



Morinaga Milk Industry Co., Ltd. Unveils New Research Demonstrating the Superiority of Human Residential Bifidobacteria

TOKYO (JULY 8, 2014) — [Morinaga Milk Industry Co., Ltd.](#) (TOKYO:2264), the second largest dairy product company in Japan, today announced that Morinaga has discovered differences in potential capabilities between bifidobacteria residing in the human intestinal tract (Human Residential Bifidobacteria ; HRB) and the other bifidobacteria (Non-Human Residential Bifidobacteria ; nHRB), and confirmed these differences on compatibility with mother's breast milk and vitamin production by performing *in vitro* and animal studies.

The genus *Bifidobacterium* is currently classed into more than 40 species or subspecies. While bifidobacteria are widely known to be the flagship of beneficial bacteria, the difference among each species is not well understood. First, the species are clearly different with regard to the habitats in which they reside. For example, *Bifidobacterium longum* and *Bifidobacterium breve* inhabit the human intestinal tract, while *Bifidobacterium animalis* is most often found in the intestines and other sites in animals. Only approximately 10 species reside in the human intestinal tract, and these species also differ between infants and adults.

This phenomenon is thought to be the result of evolution of individual species in adapting to their respective habitats. However, the actual differences of mechanism of action among these species have not been adequately evaluated to date.

Morinaga investigated the potential capabilities of HRB and nHRB from genomic information, and validated the actual capabilities by performing *in vitro* and animal experiments, results of which were presented in March 2014 at the Annual Meeting of the Japan Society for Bioscience, Biotechnology and Agrochemistry.

Study 1: Comparative Genomic Analysis

At first, Morinaga compared the genomes of a total of 68 strains belonging to 12 species of *Bifidobacterium*, including the publicly available genome sequences as well as newly analyzed genome sequences. The results indicated clear differences in conserved gene clusters specific to habitats. Detailed analysis was performed on three species which are commercially used as probiotics in the market [HRB: *B. longum* (subsp. *longum* and subsp. *infantis*) and *B. breve*; nHRB : *B. animalis* (subsp. *animalis* and subsp. *lactis*)]. The analysis showed that *B. longum* and *B. breve*, both of which are HRB, share many common gene clusters. Comparing these HRB with *B. animalis* (nHRB), the following four possibilities are suggested:

1. Only HRB can utilize specific oligosaccharides present in mother's breast milk, so that they can grow in the intestinal tract of infants.
2. HRB can produce more vitamins compared to nHRB.
3. HRB differ from nHRB in the ability to colonize the intestinal tract.
4. HRB is superior to nHRB in responding to rapid environmental changes.

Study 2: Compatibility With Human Breast Milk

Next, Morinaga examined whether the differences predicated from the genome are reflected in actual functions. Various Bifidobacterial strains were inoculated into mother's breast milk donated by healthy mothers, and cultured at 37C degrees. The results clearly showed that while most of the HRB (*B. longum*, *B. breve* and *B. infantis*) isolated from infants grew well in breast milk, nHRB failed to grow and even died out after overnight incubation in the breast milk. As for the mechanism of action, it has been suggested that the selective growth of bifidobacteria is related to the utilization of oligosaccharides (pre-biotics) in breast milk. In addition, Morinaga confirmed that resistance to lysozyme, an antibacterial active substance that exists in breast milk, is also involved in the selective growth of bifidobacteria in breast milk; HRB are tolerant to lysozyme; on the other hand, nHRB are highly susceptible to lysozyme.

Study 3: Difference in Folate Production Capability

Among the gene distribution for producing vitamins, the genes involved in the *de novo* synthesis of folate showed the greatest differences between HRB and nHRB. Morinaga therefore examined the *in vitro* folate production capability of various bifidobacterial strains.

High folate concentrations were detected in the culture media of HRB, compared with nHRB.

Further studies were conducted using germ-free mice by mono-associating with *B. longum* (subsp. *longum*), *B. breve*, and *B. animalis* (subsp. *lactis*). In mice mono-associated with HRB, especially with *B. longum*, fecal folate concentrations were higher and hematological indicators related to anemia were improved as compared to nHRB (*B. animalis*). Since folate is related to anemia prevention, it is possible that intestinal folate levels affect hematological indicators.

The results obtained from these studies indicate that the different residential features may be the cause of adaptation to the diet and environment. HRB may be more compatible with infant intestines and potentially more beneficial to the hosts.

"We were quite surprised at the result of the study on the compatibility with mother's breast milk, which showed that nHRB could not grow in the breast milk," stated Dr. Jin-zhong Xiao, Ph.D., Department Manager of Food Science & Technology Institute, Biological Function Research Department at Morinaga Milk Industry.

"It has been suggested that the selective growth of bifidobacteria observed in breastfed newborn infants is related to the Human Milk Oligosaccharides (HMOs) in human milk, however, in the *in vitro* experiments for growth in breast milk, undigested lactose should be sufficient for growth of *Bifidobacterium* even without assimilating HMOs."

"The fact that HRBs possess high compatibility with human breast milk suggests how natural and more suitable HRBs are for consumption by human and infants. We will continue our research on bifidobacteria in order to understand the mechanisms of residential features of each species and evaluate the superiority of HRB for human health," Dr. Xiao added.

About Morinaga

For over 40 years, Morinaga has conducted research and development in Human Residential Bacteria, especially *Bifidobacterium longum* BB536, which naturally live in the human intestine and are one of the most thoroughly researched probiotic strains in the world. Morinaga's first flagship probiotic, *Bifidobacterium longum* BB536, achieved FDA-notified GRAS in 2009 (GRAS Notice No. GRN 000268), and the second flagship stain, *Bifidobacterium breve* M-16V, achieved FDA-notified GRAS and infant GRAS in 2013 (GRAS Notice No. GRN 0000453/000454).

Morinaga Milk Industry Co., Ltd. is the second largest dairy product company in Japan, employing 3,122 people. Founded in 1917, Morinaga exhibit excellence in the field of technology and sell not only dairy products but also the beneficial functional ingredients isolated from milk components. Morinaga has been exporting its products and technology to around 30 overseas countries for more than 35 years.

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IF YOU WOULD LIKE MORE INFORMATION ABOUT MORINAGA'S PROBIOTIC RESEARCH AND PRODUCTS, OR TO SCHEDULE AN INTERVIEW, PLEASE SEND AN E-MAIL: INTERNTL@MORINAGAMILK.CO.JP

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