

HRB or Non-HRB, that is the question

MORINAGA MILK INDUSTRY CO., LTD.



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Human-Residential Bifidobacteria (HRB): natural inhabitants of the human gut.

Bifidobacteria are resident microbiota members of the human gastrointestinal tract throughout life. They are among the first microbes to colonise the gut for which the populations of bifidobacteria are the most abundant genus present in the healthy infant gut. As the infant begins to wean, the abundance of bifidobacteria starts to decrease. The levels continue to fall gradually but remain relatively stable during adulthood. A further decline is then seen in old age¹. Evidence is accumulating that higher bifidobacteria levels in the elderly are correlated with health and longevity. Notably, bifidobacteria levels across the life span align with key stages in immune maturation and are associated with improved host well-being.

The genus *Bifidobacterium* has been shown to play important roles in regulation of host intestinal, immunological and metabolic activities. They are purportedly beneficial for human health and certain strains of *Bifidobacterium* have been integrated into a range of products including foods, infant formula, dietary supplements, and pharmaceutical products as functional probiotics ingredients. It is widely recognised that not all probiotics are equally safe, and the effects demonstrated in one strain cannot be extrapolated to another strain, even if they belong to the same species². In recent years, scientific literature has accumulated supporting the residential origin as another important aspect that contributes to health-associated physiological differences between bifidobacterial species³.

Bifidobacterial species are widely distributed in a range of ecological niches including the gastrointestinal tract of humans and animals as well as a few that have been isolated from human vagina, oral cavity, breast milk, sewage and foods. Bifidobacteria are purported to have coevolved with their respective hosts and could be categorised into two major groups; those that naturally reside in the human intestines are referred to as human-residential bifidobacteria (HRB), whereas other species which are the natural inhabitants of animals or environment as non-HRB³(Figure 1). New research by Morinaga Milk Industry Co., Ltd. show that HRB have some traits that non-HRB do not have (Table 1). Such traits are likely to contribute to the adaptability and functionality of HRB in the human gut.

Figure 1 Bifidobacterial species and their habitat

Human-Residential Bifidobacteria (HRB)

B. breve Infant B. longum subsp. infantis B. bifidum B. longum subsp. longum

B. adolescentis B. pseudocatenulatum B. angulatum B. dentiumn etc. Ad

- B. animalis subsp. lactis
- B. animalis subsp. animalis
- B. pseudolongum
- B. thermophilum B. pseudolongum subsp. globosum
- B. magnum etc.

Representative source: bovine, pig, chicken, rat, guinea, rabbit, fermented milk, etc.

Table 1 Physiological differences between HRB and non-HRB

Physiological traits	HRB (infant-type)	Non-HRB	References
Growth in human milk	0	×	4
HMOs utilisation	$\Delta \sim 0$	×	4,5
Tolerance to lysozyme	0	×	4,6
Folate production	0	×	5,7
Degradation of food- derived opioid peptides	0	×	8
Indole-3-lactic acid (ILA) production	0	×	9,10

A key finding is that only those HRB species commonly found in the infant gut microbiome can utilise oligosaccharides found in human breast milk and are tolerant to a natural antibacterial factor in human breast milk called lysozyme^{4–6} (Figure 2). These characteristics allow HRB species to colonise the infant gut, contributing to the development and maturation of healthy gut microbiome. This point to the role of human breast milk in the selective colonisation of the infant gut by *Bifidobacterium* species. The findings show that HRB species are natural and ideal for consumption by infants and adults, and this concept of 'HRB are suitable for human consumption' is Morinaga Milk's philosophy.

In addition, the research also discovered that only HRB species are capable of certain physiological functions. Unlike non-HRB species, HRB species are particularly superior at producing folate, a critical cofactor for cell growth and metabolism, which, in turn, can aid in the prevention of anaemia and improvement of well-being in the human host⁷. Certain HRB species are also effective at eliminating the potentially harmful food-derived opioid peptides such as human milk- and bovine milk-derived β -casomorphin-7 and wheat gluten-derived α -gliadin-7⁸. Food-derived opioid peptides are associated with many health complications in infants and children, such as sudden infant death syndrome, atopic dermatitis, autism, celiac disease, and type-1 diabetes.

The latest research also discovered that certain strains of HRB, particularly infant-type HRB, are superior at producing indole-3-lactic acid (ILA)⁹, a unique tryptophan metabolite that could potentially act as a mediator in bifidobacteria-host-microbiota crosstalk and involves in infant immune and neuronal development¹⁰. The findings show that HRB species are ideal probiotics for consumption by humans at all ages, especially infants, and are more beneficial for promoting human health.

Morinaga Milk has developed a premium line of probiotic strains that are highly compatible with the human gut. The four main HRB probiotic strains are *Bifidobacterium longum* subsp. *longum* BB536, *B. breve* M-16V, *B. longum* subsp. *infantis* M-63, and *B. breve* B-3, all of which are from the gut of infants and possess a proven record of safety and clinical efficacy on human health.

Figure 2 Growth of bifidobacteria in human breast milk



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BB536: HRB Probiotic with 50-Year History of Embracing Human Well-Being

Bifidobacterium longum subsp. longum BB536 (designated as BB536) is a clinically effective, well-established, multifunctional probiotic strain that has a long history of human use in alleviating gastrointestinal, immunological and infectious diseases¹¹. For half a century, BB536 has been the superior HRB probiotic strain that helps people achieve optimal health from the inside out. Ingestion of BB536 can provide a consistent beneficial effect in the improvement of gastrointestinal conditions, maintenance of intestinal microflora balance, regulation of immune response, anti-allergy, and protection against microbial infections (Figure 3). It is evident that BB536 acts in concert with the gut microbiota to drive a fine-tuned intestinal and immune balance.

BB536 has gained long-standing acceptance in the marketplace. Due to its robust safety, efficacy and stability profile, BB536 has been used as a functional food ingredient in various products such as milk-based drink, yoghurt, infant formula, and nutritional supplements and has been marketed in over 30 countries for more than 40 years.

Figure 3 Key beneficial effects of *Bifidobacterium longum* BB536 on human health.



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M-16V: Ideal HRB Probiotic for Infant Heath

Bifidobacterium breve M-16V (designated as M-16V) is a clinically effective, well-established probiotic strain that exerts positive effects on infant health¹². M-16V has been incorporated as a functional probiotic ingredient in various products such as nutritional supplements, infant formula, and oil drop products for promoting infant health. Supplementation with M-16V is specifically effective at protecting the vulnerable premature infants against the development of the devastating necrotising enterocolitis (NEC) and sepsis, and alleviating allergic disorders in infants¹². M-16V is proven effective at promoting healthy gut microbial colonization and maturation as a means to support healthy growth and to shape a healthy gastrointestinal and immune system.

M-16V has a long history of safe use in infants, for which it is proven to be well-tolerated by both premature neonates and term infants with no adverse effects, including probiotic sepsis and deaths. To date, M-16V administered to low-birth-weight infants to support healthy growth in more than 120 neonatal intensive care units (NICUs) in affiliated hospitals in Japan, Australia, New Zealand, and Singapore. With such a strong safety profile, M-16V is well-positioned as a trustworthy and suitable probiotic ingredient for routine supplementation in infants.

M-63: HRB Probiotic with Superior Potential for Infant Use

Bifidobacterium longum subspecies infantis M-63 (designated as M-63) is unique among gut bacteria in its immense capacity to utilize human milk oligosaccharide (HMOs), the component that is highly abundant in human breast milk. HMOs offer no direct nutritive value for infants, but they function in shaping the infant gut microbiota with life-long impacts. M-63 possesses the superior potential to colonise and adapt to the intestinal environment of humans, especially infants. Ingestion of M-63 is specifically effective at improving the colonisation of bifidobacteria in the infant gut¹³. In addition to its beneficial effects on infant health, recent clinical study shows the potential of M-63 in improving mental state in individuals with irritable bowel syndrome (IBS)¹⁴, suggesting its beneficial role in gut-brain axis.

B-3: HRB Probiotic Specialized in Weight Management

Bifidobacterium breve B-3 (designated as B-3) is a unique probiotic strain that is specialized in maintaining a healthy body weight that makes peoples' lives a little more stylish and comfortable. Changes in gut microbiota composition have been commonly observed in high fat diet-triggered metabolic endotoxemia, intestinal barrier dysfunction, and metabolic disorders. In a randomized, double-blind, placebo-controlled trial, ingestion of B-3 capsule (2 x 10¹⁰ CFU) for 12 weeks significantly reduced percentage of body fat and body fat mass in pre-obese individuals¹⁵. Numerous preclinical and clinical studies indicate that B-3 could improve intestinal barrier function, repress inflammation and improve fat metabolism, leading to significant body fat reductions and prevention of metabolic disorders in pre-obese individuals.

Triple Bifidus, Triple Synergies

Studies suggest that probiotics work better as a team. Morinaga Triple Bifidus, a probiotic mixture containing the three HRB strains, BB536, M-16V, and M-63, is ideal multiple strains probiotic. When combined, the Triple Bifidus probiotics exert triple synergistic effects to promote early colonisation of bifidobacteria in premature infants and prevent against the development of allergic disorders in infants.

In a key clinical study involving 44 low birth weight infants, who were ready for feeds within seven days of birth, administration of Triple Bifidus for six weeks significantly increased the detection rates and cell numbers of bifidobacteria in the faeces¹³. Triple Bifidus supplementation resulted in an earlier formation of a bifidobacteria-dominant microbiota and a significantly lower level of *Enterobacteriaceae*, which may, in turn, supporting healthy growth in premature infants. This finding suggests that when combined, HRB probiotics could act synergistically and cooperatively with each other to confer a more remarkable beneficial effect in premature infants.

In addition, the preventive effects of Triple Bifidus on allergic disorders have been exemplified in a remarkable clinical study involving 40 children treated with Triple Bifidus for four weeks¹⁶. Administration of Triple Bifidus protected the children against pollen-induced IgE-mediated allergic rhinitis and intermittent asthma and improved their quality of life, for which these parameters were worsened in the placebo group. This study implies that when combined, HRB probiotics confer a triple synergistic effect to dampen allergic disorders in children.

Conclusion

Although bifidobacteria may have traits that are beneficial for human health, not all Bifidobacterium species possess such traits. The results obtained from these studies introduced here indicate that the different residential features would contribute to the physiological differences between bifidobacterial species. Complementing the law of nature, the compatibilities of HRB, particularly those of the natural inhabitants of infant's intestine, with human milk as well as their superiority in certain health-associated physiological functions have drawn attention to the possibility that HRB are better probiotics for human use. Among HRB strains, BB536, M-16V, M-63 and B-3 deserve special attention due to their prevalent beneficial effects in promoting human well-being. Key clinical findings imply that in addition to their effectiveness as a single organism, these HRB strains could also act synergistically to confer a more remarkable beneficial effect in humans. Given these facts, Morinaga HRB probiotics could serve as useful and worthy probiotic strains for the improvement of human well-being.

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About Morinaga Milk

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, offering a premium line of probiotics and functional ingredients to customers throughout the world. 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 Bifidobacteria, please visit us at

www.bb536.jp/english/index.html





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Multifunctional Human Bifidobacteria Strain

20

0____

12

Storage period (Month)

6

BB536 is highly stable due to Morinaga's unique

culturing method and advanced production technology.

36-month real-time stability study on BB536 powder

24

18

36

Bifidobacterium longum subsp. *longum* BB536 is one of the most well-established, clinically effective probiotic strains that confers numerous profound beneficial effects on humans. BB536 possesses a proven track record of safety and clinical efficacy in improving gastrointestinal, immunological and infectious conditions, as demonstrated in more than 180 scientific studies.



Chocolate

Cereal

Yogurt

Granola bar

These statements have not been evaluated by the Food and Drug Administration. This product is not intended to diagnose, treat, cure, or prevent any disease.

Protein powder

Ice-cream

Clinical Efficacy

Improvement of Gastrointestinal Conditions

BB536 relieved constipation in subjects with infrequent bowel movements (<4 times/week)



BB536 restored regular defecation frequency in subjects with frequent bowel movements (≥10 times/week)



Improvement of Intestinal Microenvironment

Enterotoxigenic Bacteroides fragilis (ETBF) is associated with colitis

BB536 significantly reduced ETBF numbers



Modulation of Immune Response

Number of subjects contracted influenza infection



Effect on NK Cell Activity



This graph was reproduced from Namba et al., 2010. Bioscience, Biotechnology and Biochemistry

Alleviation of Allergic Disorders

Allergic symptom scores

Rhinorrhea





Nasal blockage





Effect on Th2-skewed immune balance

This graph was reproduced from Xiao et al., 2006. Clinical and Experimental Allergy.



Ideal Probiotic for Infant Health

Bifidobacterium breve is one of the predominant species present in the infant gut and is widely recognized for its beneficial roles in maintaining infant health. *B. breve* M-16V has emerged as one of the best studied clinically effective probiotic strains that exerts positive effects, particularly in infants, to support healthy growth and promote well-being.



M-16V powder with excellent survival rate Study by Morinaga



36-month real-time stability study on M-16V powder

M-16V is highly stable due to Morinaga's unique culturing method and advanced production technology.



Oil drop



Sachet

Capsule

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Protection Against Premature Birth Complications in Low Birth Weight Infants

Prevalence of premature neonates colonized with B. breve

Prevalence of premature birth complications in preterm VLBW neonates born < 34 weeks



This graph was reproduced from Patole et al., 2014. PloS one

Significant reduction after M-16V supplementation * P < 0.05 vs control

0.53

NEC / Mortality

This graph was reproduced from Patole et al., 2016. PloS one.

0.57

Late onset sepsis

NS

2

3

Reduction in the Prevalence of Atopic Dermatitis

Prevalence of Premature Complications (Adjusted Odd Ratio)

0.5

0

Study protocol (Single strain of M-16V)



Prevalence of Atopic Dermatitis

0.43

NEC



Significant reduction after M-16V supplementation

This graph was reproduced from Taniuchi et al., 2005. The Journal of Applied Research

Effect of M-16V Prenatal and Postnatal Supplementation on Allergic Disorders





Prevalence of eczema/atopic dermatitis (AD)



Significant reduction after M-16V supplementation

This graph was reproduced from Enomoto et al., 2014. Allergology International.





Superior Potential for Infant Use

Bifidobacterium longum subsp. *infantis* M-63 is unique among gut bacteria in its immense capacity to utilize human milk oligosaccharides (HMOs), the components that are highly abundant in human breast milk. HMOs offer no direct nutritive value for infants, but they function in shaping the infant intestinal microbiota with life-long impacts.



M-63 is highly stable due to Morinaga's unique culturing method and advanced production technology.

Effect of M-63 on Intestinal Microbial Colonization in Low Birth Weight Infants

Prevalence of bifidobacteria colonization



- Control group
- Single strain group (M-16V)
- Triple strains group including BB536, M-16V, and M-63
- Subjects: 44 low birth weight infants were divided into three groups (13-16 subjects per group).
- *Bifidobacterium* administration: Single strain (*B. breve* M-16V) or a combination of triple strains including *B. longum* BB536, *B. breve* M-16V, and *B. infantis* M-63, each strain at 5 billion CFU/day/infant, for 0-6th week after birth.
- Administration of the triple bifidobacteira enhanced earlier colonization of bifidobacteria.

This graph was reproduced from Ishizeki et al., 2013. Anaerobe.

High Compatibility with Human Breast Milk

What is in Human Breast Milk?

Human breast milk is the gold standard in infant nutrition. It plays an enormous role in infant health development. Its secret is its ingenious and unique composition.

Human Breast Milk Contains HMOs

Human milk oligosaccharides (HMOs) are unconjugated complex carbohydrates that are highly abundant in and unique to human milk. HMOs exhibit bifidogenic effect that selectively stimulate the growth of bifidobacteria and contribute to gut colonization.



Growth of M-63 in human breast milk containing different structures of HMOs and its tolerance to lysozyme



This graph was reproduced from Minami et al., 2016. Beneficial Microbes

Improvement of Mental State in Subjects with Irritable Bowel Syndrome





This graph was reproduced from Ma et al., 2019. Beneficial Microbes.





Weight Management Probiotic Strain

Bifidobacterium breve B-3 is a unique probiotic strain, developed by focusing on the relationship between gut microbiota and metabolic syndrome. B-3 possesses an attractive effect in maintaining healthy body weight and ultimately improving one's lifestyle.



Clinical Efficacy

Reduction of Body Fat

Administration of B-3 (20 billion CFU/day) reduced body fat in pre-obese adults.



Intervention period (Week) Comparison between groups: Covariance analysis adjusted with the value of 0 week, †P < 0.05 Intragroup comparison: paired t test, * P < 0.05

This graph was reproduced from Minami et al., 2018. Bioscience of Microbiota, Food and Health

Proposed Mechanism of Action of B-3

High Fat Diet

Intervention period (Week)

Intake of B-3

