Bifidobacterium longum
subspecies *infantis*

B. infantis

Human Milk & *B. infantis*: Nature's Pre- and Pro-biotic for Infants

Anthony P. Thomas, PhD

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GOT (BREAST) MILK?

Human Milk & B. infantis: Nature's Pre- and Pro-biotic for Infants

This booklet provides a brief scientific review of the evidence revealing a critical relationship between complex, non-digestible sugars in mother's milk and the selective enrichment of beneficial bifidobacteria, particularly *Bifidobacterium longum* subspecies *infantis* (*B. infantis*), to establish a highly adapted gastrointestinal (gut) microbiota to optimally support growth, development, and protection of infants. The unique metabolic capacity of *B. infantis* to comprehensively utilize the diverse array of these complex sugars, to the exclusion of other bacteria, and the benefits conveyed to the developing infant highlights a key role for this single bacterial subspecies in laying the foundation for life-long health. Consequently, absence of *B. infantis* within the infant gut microbiota coincides with diminished bifidobacteria abundance and elevated levels of undesirable bacteria, which is associated with adverse health outcomes. Sadly, evidence suggests this has occurred over the last century as an unintended consequence of commonly employed medical practices in developed nations.

An opportunity exists to ensure the presence of this key beneficial infant colonizer via probiotic supplementation, but research has revealed *B. infantis* is commonly misidentified in commercially available products. Furthermore, there is widespread confusion among both consumers and healthcare practitioners with regard to probiotics and their appropriate, evidence-based clinical applications. Thus, education is vital to guide the recommendations and use of probiotic *B. infantis* strains in pregnant women and infants, which supplementation should be restricted only to strains that have been scientifically characterized with research and have demonstrated probiotic attributes and health benefits.

Anthony P. Thomas, Ph.D.

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Introduction

It is becoming increasingly clear that the bacterial ecosystem residing in our gut has a profound influence on our health. Bacterial colonization of the infant gut occurs during a critical period of gastrointestinal, immune, and brain development, with important short- and long-term health implications.

Bifidobacterium is a genus of bacteria well recognized for health-promoting functions. Bifidobacteria typically predominate in the intestinal microbiota of breastfed infants. However, recent evidence has revealed a general decline in bifidobacteria abundance and specifically the loss of the keystone infant colonizer, *Bifidobacterium longum* subspecies *infantis* (*B. infantis*), in breastfed infants, suggesting modern barriers to their natural evolutionary enrichment of the infant gut [1, 2].

Mom: Seeds & Feeds

The mother is the main source of bacteria for newborn colonization including those encountered in the birth canal via vaginal delivery, ingested bacteria from the mother's skin during breastfeeding, and bacteria derived from the maternal gut microbiota that have been incorporated into the breastmilk ("seeds") (**figure 1**). Mother's milk, shaped through evolution, is considered the perfect nutrition for a developing newborn.

Human milk is rich in unique, complex sugars called human milk oligosaccharides (HMOs), which contains between 12 - 14 g/L (in mature milk, but higher concentrations in early milk and preterm milk) of over 200 structurally different HMOs [3]. These sugars comprise the third largest component of milk, after lactose and fat, even in times of famine, yet provide no direct nutritive value to the infant since their structural complexity renders them non-digestible (**figure 2**). This apparent conundrum begs the evolutionary question, "Why would significant maternal energy be spent to produce HMOs in breast milk that cannot be used by the infant?"

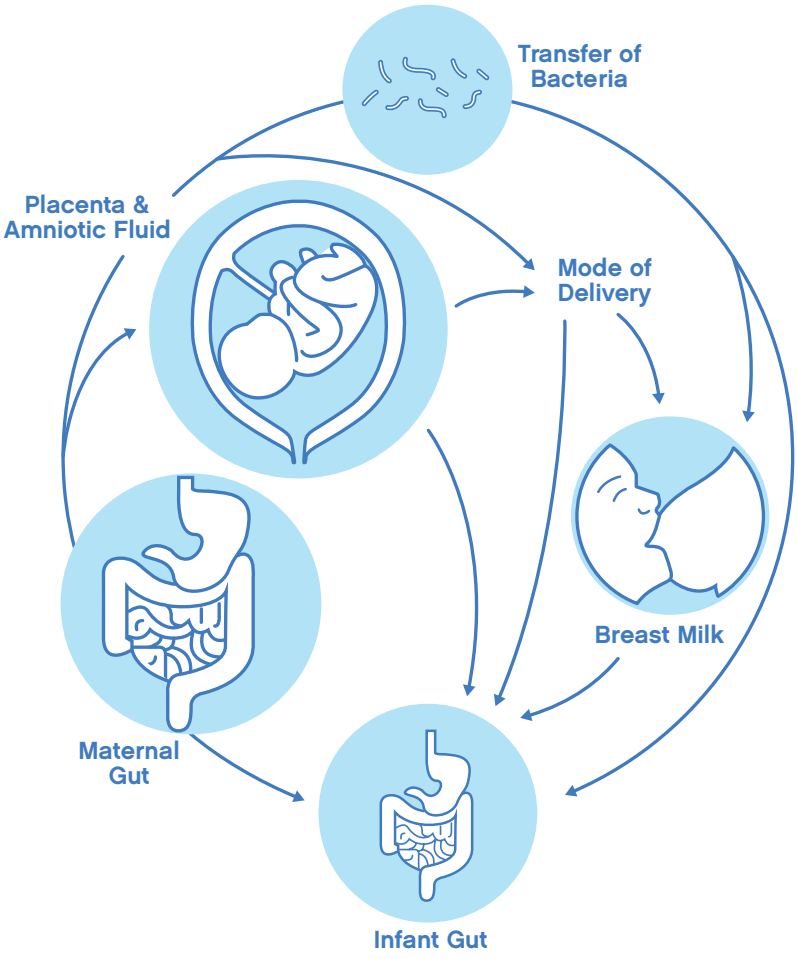


Fig 1. The gut microbiota programs host health – from mother to infant. Adapted from S. Rautava et al. Nature Rev Gastroenterol Hepatol 2012

Mom: Seeds & Feeds (cont'd)

As it turns out, HMOs are not food for the baby, but food for good gut bacteria. Yet, their structural complexity is also a barrier for use by most bacteria. Among the many bacterial species of the intestinal microbiota studied, only those of the *Bifidobacterium* and *Bacteroides* genera are able to utilize HMOs as a primary food source [4, 5]. Thus, a primary function of HMOs is to serve as a prebiotic substrate to selectively feed and promote the growth of select, beneficial bacteria, mainly bifidobacteria, residing in the colon ("feeds") (**figure 3**). However, HMO utilization is not equally observed across all bifidobacteria as many strains are not able to grow well on HMOs [6].

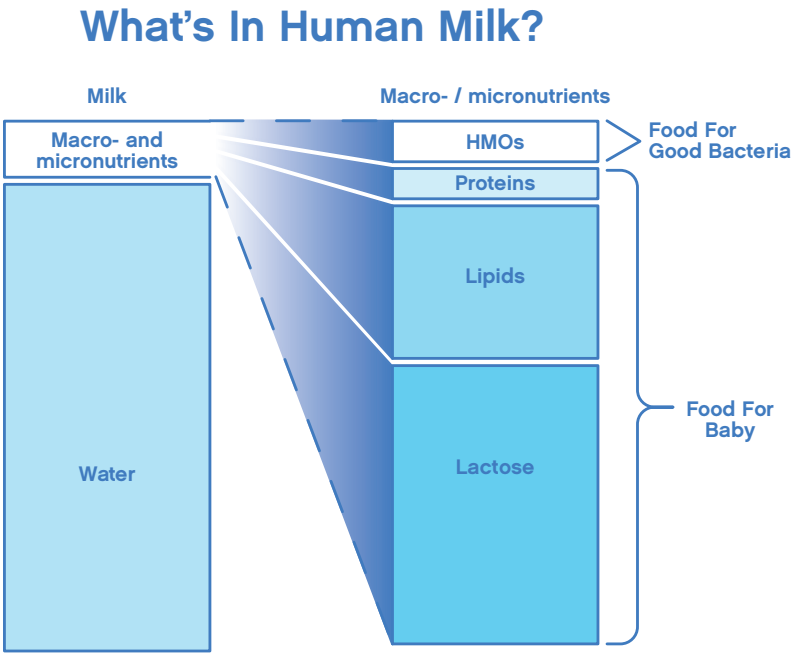


Fig 2. Human milk is uniquely rich in non-digestible oligosaccharides, which comprise the third largest component after lactose and fat. Adapted from A. Petherick et al. Nature 2010

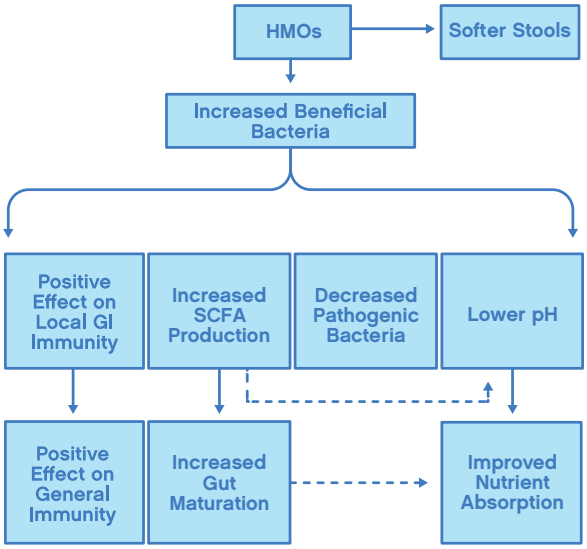
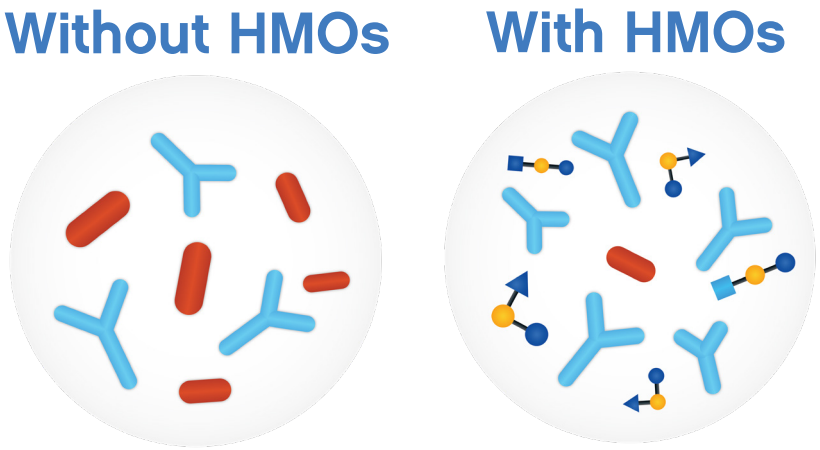
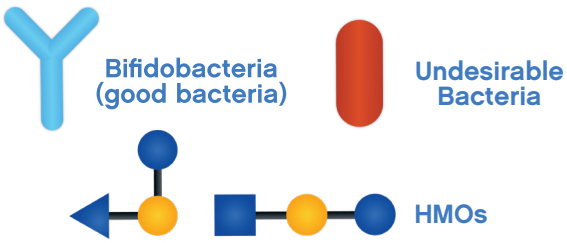


Fig 3. Beneficial effects of HMO consumption.





B. infantis
Bifidobacterium longum* subspecies *infantis

B. infantis:
"Champion Colonizer of the Infant Gut"

B. infantis is unique as the sole bacterium with the ability to import, digest, and consume all HMOs, which is the result of specific genes coding for carbohydrate transporters and enzymes to breakdown these complex sugars that are not found in other bacterial species [7, 8]. The genome and metabolic strategy adopted by *B. infantis* suggests it co-evolved with human milk to gain a competitive growth advantage. Thus, if *B. infantis* is present alongside HMOs, it will outcompete any other bacterium residing in the infant gut, thereby limiting their growth. Not surprisingly, *B. infantis* is often the dominant bacteria in the guts of healthy breastfed babies. Hence its name!

The competitive growth advantage of *B. infantis* in breastfed infants extends beyond free HMOs to other complex sugars attached to human milk proteins (glycoproteins) and lipids (glycolipids). For example, *B. infantis* produces an enzyme (endo-β-N-acetylglucosaminidase) that is able to cleave N-linked sugars associated with lactoferrin and immunoglobulins A and G [9]. Additionally, *B. infantis* has been shown to digest acidic glycolipids (sialic acid-containing gangliosides) [10] present on the surface of fat globules in human milk (the milk fat globule membrane) that are important for brain development [11] and shaping the composition of the gut microbiota in favor of beneficial bacteria [12].

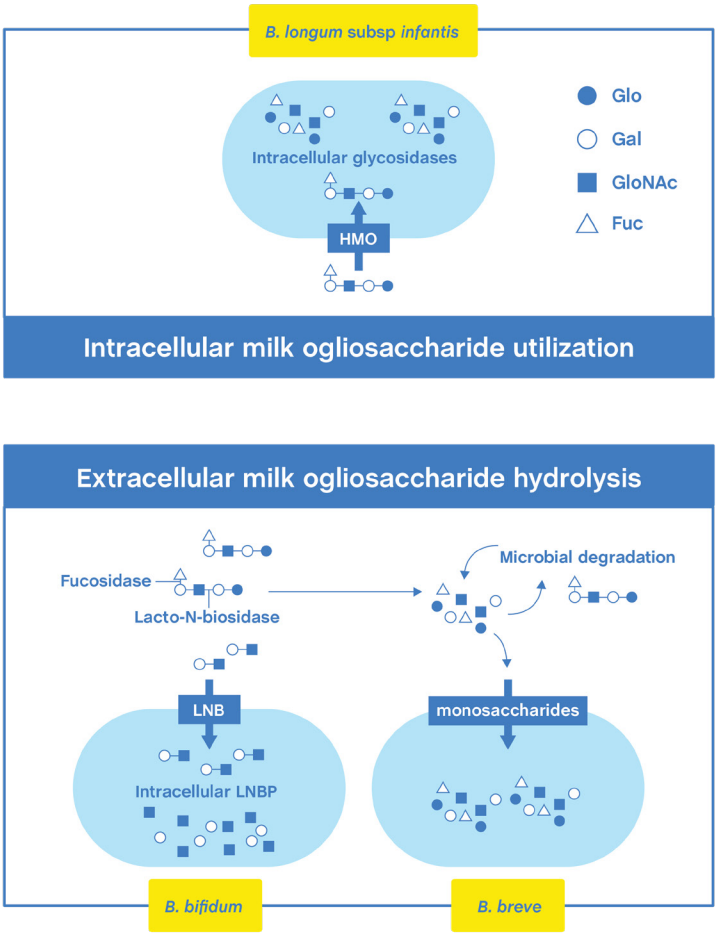


Fig 4. Species/strain-specific strategies for HMO import and catabolism. Adapted from A.M. Zivkovic et al. Proc Nat Acad Sci 2011

B. infantis specializes in the import of HMOs to completely digest and use within the cell ("inside eater") [13]. *Bacteroides* species and other prominent infant associated *Bifidobacterium* species such as *B. bifidum*, potentially rely on enzymes located on the exterior of cells to deconstruct portions of HMOs outside the cell [14]. Some of those deconstructed components are imported and utilized by the bacteria, while some components remain outside the cell ("outside eaters") (**figure 4**). This "outside consumption" mode has been shown to liberate sugar components that can promote the growth of non-beneficial bacteria that would otherwise be unable to utilize HMOs [15].

The gut colonization capacity of *B. infantis* is enhanced with exposure to HMOs. HMO-grown *B. infantis* demonstrated higher binding efficiency to intestinal cells than when grown on lactose or glucose, whereas *B. breve*, another prominent *Bifidobacterium* species of the infant gut, exhibited low adhesive capacity regardless of carbon source (**figure 5**) [16].

B. infantis:
"Champion Colonizer of the Infant Gut" (cont'd)

B. longum subspecies *longum* (*B. longum*), the closely related subspecies with substantially different metabolic capacity specialized for plant-derived carbohydrate metabolism [17], grows poorly on HMOs [18]. Among three probiotic *Bifidobacterium* strains (500 million live each of *B. infantis* M-63, *B. breve* M-16V, and *B. longum* BB536) administered orally to low birth weight babies, *B. infantis* showed the highest capacity for colonization of the infant gut (**figure 6**) [19].

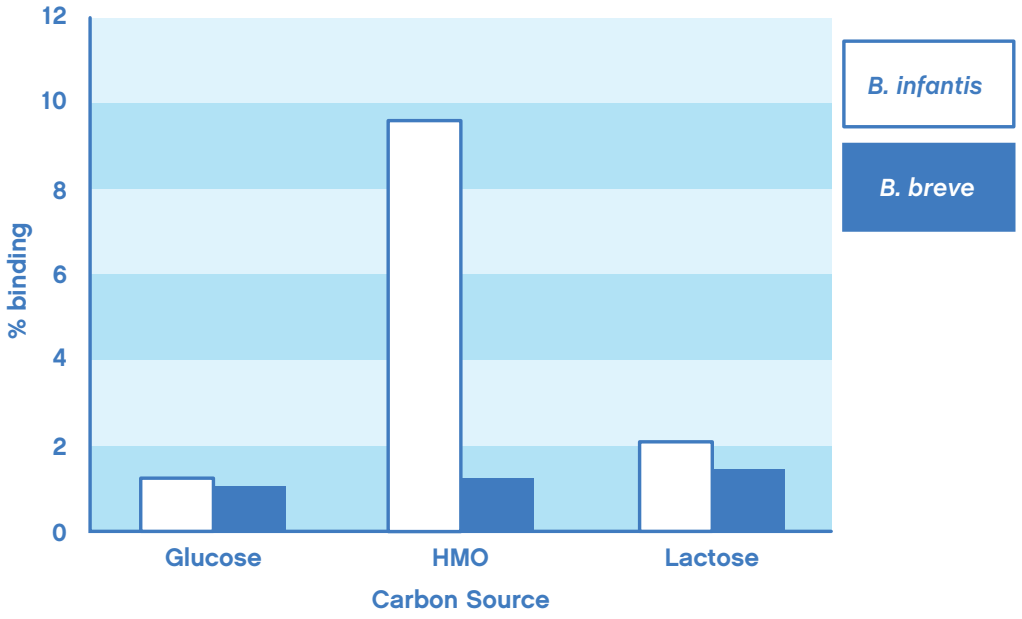


Fig 5. Binding of *B. infantis* (ATCC 15697) and *B. breve* (SC95) to human enterocyte-like cells (Caco-2). Adapted from S. Wickramasinghe et al. BMC Microbiology 2015

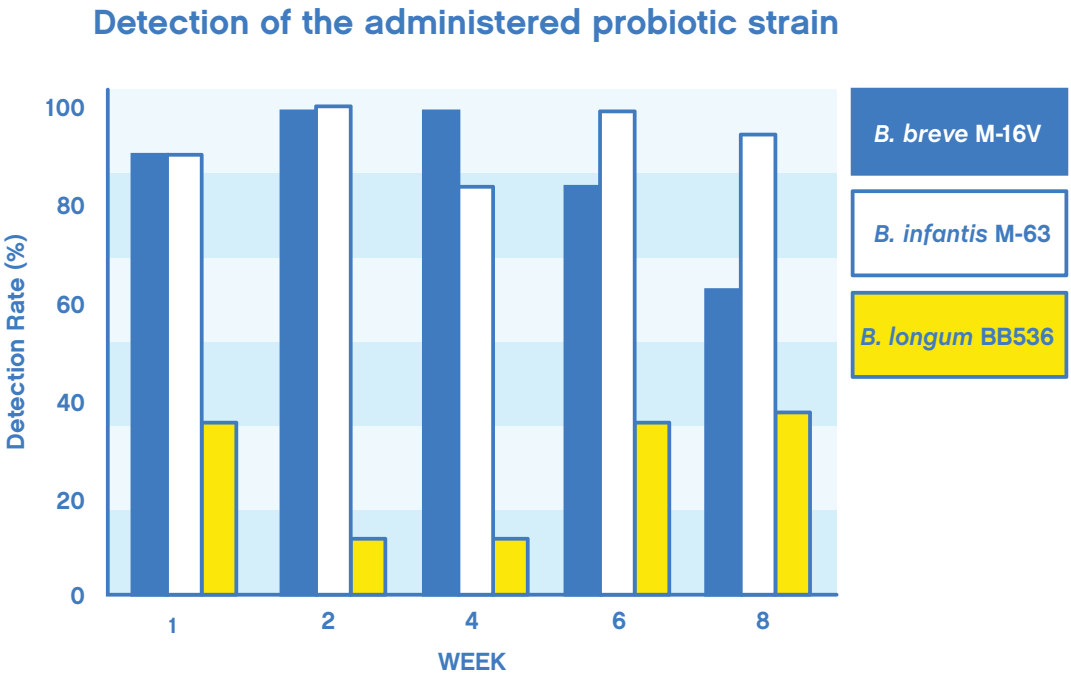


Fig 6. Colonization capacity of orally administered probiotic *Bifidobacterium* strains in low birth weight infants. Adapted from S. Ishizeki et al. Anaerobe 2013

A Win-Win Relationship

Human colostrum and early milk contain large amounts of free HMOs and glycoproteins to promote an ideal dietary niche within the intestinal tract of infants for colonization by *B. infantis*. Increased gut microbial diversity is generally considered beneficial in adults, but this is unlikely the case in newborns where predominance of relatively few *Bifidobacterium* species, of which are dominated by *B. infantis*, is associated with improved health outcomes (e.g., growth, immune development) [20].

An imbalance between the levels of good and bad bacteria in the infant gut, with increased levels of undesirable bacteria and less beneficial bacteria (dysbiosis), can increase risk for adverse immune- or metabolic-related health outcomes later in life. Acutely, infant gut dysbiosis can lead to gas, bloating, and discomfort that contributes to pain, fuzziness, and crying (colic). Increased levels of *B. infantis* in the infant gut have been associated with lower levels of non-beneficial bacteria and increased levels of total bifidobacteria, improved growth, gut maturation (e.g., reduce intestinal permeability or "leaky gut"), and vaccine responsiveness, as well as modulation of immune development and function [13].

Most HMO structures contain the sugars fucose or sialic acid: ~70% of HMOs in pooled milk are fucosylated and ~20% are sialylated [21]. Among bifidobacteria, other than *B. infantis*, only *B. breve* and *B. bifidum* produce a few of the enzymes needed to cleave some of the linkages (glycosidic bonds) between fucose or sialic acid with other sugars in HMOs, whereas *B. infantis* produces all enzymes needed to deconstruct all such bonds within complex HMO structures (**figure 7**) [8].

Of 24 tested human-isolated and commercial probiotic *Bifidobacterium* and *Lactobacillus* strains (e.g., *B. lactis* BB-12®, *L. rhamnosus* - LGG®, *L. reuteri* DSM 17938/*L. reuteri* Protectis®), only the *B. infantis* strains (*B. infantis* M-63 and *B. infantis* ATCC 15697) were able to ferment common fucosylated and sialyated HMOs: 2'-fucosyllactose (FL), 3'-FL, 3'-sialyllactose (SL), and 6'-SL [22].

Species / Subspecies	Total glycoside hydrolases	α-Sialidase	α-L-Fucosidase
B. adolescentis	22	0	0
B. angulatum	13	0	0
B. bifidum	17	2	2
B. breve	19	1	1
B. catenulatum	21	0	0
B. dentium	31	0	1
B. longum subsp *longum*	26	0	0
B. longum subsp *infantis*	24	2	5
B. minimum	2	0	0
B. psudocatenulatum	25	0	1
B. psudolongum	14	0	1
B. subtile	3	0	0
B. thermacidophilum	9	0	0

Fig 7. Fucosidases and sialidases in *Bifidobacterium* species. Adapted from A. Zivkovic et al. Proc Nat Acad Sci 2011

The short-chain fatty acid, acetic acid (acetate), is produced from the metabolism of fucose by bifidobacteria that are able to utilize fucosylated HMOs such as 2'- FL, typically one of the most abundant HMOs (**figure 8**). Production of acetic acid by *B. infantis* from the utilization of fucosylated HMOs acidifies the gut lumen to lower the pH, which is not conducive to the growth of many non-beneficial bacteria. Research has shown infant gut microbiotas dominated by *B. infantis* have higher acetic acid concentrations, lower pH, lower residual HMOs lost in the feces (i.e., increased utilization of HMOs), with significantly more bifidobacteria and fewer undesirable bacteria [23]. Specifically, Matsuki et al. [23] observed significantly lower Enterobacteriaceae abundances from infants with FL-utilizing bifidobacteria-

A Win-Win Relationship (cont'd)

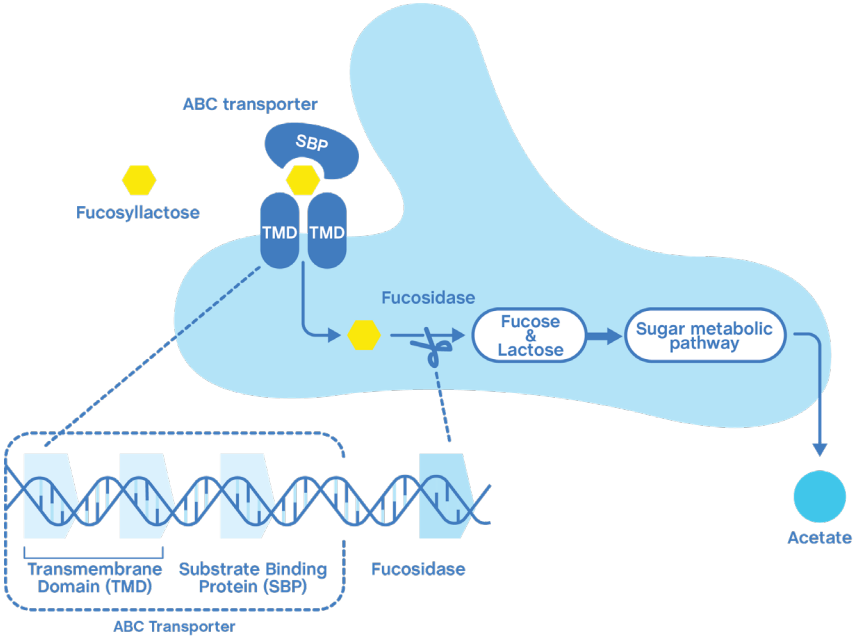


Fig 8. Molecular mechanisms of fucosyllactose utilization by select *Bifidobacterium* species. Adapted from T. Matsuki et al. Nature Comm 2016

dominated gut microbiotas compared to those with non-FL-utilizing bifidobacteria dominated and Enterobacteriaceae-dominated gut microbiotas. Correlations between reduced Enterobacteriaceae abundance and lower susceptibility to infection have been demonstrated in both animal models [24] and humans [25].

Acetic acid not only contributes to a lower luminal pH but also serves as a nutrient source for host colonocytes without releasing oxygen into the lumen. Thus, discouraging growth of facultative anaerobes (e.g., Enterobacteriaceae), whereas, in the absence of short chain fatty acids, the luminal pH is elevated and colonocytes must utilize other fuel sources (e.g., lactose) that can increase gut luminal oxygen, which is more favorable to the growth of facultative anaerobes (e.g., *E. coli*) and less to strict anaerobes (e.g., bifidobacteria) [26].

Fukuda et al. [27] showed increased production of acetate by certain protective *Bifidobacterium* strains (that possessed a specific carbohydrate transporter; i.e., *B. infantis* and *B. longum*, but not *B. adolescentis*) prevented translocation of Shiga toxin from the gut lumen to the blood (i.e., reinforced intestinal barrier function), thereby protecting germ-free mice against death from an otherwise lethal oral dose of enterohemorrhagic *E. coli* O157:H7 (**figure 9**). Furthermore, the researchers showed acetate treatment of human colonic epithelial cells (Caco-2) prevented the *E.coli* O157-induced reduction in intestinal barrier function and translocation of Shiga toxin from the apical to the basolateral side of the cells.

Sialic acid is a critical nutrient for optimal brain development as a vital component of brain gangliosides that play an essential role in the transmission and storage of information in the brain. The rate of human brain growth in the first year of life is greater than any other organ or body tissue [28]! Neurons are already formed at birth, but the synaptic connections between these cells are primarily established and expanded after birth, creating a high nutritional demand for the production of gangliosides [29]. Notably, there is up to four times more sialic acid in the brain cortex of humans vs. several other tested mammals [30]. The concentration of sialic acid in the brain of breastfed babies is higher than their formula-fed counterparts [31]. Sialic acid can be released during the degradation of sialic acid-containing HMOs, glycoproteins, and glycolipids by *B. infantis* to support this accelerated brain development.

A Win-Win Relationship (cont'd)

Essentially, colonization of the infant gut by *B. infantis* is required to exploit the full benefits of nature's perfect nutrition for developing infants, which selectively reinforces this beneficial bacterium during this vulnerable period. It is anticipated that *B. infantis* would predominate, or at least be present at high levels, within the intestinal microbiota of all breastfed infants. However, recent comparisons of the infant gut microbiota across diverse demographic regions has revealed loss of *B. infantis* and reduced bifidobacteria abundance within the intestinal microbiota of infants in resource-rich nations/regions, such as the U.S. and Europe, compared to relatively resource-poor areas such as South Asia and sub-Saharan Africa [8, 20].

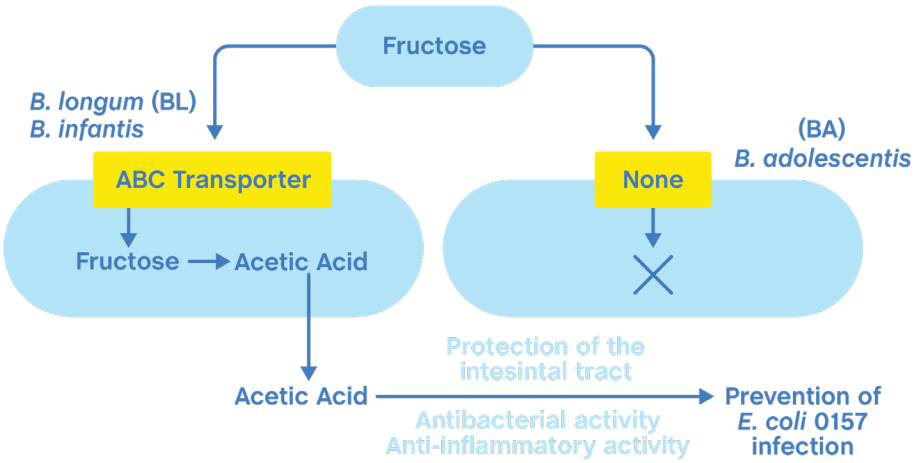
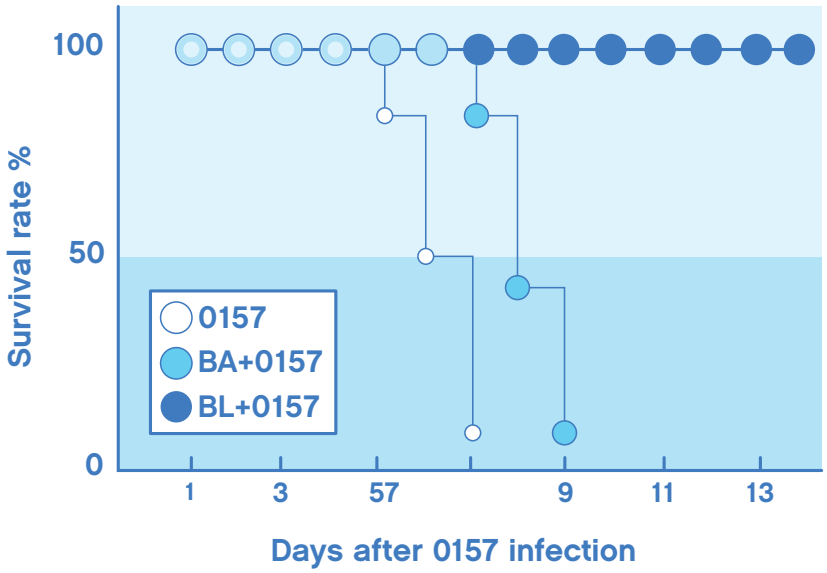


Fig 9. Acetic acid is more potent than other acids for inhibiting growth of pathogenic *E. coli*. Adapted from S. Fukuda et al. Nature 2011



Missing in Action?

Recent evidence suggests that infant fecal pH has significantly increased (5.0 to 6.5) over the last century coincident with loss of highly specialized infant *Bifidobacterium* species and increased levels of non-beneficial bacteria reflective of dysbiosis (**figure 10**) [2]. Infant fecal pH is strongly associated with bifidobacteria abundance in breastfed infants [23, 32], so loss or absence of keystone *Bifidobacterium* colonizers (i.e., *B. infantis*) is a likely contributor to the observed increase in fecal pH and levels of non-beneficial bacteria.

Again, the mother is the primary source of bacteria for newborn colonization. Commonly employed, modern medical practices that can interrupt the transfer of bacteria from mother to child are thought to have played a role in the loss of *B. infantis* and general decline of bifidobacteria in the infant gut across generations.

Of course, the type of infant feeding is a primary factor contributing to the development of the infant gut microbiota. Breast milk has been the principle source of nutrition for infants over the evolutionary history of humans, whereas the relatively recent introduction and rapid rise in infant formula feeding has disrupted the typical development of the intestinal microbiota.

Formula-fed infants have lower levels of bifidobacteria compared to those that are breastfed [33] as infant formula is lacking in HMOs and other human milk glycans (i.e., glycoproteins and glycolipids) that function as prebiotic substrates to shape the gut microbiota via enrichment of select *Bifidobacterium* species and restricting growth of other bacteria. *B. infantis* is the bacterium most specialized for the utilization of the diverse range of HMOs as growth substrates. Thus, increased use of infant formula may explain in part the loss of this keystone infant colonizer within the gut microbiota of infants in the U.S. and Europe, whereas *B. breve* and *B. longum*, which can utilize complex sugars in mucins and plants [34], are still relatively abundant. However, no amount of prebiotic substrate can enrich a bacterium that is not present.

Delivery via C-section worldwide has increased to unprecedented levels, particularly in the last quarter century, although there is a gap between higher- and lower-resource settings [35]. C-section delivery limits the natural fecal-oral transfer of bifidobacteria from mother to infant associated with vaginal delivery [36, 37]. Additionally, antibiotic use is common in both pregnant mothers (e.g., during labor to prevent the transmission of group B *Streptococcus*) and infants, as well as prophylactically in babies born prematurely. The infant gut microbiota is more susceptible to disturbances from antibiotics as many infant-associated bifidobacteria are sensitive to commonly used antibiotics [38]. Early-life exposure to antibiotics has been shown to diminish levels of bifidobacteria within the gut microbiota of infants [39].

B. infantis
Bifidobacterium longum* subspecies *infantis

Pleased to Make Your (Re)Acquaintance

Breast milk is the recognized perfect source of nutrition to support the growth, development, and protection of infants. According to the American Academy of Pediatrics (AAP), the World Health Organization (WHO), United Nations International Children's Emergency Fund (UNICEF), and the Center for Disease Control (CDC), exclusive breastfeeding is recommended for the first six months of life, followed by breastfeeding in combination with the introduction of complementary foods until at least 12 months of age, and continuation of breastfeeding for up to two years and beyond or as long as mutually desired by mother and baby.

B. infantis is the keystone bifidobacteria colonizer of the infant gut needed to get the most benefits out of mother's milk. The provision of HMOs and other complex sugars attached to proteins and lipids in human milk creates an ideal and unique nutrient niche for the expansion and predominance of *B. infantis* within the gut microbiota of infants, which in return, conveys various health benefits to the developing infant during this critical and vulnerable period.

However, the unintended consequences of some commonly employed modern medical practices have become a barrier to the transfer of *B. infantis* from mother to child and enrichment of the infant gut by health-promoting bifidobacteria. Thus, oral supplementation of infants with a genuine probiotic *B. infantis* strain, such as *B. infantis* M-63, is likely warranted to ensure exposure to this keystone colonizer of the infant gut as long as breast milk is on the menu.

This recommendation may be even more prudent for exclusively and primarily formula-fed infants given the inability of formulas to foster as high levels of bifidobacteria within the infant gut as breast milk. Although, some HMOs are now being synthesized and fortified into some infant formulas, such as the abundant fucosylated HMO, 2'-FL, that can serve as prebiotic support for *B. infantis* within the formula-fed infant gut.

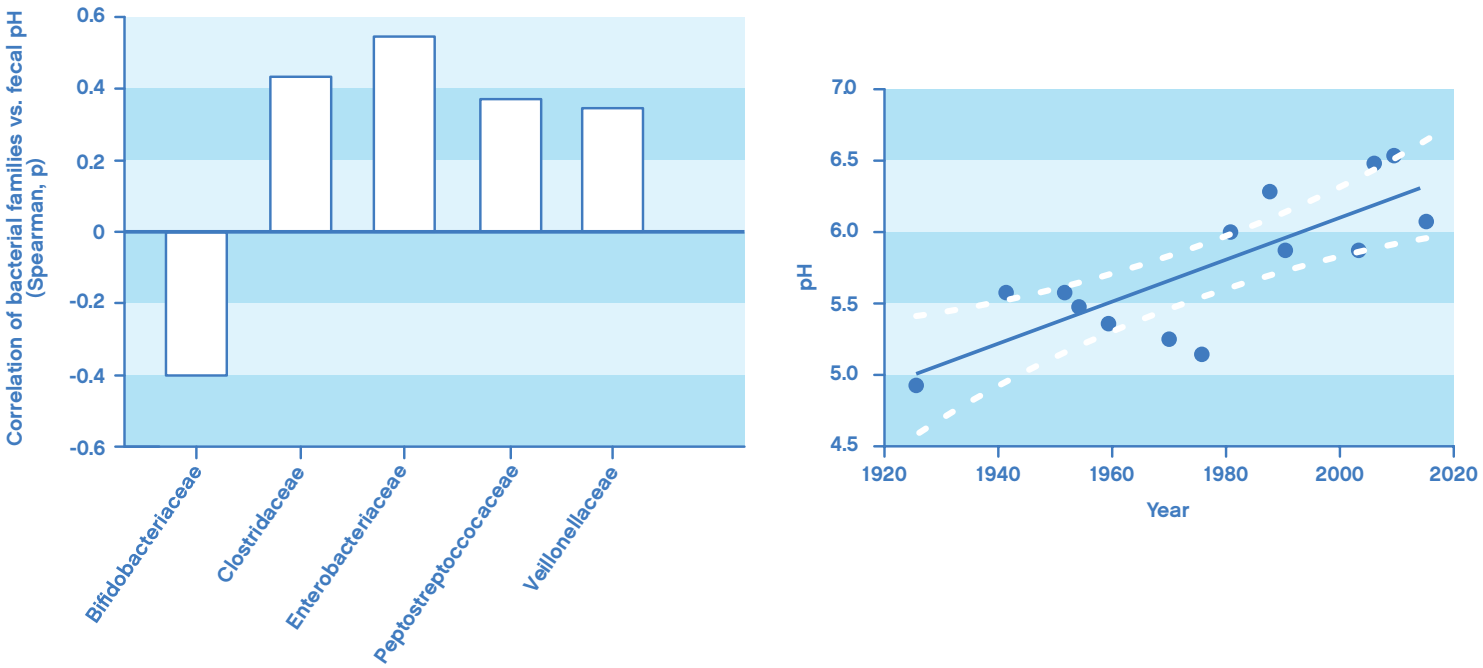


Fig 10. Correlation of bacterial families with fecal pH and positive correlation (solid line) and 95% confidence interval (dashed line) of fecal pH and publication year from clinical studies reporting from healthy, breastfed infants. Adapted from B. Henrick et al. mSphere 2018

Be Sure It's *B. infantis*

While there is good evidence to support the use of *B. infantis* as a probiotic for infants, research has revealed the advertised content of many commercial products containing bifidobacteria can vary significantly from their actual content with *B. infantis* commonly misidentified in commercial probiotics (i.e., not actually present) [40].

B. longum has two subspecies in humans (subspecies *longum* and subspecies *infantis*) that have historically been very challenging to distinguish as not distinguishable using commonly employed gene sequencing methods. Again, while closely related, *B. longum* and *B. infantis* possess very different capacities for HMO utilization [17]. Lewis et al. state, "The risk of species and subspecies misidentification is high, especially given the recently refined definition of these two *B. longum* subspecies further confirmed through genome sequencing." Therefore, it is pertinent that the identity of *B. infantis* used in a commercial probiotic is verified and best to use products manufactured by a reputable company that has a long-standing reputation for producing quality probiotic supplements.

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B. infantis

Human Milk & *B. infantis*: Nature's Pre- and Pro-biotic for Infants

by Anthony P. Thomas, PhD

Bio

Anthony Thomas, Ph.D. earned his B.A. in Nutrition, Food Science, and Dietetics from California State University Northridge, his doctorate in Nutritional Biology from the University of California at Davis, and conducted postdoctoral research at the University of California at Los Angeles Larry Hillblom Islet Research Center.

His primary research interests (via both pre-clinical and clinical studies) have focused on the influence of dietary and lifestyle factors (i.e., physical activity, circadian disruption) on the pathogenesis of chronic cardiovascular/metabolic diseases including obesity, insulin resistance syndrome, and type 2 diabetes.

He has authored/co-authored multiple peer reviewed scientific manuscripts and has served as a referee with relevant expertise in the fields of nutrition, obesity, and diabetes for multiple scientific journals.