The gut microbiome is more malleable in the first two years after birth, allowing probiotics to make their mark. Can we exploit this to improve infants' health?

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In the fall of 2018, a team of researchers from the Weizmann Institute of Science in Israel published findings that a cocktail of 11 strains of *Lactobacillus* and *Bifidobacterium* had minimal immediate impact and no lasting effect on the makeup of the gut microbiome of mice or people. In fact, the probiotic bacteria were not found in any of the fourteen adult participants after supplementation ended.

These recent findings received quite a lot of press and added to growing sentiment among the public that probiotics—live microorganisms that are purported to confer benefits on the human host—don't work. Decades of research have shown that most probiotics aren't able to colonize or exert lasting benefits in the human gut. Some critics even suggested that probiotics may not be a promising avenue for treating disease or otherwise improving health and wellness. But we thought: "Don't throw the baby out with the bathwater—our work shows that the right probiotic can work in the infant gut." Findings we published in 2017 showed that feeding breastfed babies a probiotic that included a specific strain of *Bifidobacterium longum* subspecies *infantis* (*B. infantis* EVC001) resulted in a 10,000,000-fold average increase in levels of fecal *B. infantis*. This level persisted for one month after the supplement was consumed, and levels remained elevated for up to one year after treatment.

To understand why the infant gut microbiome changed so drastically over the past century, we sought to understand how the infant gut microbiome forms.

Colonization of the infant gut by *B. infantis* had protective effects, such as lower levels of potential gut pathogens and fecal endotoxin, an outer membrane component of Gram-negative organisms known to trigger inflammation. We also found that infants given the *B. infantis* probiotic had reduced intestinal inflammation compared with breastfed infants who did not receive the probiotic. The gut microbiomes of *B. infantis* supplemented babies harbored fewer antibiotic resistance genes—a sign of fewer pathogens—and showed less degradation of mucin, a glycoprotein secreted by the intestinal epithelium that protects epithelial cells from direct contact with gut microbes. These data support earlier findings from Mark Underwood and colleagues at the University of California, Davis. In 2013, Underwood's team showed that feeding preterm infants a different strain, *B. infantis* ATCC15697, resulted in greater increases in fecal *Bifidobacterium* and reduced levels of potential pathogens compared with infants given a probiotic containing *B. lactis*.

While the scientific community and the public grappled with repeated findings that probiotic supplements taken by adults are not consistent in effectively colonizing the gut or conferring benefit, we now had convincing evidence that babies' gut microbiomes responded incredibly well to specific strains of *B. infantis*. The question was why.

Microbiome origins

Hints about the infant microbiome can be found in century-old articles on commensal bacteria in infant feces. W. R. Logan, a clinical pathologist at the Research Laboratory of the Royal College of Physicians in Edinburgh, was the first to report, 100 years ago, that bacteria in fecal smears from breastfed infants were a near monoculture of *Bacillus bifidus*, which is today known as the genus *Bifidobacterium*. Fecal smears from formula-fed infants of that time, by contrast, had a diversity of bacteria, with relatively few *Bifidobacterium*—more similar to the microbial diversity found in today's breastfed infants.

These striking changes in the gut microbiome composition seen over the past century were consistent with our recent finding that the fecal pH in breastfed infants dramatically increased from pH 5.0 to 6.5 within the past 100 years, a change associated with an apparent generational loss of *Bifidobacterium* and concomitant increase in potential pathogens. The reduction in *Bifidobacterium* in the gut microbiome of breastfed infants is likely an unintended consequence of medical practices that can save lives but do not support the growth of *Bifidobacterium*. Such medical practices include treatment with antibiotics to which *Bifidobacterium* are sensitive; infant formula that doesn't provide the specific food the bacterium requires; and greater numbers of cesarean section deliveries, which bypass the route by which the bacterium is transferred from mother to baby. These medical practices have been implicated in the increased risk for allergic and autoimmune diseases prevalent in resource-rich nations. The reduction in *Bifidobacterium* and increase in proinflammatory microbes in early infancy is proposed to occur during the critical window of immune system development, and thereby may increase the risk for immune disease later in life.

To understand why the infant gut microbiome changed so drastically over the past century, we sought to understand how this community forms. Infant gut microbiome colonization begins at delivery with exposure to maternal microbes—mostly vaginal and fecal microbes for vaginally delivered babies or predominately microbes from the skin, mouth, and surrounding environment in infants born by cesarean delivery. After birth, infants are bombarded by a vast array of microbes found in the environment, including in breast milk, but the species that go on to become durable members of the microbial community are often those transmitted by the infants' mothers through physical contact.

Children continue to acquire gut microbiome species from their mothers and others in the community during early life. This stands in contrast to an adult's gut microbiome, which is stable and resists change largely because the available space and food is already used by established microbes—the ecological niches are simply occupied in adult guts. Thus, it makes sense that a probiotic has a better chance of persisting in the infant gut, where it faces less competition, and therefore is more likely to have food it can consume and a location where it can grow. A probiotic serves as just one more source of exposure to new bacteria for the infant.

Recognizing this, we began to wonder: In our studies, what ecological niche did *B. infantis* fill that supported its persistence in infants long after probiotic administration stopped.

Setting the stage

A major factor in determining which bacteria thrive in the gut is the availability of their carbohydrate food sources. Thus, for a probiotic to work in an infant, microorganisms should be selected so that the food source they use most efficiently matches what's available—a food that is present and not already being

consumed by other bacteria. We set out to determine what carbohydrates *B. infantis* consumes in the infant gut.

Naturally, we turned to breast milk, which for millions of years has been the single food that can exclusively nourish and protect babies for the first six months of life. Human milk delivers nutrients as well as non-nutritive, bioactive molecules, including carbohydrates known as human milk oligosaccharides (HMOs). Back in the mid-1900s, Paul György, a world-renowned biochemist, nutritionist, and pediatrician from the Hospital of the University of Pennsylvania, and colleagues unknowingly referred to HMOs when they proposed the existence of a "bifidus factor," something unique in breast milk that fed *Bifidobacterium*. While humans cannot digest HMOs, it turns out that *Bifidobacterium*, especially *B. infantis*, can. In 2007, our group at UC Davis used mass spectrometry–based tools coupled with microbiology to show that *B. infantis* gobbles up HMOs as its sole energy source, while other species of *Bifidobacterium* consume only some HMOs in addition to plant-, animal-, and host-derived carbohydrates.

HMOs are a diverse class of complex carbohydrate molecules synthesized by the mammary gland. With approximately 200 different molecular species, they represent the third most abundant solid component in human milk following lactose and fat. Because HMOs are complex and vary in structure, they are expensive to manufacture. Current infant formulas may contain one or two simple HMO structures, but at a fraction of the concentration found in breast milk. Infant formulas lack the abundance and complexity of HMOs to selectively feed beneficial gut microbes and to bind and neutralize pathogens from the gut.

The bacterial species in the infant gut capable of consuming HMOs can be considered the milk-oriented microbiome (MOM). Although *B. infantis* appears to be the most efficient consumer of HMOs, other species of *Bifidobacterium*, in particular, *B. breve* and *B. bifidum*, can and do consume some HMOs but also consume plant-, animal-, and host-derived carbohydrates. The *Bifidobacterium* species that colonize the gut change throughout life in response to available carbohydrates in the host diet. For instance, *B. infantis*, *B. breve*, and *B. bifidum* are MOM bifidobacteria typically found in the stool of exclusively breastfed infants, while *B. longum* and *B. adolescentis*, which preferentially consume plant- and animal-derived carbohydrates, are typically found in the stool of adults. Yet there is variation and overlap in the species present at different life stages.

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Of the MOM bifidobacteria found in the infant gut microbiome, different species may have different implications for the microbiome. For example, when we gave exclusively breastfed infants a supplement with the probiotic *B. infantis* EVC001, their gut became dominated by the genus *Bifidobacterium*—upwards of 80 percent relative abundance of the gut microbiome—and potential pathogens made up less than 10 percent of the community. On the other hand, the gut microbiomes of exclusively breastfed infants who were not supplemented with *B. infantis* EVC001 had much lower levels of *Bifidobacterium*, with only about 30 percent relative abundance, and potential pathogens constituted about 40 percent of the microbes in their gut, findings that are consistent with previous work from our group and others. This near-monoculture of *Bifidobacterium* appeared to be driven by *B. infantis*, which represented about 90 percent of the total *Bifidobacterium* in infants fed the probiotic. In contrast, *B. longum* was the predominant gut *Bifidobacterium* in the control group, followed by *B. breve* and *B. bifidum*. These data highlight the vital importance of strain specificity in probiotics, and the combination of the presence of *B. infantis* and breastfeeding to support a protective gut environment in infants.

To understand how supplementary *B. infantis* can so successfully outcompete other microbes in the infant gut, we took a deep dive into its feeding strategy. Turns out it is a picky eater, exclusively dining on HMOs, and when HMOs are abundant, *B. infantis* gobbles them up ravenously. Unlike other MOM bifidobacteria, *B. infantis* possesses all the genes necessary for the complete, internal degradation of HMOs and preferentially uses HMOs over any other carbohydrate source. Other MOM bifidobacteria such as *B. bifidum* and *B. breve* strains display growth capabilities with only a subset of HMOs. *B. infantis* thus has a competitive advantage when breast milk makes up the entire diet.

A 2008 study from colleagues at UC Davis and their collaborators showed how *B. infantis* makes quick use of HMOs: with binding proteins to grab HMOs from the gut lumen and transporters to usher them into the cytoplasm, breaking them down into monosaccharides that are then fermented into lactate and the short-chain fatty acid acetate that are secreted from the cell. These end products maintain a lower pH in the intestinal milieu, supporting the transport of these compounds into the intestinal epithelium for use by the host and creating an undesirable environment for potential pathogens. The production of acetate also blocks the infiltration of toxic molecules produced by pathogenic bacteria by enhancing intestinal barrier function and inhibiting pro-inflammatory and apoptotic responses. Recent findings from one in vitro study have shown that the amount of acetate and lactate produced by different bifidobacterial species is dependent on how well they consume the carbohydrates available to them. Hence, feed a carbohydrate-consuming microbe its preferred carbohydrate, and it has greater potential to produce more of its protective end-products.

Another reason why *B. infantis* outcompetes other bifidobacterial strains in the gut of breastfed infants is that all of its HMO digestion happens inside the bacterial cell. *B. bifidum*, on the other hand, digests HMOs externally. This extracellular digestion liberates simple carbohydrates and may cross-feed other species of *Bifidobacterium*, but also cross-feeds and thus opens an ecological niche for other, perhaps less beneficial microbes. Cross-feeding among microbes diversifies the gut microbiome, which is considered to be generally beneficial in adults.

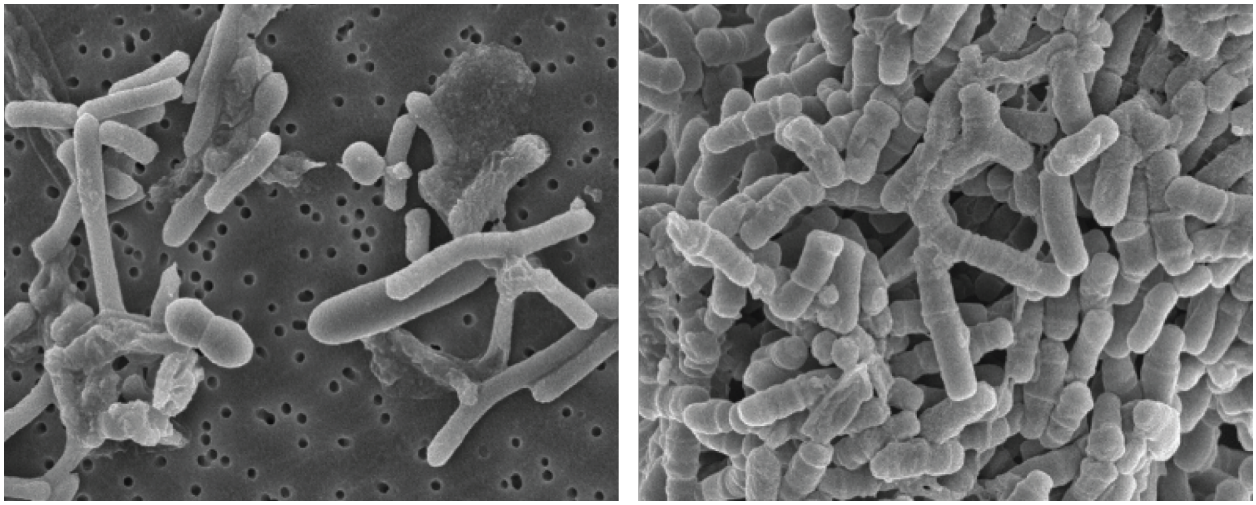
But is there an advantage to having a near monoculture of *Bifidobacterium* in infants? By asking this question, our focus turned to immune development.

Benefits of a *Bifidobacterium*

The decline of *Bifidobacterium* in infant gut microbiomes and the associated dysregulation of the microbial community, with more numerous potential pathogens, has been suggested as one possible contributor to the increased incidence of autoimmune diseases that plague residents of resource-rich nations. Conversely, observational studies have shown beneficial immune effects of having a fecal microbiome dominated by *Bifidobacterium*. In two studies in Bangladeshi infants and young children, fecal *B. infantis* and *Bifidobacterium* abundances at two months of age were strongly correlated with improved vaccine responses at six months and two years old compared with infants not colonized by *B. infantis* or with low relative abundances of *Bifidobacterium*.

Additionally, bifidobacteria are less likely than other microbes, especially potential pathogens, to carry and share antimicrobial resistance genes, which can lead to a higher risk of antibiotic-resistant infections. In an observational study of Bangladeshi and Swedish infants, a dominance of intestinal *Bifidobacterium* was associated with a significant reduction in both the number and the abundance of antibiotic resistance genes. Moreover, compared with matched-control breastfed infants, supplementation with *B. infantis* EVC001 led to a reduction of antibiotic resistance genes by 90 percent, a drop largely driven by a reduction in levels of *Escherichia*, *Clostridium*, and *Staphylococcus*—potentially pathogenic bacteria that play a major role in the evolution and dissemination of antibiotic resistance genes.

In an effort to restore the *Bifidobacterium*-dominated infant gut microbiome that was typical of breastfed babies 100 years ago, we decided to conduct a randomized, controlled trial using the *B. infantis* EVC001 probiotic. Given that not all *B. infantis* strains consume all HMOs efficiently, we selected *B. infantis* EVC001 because we knew this strain had the full cassette of genes needed to fully digest all HMOs. Healthy, full-term, breastfed infants were randomized to consume *B. infantis* EVC001 for 21 consecutive days starting on day 7 postnatal or to not receive the probiotic.



A PROBIOTIC THAT STICKS: Scanning electron micrographs of infant fecal samples show a large increase in the number of *Bifidobacterium* microbes in those treated with a probiotic called EVC001 (right) compared with controls (left).
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Compared with breastfed control infants who did not receive the probiotic, supplementation resulted in a 10,000,000-fold average increase in levels of fecal *B. infantis* and increased fecal *Bifidobacterium* by 79 percent during the supplementation period, and this was still true at one month post supplementation. This means *Bifidobacterium* colonization persisted without the continuation of probiotic supplementation. Additionally, colonization of *B. infantis* persisted until one year of age if infants were continuing to consume any breast milk and were not exposed to antibiotics. Importantly, the supplemented infants exhibited an 80 percent reduction in potential gut pathogens belonging to the families *Enterobacteriaceae* and *Clostridiaceae* and reduced fecal endotoxin. Additionally, we saw a 2-fold increase in fecal lactate and acetate and a 10-fold decrease in fecal pH. The supplemented infants' gut microbiomes and biochemistry resembled norms observed a century ago.

We also identified some clues about the consequences of the gut microbiome's "modernization." Breastfed infants with low fecal *Bifidobacterium* had excreted 10-fold more HMOs in their stool throughout the two-month study period than infants supplemented with *B. infantis* EVC001, indicating that HMOs—the third most abundant component in breast milk—were going to waste. We also found that infants with low fecal *Bifidobacterium* had several-fold higher levels of fecal proinflammatory cytokines compared with infants whose gut microbiomes were dominated by *Bifidobacterium* post supplementation with *B. infantis* EVC001.

Taken together, these data demonstrate that this particular strain of *B. infanti*s, provided as a probiotic to breastfed infants, dramatically colonized the infant gut microbiome during and after supplementation, and beneficially remodeled the microbial, biochemical, and immunological environment in the infant gut. Many infants around the world never acquire *B. infantis*, but the combination of breastfeeding and probiotic supplementation with this bacterium seems to lead to a nourishing and protective gut environment.

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Our findings also support the hypothesis that the ineffectiveness of some probiotics in adults is due in part to the fact that they are introducing a new species to an established community with few ecological niches still open. Probiotics may not work in infants when there is a mismatch between the carbohydrate needs of the probiotic and the availability of highly specific carbohydrates such as HMOs in breast milk.

Because *B. infantis* efficiently consumes almost all HMOs found in breast milk, it is likely to find an open ecological niche and then outcompete other microbes, especially proinflammatory pathogens.

Many scientists are working to understand what the infant gut microbiome really means for health across the lifespan. Meanwhile, we are turning our attention to other questions: How do colonization patterns of *Bifidobacterium* differ in infant populations around the world from infancy to weaning? And what solid foods support a healthy gut and immune system? Working with funding from the National Institutes of Health, we are now conducting a study designed to understand how the carbohydrate structures of complementary foods influence microbial function that will support a healthy gut microbiome and immune system development in late infancy and early toddlerhood. The ultimate goal is to identify specific carbohydrate structures in the diet that selectively feed beneficial gut microbes in children during the critical window of immune development for lifelong health.