Review

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Bifidobacterial strains in the intestines of newborns originate from their mothers

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The gastrointestinal tract is believed to be colonized rapidly with bacteria immediately from birth. The source of these intestinal microbes is an ongoing topic of interest because increasing evidence suggests that the composition of the initial intestinal bacterial colonization strongly affects health. In particular, the source of bifidobacteria has received marked attention because these bacteria are suggested to play a crucial role in protecting against susceptibility to diverse diseases later in life. However, the source of these microbes has remained unclear. Recently, it was confirmed that mothers transmit their unique bifidobacterial strains to their children shortly after birth. The transmitted strains predominate during early infancy, suggesting that maternal intestinal bifidobacteria are an important source of the infant gut microbiota. Accordingly, maintenance of a healthy, balanced gut microbiota during pregnancy has an important positive influence on the newborn gut microbiota.

Key words: bifidobacteria, human milk, infancy, intestinal microbiota, mother-to-infant transmission

INTRODUCTION

The intestinal microbiota is a complex ecosystem with extensive metabolic activity; it comprises more than 1,000 bacterial species and accounts for 10^{11} to 10^{12} bacterial cells per gram of feces [1, 2]. These bacterial species are considered to play a role in their host's health and disease states by protecting against pathogens, processing nutrients, regulating fat storage, and stimulating angiogenesis [3–6]. The composition of the adult human intestinal microbiota is thought to be stable over a long period, although it can differ markedly among individuals [7]. In contrast, the gastrointestinal tract in infants is rapidly colonized with bacteria immediately after birth [8–10]; the composition of the intestinal microbiota is comparatively simple during breastfeeding, becomes complex after weaning starts, and remains stable into old age [11]. Accumulating evidence suggests that the initial intestinal colonization provides a vast microbial stimulus that leads to profound changes in the development of the gut and the mucosal immune system [12, 13]. Therefore, microbial colonization during infancy is thought to be essential for a lifetime of good health.

Bifidobacteria are among the most important and beneficial bacteria in the intestine, not only for adults but also for infants. Generally, bifidobacteria become the predominant microorganisms in the intestine within a week after birth, and they remain dominant until weaning [11]. Moreover, bifidobacteria seem to play a crucial role in protecting the host against pathogenic bacteria by helping to prime the mucosal immune system and consequently protecting against susceptibility to diverse diseases later in life [14].

Little information is available regarding the source of these intestinal bacteria. *Bifidobacterium* species are classified as typical anaerobic bacteria [15], and it has been hypothesized that the infant's intestinal microbes are acquired during transit through the birth canal [16]. Several studies have reported the isolation of *Bifidobacterium* species—including *B. adolescentis*, *B. bifidum*, *B. breve*, *B. catenulatum*, and *B. longum*—from vaginal swabs [17, 18]. However, little is known about the growth environment in the birth canals of pregnant women and the composition of the vaginal microbiota. Therefore, the origin of these intestinal microbes continues to draw attention.

Several recent studies using strain-level analyses have confirmed that mothers transmit their unique bifidobacterial strains to their infants shortly after birth [19–21]. In this review, I summarize those studies that have investigated the relationships between the maternal intestinal microbiota, the infant intestinal microbiota, and human breast milk.

MATERNAL INTESTINAL BIFIDOBACTERIA AND INFANT INTESTINAL BIFIDOBACTERIA

Several studies employing molecular biological techniques have suggested the possibility of intestinal microbiota being transmitted from mother to infant [18, 22–25]. The molecular methods used in those studies, such as real-time quantitative PCR (qPCR), are effective in identifying microorganisms at the species level but do not allow comparisons at the

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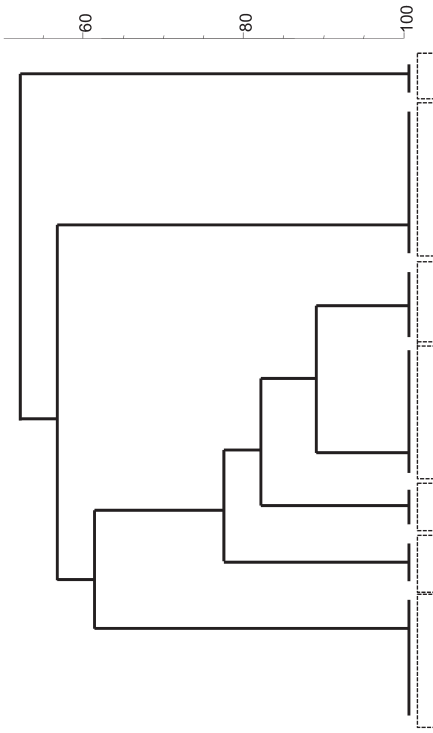

Strain no.	Family no.	Isolate source	Isolate period	Sequence type
2134	87	Mother	28 days before delivery	BIF-A*
2140	87	Infant	Day 3	BIF-A*
1487	76	Mother	17 days before delivery	BIF-B*
1481	76	Mother	10 days before delivery	BIF-B*
1469	76(A)	Infant (A)	Day 3	BIF-B*
1457	76(A)	Infant (A)	Day 7	BIF-B*
1475	76(B)	Infant (B)	Day 3	BIF-B*
1466	76(B)	Infant (B)	Day 7	BIF-B*
2480	134	Mother	64 days before delivery	BIF-C*
2482	134	Mother	54 days before delivery	BIF-C*
2491	134	Infant	Day 3	BIF-C*
2257	105	Mother	38 days before delivery	BIF-D*
2262	105	Mother	30 days before delivery	BIF-D*
2270	105	Infant	Day 3	BIF-D*
2275	105	Infant	Day 7	BIF-D*
2283	105	Infant	Day 90	BIF-D*
337	12	Mother	51 days before delivery	BIF-E*
1094	12	Infant	Day 30	BIF-E*
2338	96	Mother	36 days before delivery	BIF-F*
2356	96	Infant	Day 90	BIF-F*
175	14	Mother	53 days before delivery	BIF-G*
259	14	Mother	44 days before delivery	BIF-G*
649	14	Infant	Day 3	BIF-G*
690	14	Infant	Day 7	BIF-G*
1832	14	Infant	Day 30	BIF-G*

Fig. 1. Dendrogram of 25 individual *B. bifidum* strains (BIF) (modified from the study by Makino *et al.* [21]). *Isolates from both members of a mother–infant pair shared the same sequence type and cluster. Note that mother No. 76 gave birth to twins (A, B). The original publication is available at https://doi.org/10.1371/journal.pone.0078331.

strain level. Moreover, randomly amplified polymorphic DNA (RAPD) and pulsed-field gel electrophoresis (PFGE) analyses have been questioned as highly sensitive strain-level typing techniques due to factors in their basic designs that can potentially produce spurious bands [26, 27]. Therefore, further, more highly sensitive analyses are required to confirm what, when, and how bacteria are transferred from the mother or any other environmental sources to the infant.

Makino *et al.* investigated the relationship between maternal intestinal bifidobacteria and infant intestinal bifidobacteria by using multilocus sequence typing (MLST) [19, 20]. MLST uses sequence polymorphisms of a set of seven genes in the genome to generate data that can be used to differentiate between bacterial strains [28]. This method yields high-resolution, reproducible data and is, therefore, suitable for both species identification and strain typing [29].

Bifidobacterial strains were isolated from fecal samples that were collected from 17 healthy mother–infant pairs (vaginal delivery, 12 pairs; cesarean delivery, 5 pairs), living in Antwerp (Belgium) [20]. Fecal samples were taken from the mothers twice (at least 1 week apart) before delivery and from the infants at 0 (meconium), 3, 7, 30, and 90 days of age. Bifidobacterial strains were isolated from these samples and categorized by using MLST. In total, 273 bifidobacterial isolates were obtained, and five *Bifidobacterium* species (*B. adolescentis*, *B. bifidum*, *B. catenulatum*, *B. longum* subspecies *longum*, and *B. pseudocatenulatum*) were found to be monophyletic between individual mother and infant pairs. These findings confirmed that mother-to-infant transmission of several *Bifidobacterium* species occurs. Mother–infant monophyletic strains were continuously detected over time in the infant fecal samples (Fig. 1). These results suggested that the predominant strains in the intestines of the pregnant mothers were transferred to the intestines of their infants, increased in numbers soon after birth, and subsequently colonized the infants. These findings confirmed the initial studies that suggested the importance of mother-to-infant transmission of bacteria in the colonization of the gastrointestinal tract of neonates [22–25]. Moreover, in several families, two strains from different species were monophyletic, confirming that mother-to-infant transmission of several *Bifidobacterium* species can occur in parallel within a single family. These results further suggest that other commensal bacterial species might also be transmitted from the mother and colonize the intestine of the infant soon after birth.

Mother–infant monophyletic *Bifidobacterium* strains were obtained from 11 of 12 vaginally born infants (Table 1). Of note, monophyletic *Bifidobacterium* strains were not observed among the five infants delivered by C-section, verifying that mother-to-infant transmission of these strains occurred only among the vaginally born infants. These results suggest that the delivery mode may influence the occurrence of mother-to-infant transmission.

Table 1. Detection of mother–infant monophyletic *Bifidobacterium* strains among 17 mother–infant pairs (modified from the study by Makino *et al.* [21])

Mode of delivery	Family no.	Mother–infant monophyletic *Bifidobacterium* strains obtained from mother–infant pairs				
		B. adolescentis	*B. bifidum*	*B. catenulatum*	*B. longum* subsp. *longum*	*B. pseudocatenulatum*
Vaginal	12		●			
	14		●			
	16	●			●	
	66					
	76 Twin (A)[a]		●			
	76 Twin (B)[a]	●	●			
	87		●	●		
	96		●			●
	105		●			
	87				●	●
	121	●			●	
	134		●		●	
Cesarean	10					
	19					
	30					
	31					
	48					

Isolation of at least one mother–infant monophyletic *Bifidobacterium* strain from a family is indicated by a filled circle (●).
[a] Mother No. 76 gave birth to twins (A, B).
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The bifidobacterial counts of the monophyletic strain species were analyzed by qPCR. Among vaginally delivered infants, mother–infant monophyletic strain species in the intestines increased in number to become the predominant *Bifidobacterium* strains within 3 days of birth. In contrast, among infants delivered via C-section, the total number of bifidobacteria remained significantly lower than those for vaginally delivered infants until 7 days of age. This finding offers confirmatory evidence that intestinal colonization with bifidobacteria starts earlier among vaginally delivered infants than among those delivered by C-section [30]. Among infants born vaginally, the increase in the number of bifidobacteria within a few days of birth may be correlated with the occurrence of mother-to-infant transmission. Moreover, given that mother–infant monophyletic strains were not observed in infants delivered via C-section, the maternal strains were likely transmitted during transit through the birth canal.

Interestingly, not all mother–infant monophyletic *Bifidobacterium* strains were isolated throughout the sampling period. Mother–infant monophyletic *B. bifidum* and *B. longum* subsp. *longum* strains were isolated from the infant fecal samples for as long as 90 days after birth. The bacterial count showed that these were the predominant *Bifidobacterium* species from 3 until 90 days after birth (Fig. 2). In contrast, monophyletic strains belonging to *B. adolescentis* and *B. catenulatum* were not found in the infant fecal samples after 7 days of age. Moreover, although several vaginally delivered infants harbored these species at concentrations as high as 10^{10} cells/g of feces, these species were not dominant in the intestines of vaginally delivered infants during early infancy. These results suggest that some bifidobacterial species predominantly proliferate in, and colonize, the guts of vaginally delivered infants.

All infants in the study were exclusively breastfed for at least 2 months. It is widely known that human milk contains a variety of complex oligosaccharides (HMOs) that selectively stimulate the growth of specific bifidobacterial species [31, 32]. Typical infant species such as *B. bifidum* and *B. longum* effectively utilize HMOs [33–35], whereas adult-type bifidobacteria such as *B. adolescentis* are less efficient in utilizing HMOs [34, 35]. Therefore, breastfeeding and the presence of HMOs may be key factors that explain why, despite infants acquiring a wide spectrum of bifidobacterial species from their mothers, the species that are able to utilize HMOs remain as some of the predominant colonizers of the infant gut.

Several studies have suggested that environment factors, such as the presence of hospital staff or other infants, may also influence the composition of the intestinal microbiota [36–38]. Using plasmid profiling, Murono *et al.* found that hospital strains of *Escherichia coli* were transmitted horizontally among infants [38]. On the other hand, Makino *et al.* showed that none of the monophyletic bifidobacterial strains were identified in other infants born in the same hospital, and they found no evidence for the occurrence of horizontal transmission of bifidobacteria [20]. Moreover, mother–infant monophyletic strains of all five of the *Bifidobacterium* species

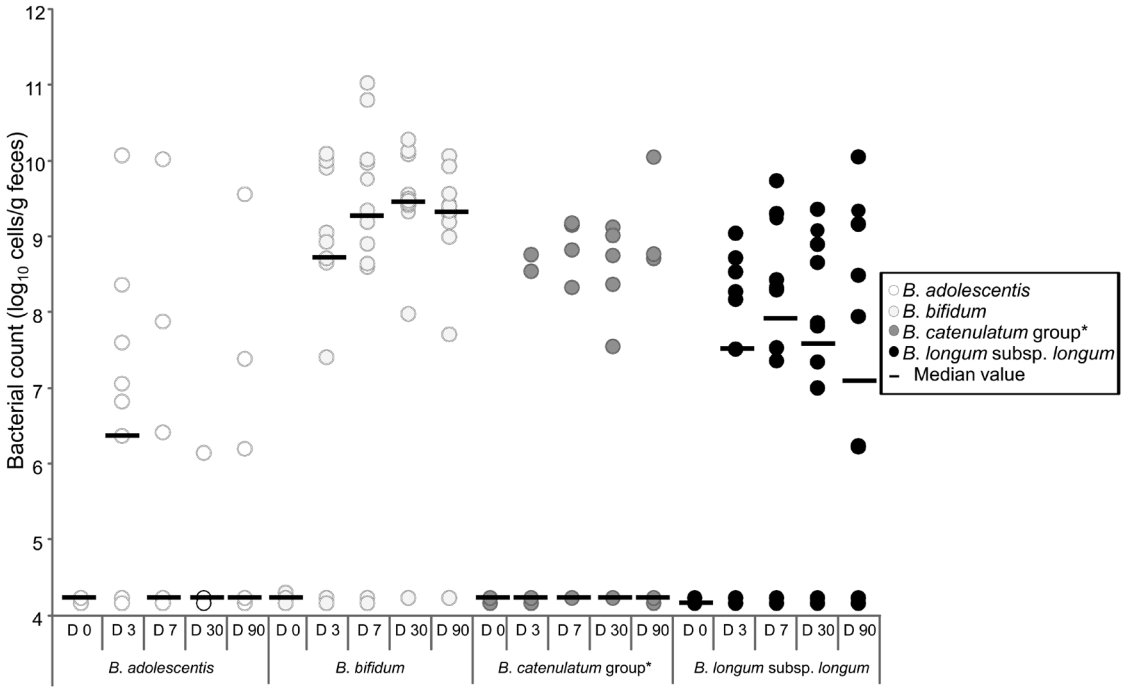

Fig. 2. Counts of each *Bifidobacterium* species in feces of infants aged 0 to 90 days (modified from the study of Makino *et al.* [21]).
*The *B. catenulatum* group comprises species of *B. catenulatum* and *B. pseudocatenulatum*. The original publication is available at https://doi.org/10.1371/journal.pone.0078331.

formed individual clusters for each family, suggesting that each family had its own unique bifidobacterial group that was transmitted from mother to infant. This vertical transfer concept is strengthened by the fact that *Bifidobacterium* strains from a mother who gave birth to twins were identified in the intestines of both infants (Fig. 1), suggesting that the host genetic background may also be a key factor that influences the composition of the intestinal microbiota. This is in agreement with the findings of previous studies reporting that each family harbors its own set of *Lactobacillus* and *Bifidobacterium* species [39, 40]. Taken together, these findings indicate that the delivery mode and the intestinal bifidobacterial strains of the mother are key determinants of the infant bifidobacterial microbiota during early infancy.

INFANT INTESTINAL BIFIDOBACTERIA AND BIFIDOBACTERIA IN HUMAN BREAST MILK

It has been suggested that bacteria from the human maternal gut reach the milk via the so-called entero-mammary pathway and thereby influence the colonization of the infant gut [41, 42]. Previous studies have demonstrated that strains of *B. breve* and *B. longum* subsp. *longum* found in the feces of human infants are identical to the strains found in their mother's milk [19, 43, 44]. Those studies hypothesized that there is vertical transmission of *B. breve* strains from maternal milk to infant, because *B. breve* strains were found to be monophyletic between the human milk and the infant gut within the same mother–infant pairs [43, 44]. However, given that detailed time series data of the isolates (including isolation periods) were not published in those studies, the question remains as to whether these shared bifidobacteria were transiently present or whether they colonized the infant's gut.

Makino *et al.* investigated whether the sharing of bifidobacterial strains between maternal milk and the infant gut is sustained over the period of breastfeeding [21]. In their study, 283 bifidobacterial strains were isolated from maternal human milk and infant feces collected at various time points from 102 healthy mother–infant pairs (for human milk, once before delivery, at delivery [colostrum], and at 7 and 30 days after delivery; for infant feces, at birth [meconium] and at 7 and 30 days of age). Isolates were identified by 16S rRNA gene sequencing and classified by using MLST. Bifidobacterial strains were obtained from human milk collected at 7 and 30 days after delivery, and in agreement with previous studies [45, 46], the most commonly isolated *Bifidobacterium* species was *B. breve*. In contrast, no bifidobacteria were isolated from any of the human milk samples taken before delivery or from colostrum. On the other hand, isolation from infant feces was confirmed sometimes as early as the day of birth (meconium).

Interestingly, strains belonging to infant-type bifidobacteria such as *B. breve*, *B. longum* subsp. *longum*, and *B. bifidum* species were the only species to be identified as monophyletic between infant feces and maternal milk. These strains were

Table 2. Timing of the isolation of each monophyletic *Bifidobacterium* strain from maternal human milk and infant feces (modified from the study by Makino *et al.* [22])

Species	Sequence type	Family no.	Isolate source	Time after delivery		
				Day 0	Day 7	Day 30
B. bifidum	BIF-A	105	Human milk	—	●	●
			Infant feces	—	●	
	BIF-B	12	Human milk	—	—	●
			Infant feces	—	—	●
	BIF-C	14	Human milk	—	—	●
			Infant feces	—	●	●
B. breve	BRE-A	20	Human milk	—	—	●
			Infant feces	—	●	●
	BRE-B	28	Human milk	—	●	●
			Infant feces	—	●	●
	BRE-C	130	Human milk	—	●	—
			Infant feces	—	●	—
	BRE-D	54	Human milk	—	—	●
			Infant feces	—	—	●
	BRE-G	87	Human milk	—	●	●
			Infant feces	—	●	●
	BRE-H	121	Human milk	—	—	●
			Infant feces	—	●	●
	BRE-I	32	Human milk	—	—	●
			Infant feces	—	—	●
	BRE-L	105	Human milk	—	—	●
			Infant feces	—	—	●
	BRE-M	76 (A)	Human milk	—	—	●
			Infant feces	—	—	●
		76 (B)	Human milk	—	—	●
			Infant feces	—	—	●
	BRE-N	96	Human milk	—	—	●
			Infant feces	—	●	●
B. longum subsp. *longum*	LON-A	134	Human milk	—	—	●
			Infant feces	—	●	●
	LON-B	134	Human milk	—	—	●
			Infant feces	—	●	●
	LON-C	8	Human milk	—	—	●
			Infant feces	●	●	—
	LON-F	18	Human milk	—	●	—
			Infant feces	—	●	●
	LON-G	129	Human milk	—	●	●
			Infant feces	●	●	●

Isolation of at least one monophyletic *Bifidobacterium* strain from a sample is indicated by a filled circle (●).
Non-shaded cells indicate families in which the earliest isolation of monophyletic strains was on the same day for both the infant feces and human milk.
Shaded cells indicate families in which the monophyletic strains were isolated earlier from the infant feces than from human milk.
Mother No. 76 gave birth to twins (A, B).
The original publication is available at https://doi.org/10.3920/BM2014.0082.

continuously detected in the mother's milk and infant feces throughout the period of breastfeeding, confirming that they were sustainably shared between the maternal milk and the infant gut. Moreover, the monophyletic strains were isolated from infant feces at time points the same as, or earlier than, when they were isolated from human milk; none were isolated

earlier from human milk than from infant feces (Table 2). Therefore, these results do not support those of previous reports that suggested that bifidobacteria are transmitted from human milk to the infant gut [41, 42, 45, 46]. In fact, the results of the study of Makino *et al.* suggest that bifidobacterial strains are transmitted from the infant to human milk during breastfeeding, given that infrared photography has revealed a high degree of retrograde flow back into the mammary ducts during suckling [47]. The probability of strain transmission from infant to human milk was significantly higher than from human milk to infant [21].

Because of the limitations of the methodology used by Makino *et al.* (i.e., the inherent bias that arises from the culture methods used), it remains unclear whether human milk is the first source of microbes for infants. However, the results confirm that human milk is a reservoir of bifidobacteria and that specific strains are shared between the infant intestine and human milk during breastfeeding.

CONCLUDING REMARKS AND OUTLOOK

Recent studies have confirmed that mothers who give birth vaginally transmit their unique, family-specific bacterial strains to the intestines of their infants during early infancy. These data suggest that the delivery mode and the maternal intestinal bifidobacterial strains are key factors in determining the infant bifidobacterial microbiota during early infancy. Maintenance of a healthy, balanced intestinal microbiota during pregnancy is an important factor that positively influences the newborn's intestinal microbiota. Factors such as nutrition in infants may influence the persistence of family-specific bifidobacterial strains in infants. Further advanced strain-level investigations will clarify how mother-to-infant transmission influences other components of the intestinal microbiota during infancy and hence the importance of the maternal gut, birth canal, and milk in the colonization of the neonate gut.

PRESENTATION AT A CONFERENCE

The contents of this article received the 2017 Japan Bifidus Foundation Research Encouragement Award and were presented at the Japan Bifidus Foundation's 22nd Annual Meeting of Intestinal Microbiology (May 31–June 1, 2018, Tokyo, Japan).

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