Morinaga Milk uncovers clue about infant gut microbiome assembly

The formation of Bifidobacterium communities in breastfed infant gut is associated with not only the ability to utilize human milk oligosaccharides (HMOs), but the arrival order of the species also has a significant impact.

TOKYO (JULY, 2022) — Morinaga Milk Industry Co., Ltd. (TOKYO:2264), a pioneer in bifidobacteria and gut microbiota research, uncovers a breakthrough in understanding the impact of priority effects – the arrival order of a species – on the assembly of infant-type *Bifidobacterium* communities in the infant gut. The research is a collaborative work of Morinaga Milk with Professor Takane Katayama of Kyoto University as well as research experts in the field from Georgia Institute of Technology (USA), Sanford Bumham Prebys Medical Discovery Institute (USA), Niigata University, The University of Shiga Prefecture, Kyoto Women's University, Obihiro University of Agriculture and Veterinary Medicine, and University College Cork (Ireland).

The new study published in the peer-reviewed scientific journal of *The ISME Journal* by Springer Nature on June 29, 2022, noted that the arrival order of four representative species of infant-type human-residential bifidobacteria (HRB) including *Bifidobacterium bifidum*, *B. longum* subsp. *infantis*, *B. longum* subsp. *longum*, and *B. breve* and their HMO-utilization ability significantly influenced species dominance and bifidobacterial community structure.

Whoever comes first matters the most?

The findings highlight the importance of early arriving species during bifidobacterial community assembly, whose mechanisms are influenced by species-specific competitive strategies and HMO-utilization ability. The four representative infant-type HRB species studied display variation in their ability and ways to utilize HMOs. *B. bifidum* and *B. infantis* are strong competitors due to their ability to utilize a variety of HMOs and thus could dominate through inhibitory priority effects in vitro, in which the early arriving species apparently depletes resources for later arriving species. On the other hand, *B. longum* is a modest competitor, while *B. breve* appeared to be a weak competitor with limited HMO-utilization ability. However, *B. breve* could dominate against stronger competitors (*B. bifidum* and *B. infantis*) if present in the community early, despite its considerable fitness differences in HMO-utilization ability.

The dominance of *B. breve* through priority effects was partly underpinned by its ability to utilize fucose, an HMO degradant produced by competitors like *B. bifidum* and *B. infantis*. Further fecal metagenomics analysis of a cohort of breastfed infants observed similar trends for *B. breve*. These findings suggest that the bifidobacterial species that arrive in the infant's gut soon after birth could affect the formation of gut microbiota in later life.

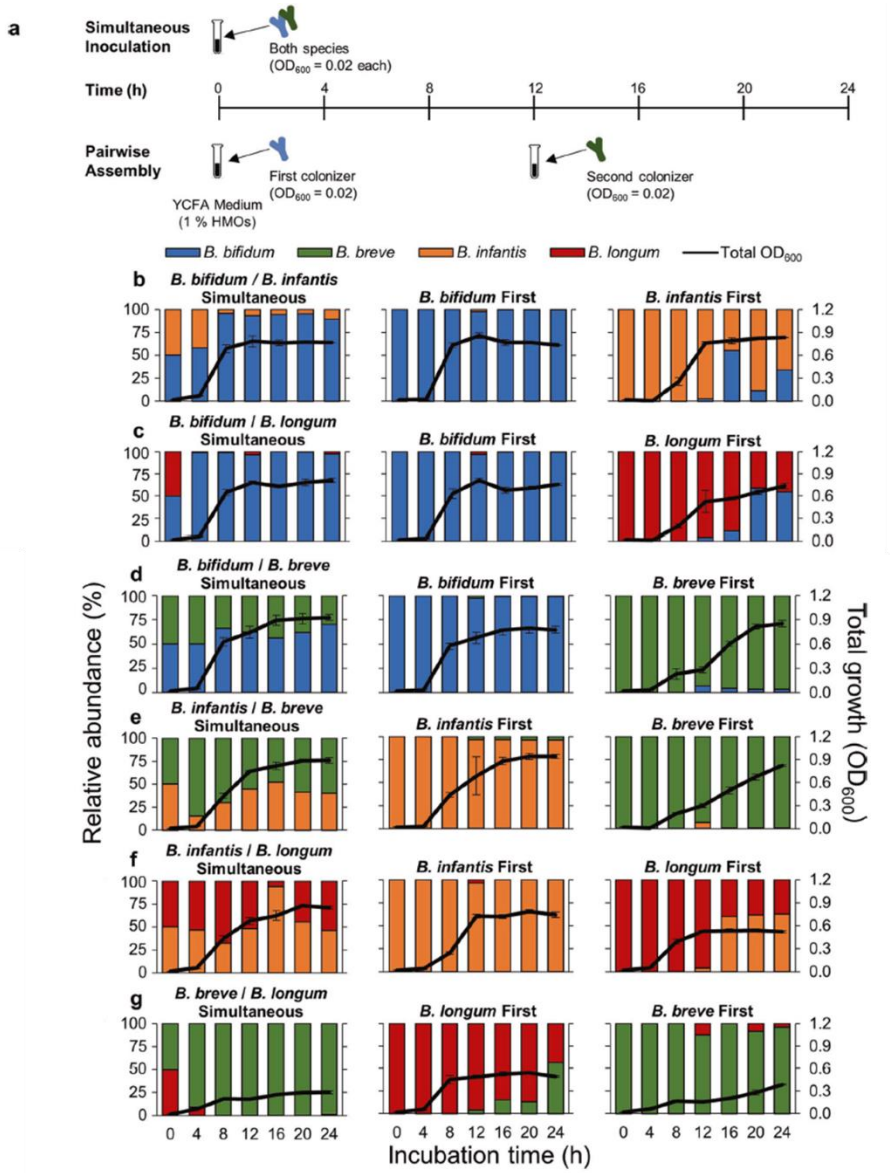
Research background

Bifidobacteria are among the first colonizers of the infant gut. Studies have shown that HMOs in breastmilk function as a bifidogenic factor that selectively promotes the dominance of *Bifidobacterium* species in the infant gut. Increasingly, several HMOs are becoming more commercially available and have been incorporated into infant formulas, mainly in Europe and the United States. The strategy and ability of infant-type HRB species to utilize HMOs differ and it has remained unclear under what circumstances certain *Bifidobacterium* species become predominant. Bifidobacterial communities in the gut of a breastfed infant often fall into one of two

groups: *B. longum*-dominant or *B. breve*-dominant. Numerous factors such as delivery mode, feeding regime, and maternal microbiota can affect the assembly of bifidobacteria in the infant gut, and the ecological mechanisms that lead to *B. longum* dominance are yet to be determined. Meanwhile, *B. breve* predominates in the guts of many breastfed infants despite its limited HMO-utilization ability. In the present study, the researchers investigated the formation process of a bifidobacteria-rich microbiota and elucidated the impact of priority effects using four representative infant-type HRB (*B. bifidum*, *B. infantis*, *B. longum*, and *B. breve*) that have different HMO utilization strategies.

The study and findings

In the present study, researchers performed in vitro cultivation experiments in HMO-containing mediums to test for priority effects in infant-gut-associated bifidobacterial communities. Researchers conducted the culturing in three phases: monocultures, pairwise cultures, and four-species assemblages in HMO-supplemented mediums. In the pairwise cultures and four-species assemblages, inoculation order was manipulated to examine its effect on community structure.



The results showed that when *B. bifidum* and *B. infantis*, which have a high ability to utilize HMOs, were added first, they dominated the culture mediums. When added first, both *B. bifidum* and *B. infantis* depleted most of the available HMOs, resulting in limited remaining resources for other species added later. Nevertheless, despite the limited HMO-utilization ability, *B. breve* dominated if added first to the culture medium for which it outcompeted other species in the mixed cultures.

The researchers then performed fecal metagenomics data mining analysis to analyze bifidobacterial communities in a cohort of European infant-mother pairs. The metagenomics datasets were available for four different time points: the mother at the time of delivery, and the infant at the age of 0, 4, and 12 months. Infants who were at least partially breastfed (exclusively breastfed or mixed-fed) at 4 months of age were chosen for analysis. The results show that if *B. breve* was present in the infant gut microbiota at birth, it was more likely to dominate in the community at 4 months of age. The observed trends in the infant's gut for *B. breve* were consistent with the in vitro data in the present study, suggesting that priority effects may have contributed to the dominance *of B. breve*.

To examine how *B. breve* dominates within bifidobacterial communities, researchers compared wild-type (WT) and ΔfucP mutant strains of *B. breve*. The results show that the ability of *B. breve* to import and utilize fucose – an HMO degradant released by *B. bifidum* and *B. infantis* – contributed to its persistence and/or dominance.

Implications

This study revealed that not only the ability of bifidobacterial species to utilize HMOs but also the arrival order of the species in the infant gut greatly influenced the infant gut microbiota assembly. In particular, the prevalence of priority effect is one of the mechanisms by which *B. breve* becomes dominant in the gut of breastfed infants, despite its limited HMO-utilization ability. The results provide insight into previously unexplained *B. breve* abundance in breastfed infants and highlight the importance and history dependency of initial community assembly and its implications for the maturation trajectory of the infant gut microbiota.

Morinaga Milk will continue its efforts to clarify the mechanism of infant gut microbiota assembly and provide the scientifically substantiated probiotic ingredients that can contribute to infant health.

About Morinaga Milk Industry Co., Ltd.

Morinaga Milk Industry Co., Ltd. is one of the leading dairy product companies in Japan with a century of history harnessing the nutritional properties of dairy products and its functional ingredients. Morinaga Milk is also a key global probiotics manufacturer that excels in innovative technology and offers a premium line of probiotics and functional ingredients worldwide. Since the 1960s, Morinaga Milk has been engaged in research on the safety, functional health benefits, and mechanisms of action of probiotic bifidobacteria to better understand their role in maintaining human health. For more information about Morinaga Human-Residential Bifidobacteria (HRB) probiotics, please visit us at https://morinagamilk-ingredients.com/.

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