB, Panchalingham A, Lee BW. Effect of a milk formula containing probiotics on the fecal microbiota of Asian infants at risk of atopic diseases. Pediatr Res 2007; 62(6):674-9; PMID:17957155; http://dx.doi.org/10.1203/PDR.0b013e31815991d5

[125] Maldonado-Lobón JA, Gil-Campos M, Maldonado J, López-Huertas E, Flores-Rojas K, Valero AD, Rodríguez-Benítez MV, Bañuelos O, Lara-Villoslada F, Fonollá J, et al. Long-term safety of early consumption of Lactobacillus fermentum CECT5716: A 3-year follow-up of a randomized controlled trial. Pharmacol Res 2015; 95-6:12-9; PMID:25697549; http://dx.doi.org/10.1016/j.phrs.2015.01.006

[126] Murakami H, Shimomura Y, Matsumoto M, Lane GJ, Yamataka A, Okawada M. Intestinal microbiota in neonates requiring urgent surgery: assessing the role of probiotics using fecal DNA sequencing. Pediatr Surg Int 2016; 32(1):37-43; PMID:26516074; http://dx.doi.org/10.1007/s00383-015-3810-0

[127] Rodenas CL, Lepage M, Ngom-Bru C, Fotiou A, Papagaroufalis K, Berger B. Effect of Formula Containing Lactobacillus Reuteri DSM 17938 on Fecal Microbiota of Infants Born by Cesarean-Section. J Pediatr Gastroenterol Nutr 2016; 63(6):681-7[Epub ahead of print]

[128] Wu BB, Yang Y, Xu X, Wang WP. Effects of Bifidobacterium supplementation on intestinal microbiota composition and the immune response in healthy infants. World J Pediatr 2016; 12(2):177-82; PMID:25846071; http://dx.doi.org/10.1007/s12519-015-0025-3

[129] Taylor AL, Dunstan JA, Prescott SL. Probiotic supplementation for the first 6 months of life fails to reduce the risk of atopic dermatitis and increases the risk of allergen sensitization in high-risk children: a randomized controlled trial. J Allergy Clin Immunol 2007; 119 (1):184-91; PMID:17208600; http://dx.doi.org/10.1016/j.jaci.2006.08.036

[130] Roos S, Dicksved J, Tarasco V, Locatelli E, Ricceri F, Grandin U, Savino F. 454 pyrosequencing analysis on faecal samples from a randomized DBPC trial of colicky infants treated with *Lactobacillus reuteri* DSM 17938. PLoS One 2013; 8(2):e56710; PMID:23468874; http://dx.doi.org/10.1371/journal.pone.0056710

[131] Ishizeki S, Sugita M, Takata M, Yaeshima T. Effect of administration of bifidobacteria on intestinal microbiota in low-birth-weight infants and transition of administered bifidobacteria: a comparison between one-species and three-species administration. Anaerobe 2013; 23:38-44; PMID:23988359; http://dx.doi.org/10.1016/j.anaerobe.2013.08.002

[132] Chrzanowska-Liszewska D, Seliga-Siwecka J, Kornacka MK. The effect of *Lactobacillus rhamnosus* GG supplemented enteral feeding on the microbiotic flora of preterm infants-double blinded randomized control trial. Early Hum Dev 2012; 88(1):57-60; PMID:22055271; http://dx.doi.org/10.1016/j.earlhumdev.2011.07.002

[133] Mohan R, Koebnick C, Schildt J, Schmidt S, Mueller M, Possner M, Radke M, Blaut M. Effects of Bifidobacterium lactis Bb12 supplementation on intestinal microbiota of preterm infants: a double-blind, placebo-controlled, randomized study. J Clin Microbiol 2006; 44(11):4025-31; PMID:16971641; http://dx.doi.org/10.1128/JCM.00767-06

[134] Rougé C, Piloquet H, Butel MJ, Berger B, Rochat F, Ferraris L, Des Robert C, Legrand A, de la Cochetière MF, N'Guyen JM, et al. Oral supplementation with probiotics in very-low-birth-weight preterm infants: a randomized, double-blind, placebo-controlled trial. Am J Clin Nutr 2009; 89(6):1828-35; PMID:19369375; http://dx.doi.org/10.3945/ajcn.2008.26919

[135] Lahtinen SJ, Boyle RJ, Kivivuori S, Oppedisano F, Smith KR, Robins-Browne R, Salminen SJ, Tang ML. Prenatal probiotic administration can influence Bifidobacterium microbiota development in infants at high risk of allergy. J Allergy Clin Immunol 2009; 123(2):499-501; PMID:19135234; http://dx.doi.org/10.1016/j.jaci.2008.11.034

[136] Rutten NB, Gorissen DM, Eck A, Niers LE, Vlieger AM, Besseling-van der Vaart I, Budding AE, Savelkoul PH, van der Ent CK, Rijkers GT. Long term development of gut microbiota composition in atopic children: impact of probiotics. PLoS One 2015; 10(9):e0137681; PMID:26378926; http://dx.doi.org/10.1371/journal.pone.0137681

[137] Rinne M, Kalliomäki M, Salminen S, Isolauri E. Probiotic intervention in the first months of life: short-term effects on gastrointestinal symptoms and long-term effects on gut microbiota. J Pediatr Gastroenterol Nutr 2006; 43(2):200-5

[138] Maldonado J, Cañabate F, Sempere L, Vela F, Sánchez AR, Narbona E, López-Huertas E, Geerlings A, Valero AD, Olivares M, et al. Human milk probiotic *Lactobacillus fermentum* CECT5716 reduces the incidence of gastrointestinal and upper respiratory tract infections in infants. J Pediatr Gastroenterol Nutr 2012; 54(1):55-61; PMID:21873895

[139] Moles L, Escribano E, de Andrés J, Montes MT, Rodríguez JM, Jiménez E, Sáenz de Pipaón M, Espinosa-Martos I. Administration of *Bifidobacterium breve* PS12929 and *Lactobacillus salivarius* PS12934, two strains isolated from human milk, to very low and extremely low birth weight preterm infants: a pilot study. J Immunol Res 2015; 2015:538171; PMID:25759843

[140] Szymań ski H, Pejcz J, Jawień M, Chmielarczyk A, Strus M, Heczko PB. Treatment of acute infectious diarrhoea in infants and children with a mixture of three Lactobacillus rhamnosus strains–a randomized, double-blind, placebo-controlled trial. Aliment Pharmacol Thern 2006; 23(2):247-53; doi:10.1111/j.1365-2036.2006.02740.x

[141] McFarland LV. Deciphering meta-analytic results: a mini-review of probiotics for the prevention of paediatric antibiotic-associated diarrhoea and Clostridium difficile infections. Benef Microbes 2015; 6(2):189-94; PMID:24889895

[142] Kalliomäki M, Salminen S, Arvilommi H, Kero P, Koskinen P, Isolauri E. Probiotics in primary prevention of atopic disease: a randomised placebo-controlled trial. Lancet 2001; 357(9262):1076-9; PMID:11297958

[143] Deshpande G, Rao S, Patole S, Bulsara M. Updated meta-analysis of probiotics for preventing necrotizing enterocolitis in preterm neonates. Pediatrics 2010; 125(5):921-30; PMID:20403939

[144] Chu DM, Meyer KM, Prince AL, Aagaard KM. Impact of maternal nutrition in pregnancy and lactation on gut microbial composition and function. Gut Microbes 2016; 7(6):459-70(in press).