Yakult

Yakult's future research into the beneficial health effects of gut microbiota and probiotics

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More than 80 years ago, Dr Minoru Shirota, the founder of Yakult, advocated two health-related principles: 'preventive medicine', which aims to protect humans against diseases rather than treating symptoms; and 'healthy intestines for longevity', which works to keep the human intestine healthy throughout life. Using these principles, Yakult scientists have been conducting research on intestinal bacteria and probiotics that are involved in homeostasis. Here we describe our efforts to address the ultimate challenge: understanding the significance of gut microbiota in maintaining human health and the results of probiotic studies.

The symbiotic relationship between humans and intestinal bacteria

Human lifespan and gut microbiota

At the Yakult Central Institute in Tokyo, we have been isolating and collecting lactic acid bacteria and bifidobacteria and studying them as part of our research and development into probiotics that are beneficial for human health. Additionally, as part of our studies on intestinal bacteria, we have isolated, identified and numerated microbes by using the culture method or YIF-SCAN® (Yakult Intestinal Flora Scan) analyses based on quantitative polymerase chain reaction (PCR) and reverse transcriptase polymerase chain reaction (RT-PCR), characterising many types of intestinal bacteria. Because a considerable number of microbes are difficult to cultivate, we have been using 16S ribosomal RNA (rRNA) gene segments analysis to comprehensively sequence specific regions of 16S rRNA genes in all faecal microbes to accumulate data on intestinal bacteria. For example, examining gut microbiota from individuals across age groups using YIF-SCAN® has shown that although the genus Enterobacteriaceae was dominant immediately after birth, in individuals over three years of age gut microbiota were dominated by obligate anaerobes including *Bifidobacterium*.

A human foetus has an axenic intestine, but a variety of microbes begin to colonise the gut shortly after birth. We asked the question: What is the source of *Bifidobacterium*, the most dominant genus in infants? Our colleagues at Yakult Honsha European Research Centre for Microbiology in Ghent, Belgium, found that strains of bifidobacteria are transferred from the intestinal tracts of mothers to their babies. To carry out the research, the team compared bifidobacteria isolated from the faeces of 12 antenatal mothers and from each newborn using multilocus sequence typing (MLST). The researchers recorded identical strains of bifidobacteria from each mother–infant pair[1]. Some of the babies had the same strain detected in their faeces from shortly after birth until they were three months old, suggesting that those strains were vertically transmitted from the intestinal tracts of their mothers. Vertical transmission was not observed in the five mother–infant pairs who had given birth by caesarean section, suggesting that transfer of intestinal microbiota occurs when an infant passes through the birth canal[2] (Figure 1). Babies delivered by caesarean section exhibited delayed colonisation of bifidobacteria compared with babies who were spontaneously delivered. In babies born by caesarean, bifidobacteria began to colonise the gastrointestinal tract one month after birth. Infants delivered by caesarean at the same hospital did not share identical strains of bifidobacteria; the origins of bifidobacteria in the caesarean section infants were not identified. The influence of bacterial colonization of the gastrointestinal tract

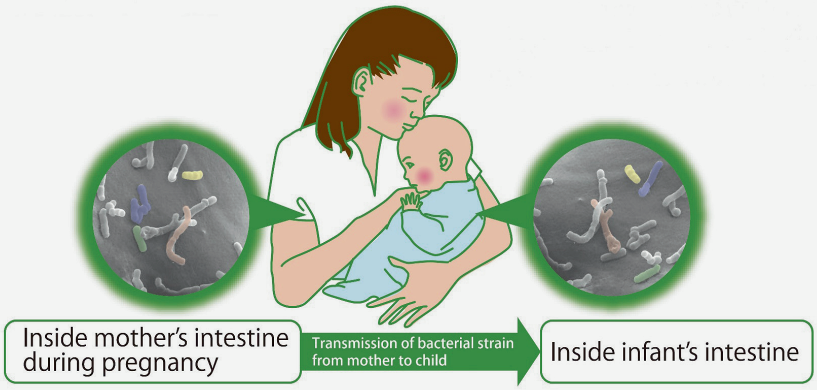

Figure 1 | Mother-to-child transmission of intestinal bacteria (bifidobacteria). Among infants born vaginally, several *Bifidobacterium* strains transmit from the mother and colonize the infant's intestine shortly after birth.

on a child's health is not yet known, and no consensus has been reached on the risks of a child developing asthma or allergies following a particular mode of delivery — these would be areas for future research.

I. B. Jeffery and colleagues at University College Cork, Ireland, reported that elderly individuals had a decreased diversity of gut microbiota compared with younger adults, and that those residing in care facilities or consuming a high-fat diet had specific modules of gut microbiota[3]. In addition, YIF-SCAN® confirmed that individuals aged over 60 had fewer *Bifidobacterium* and more Enterobacteriaceae as they grew older. Further studies are needed to monitor intestinal bacteria in different people and to assess microbiota in a cross section of age groups; constructing a database of results should help to advance research in this field.

The significance of intestinal bacteria

In living systems such as human, mouse and rat, the beneficial nutritional effects of intestinal bacteria include the production of essential amino acids, vitamins and short-chain fatty acids (SCFAs). But research in the past three years has suggested that gut microbiota might have a greater impact on the health of living systems than had previously been thought.

In particular, SCFAs, which are metabolites produced by intestinal bacteria, were found to be directly involved in energy metabolism. I. Kimura and colleagues at Kyoto University, Japan[4,5], showed that the SCFAs were used as an energy source for intestinal epithelial cells. Some of the SCFAs were absorbed into the body and were recognized by the short-chain fatty acid receptor GPR41. GPR41 activated the sympathetic nervous system and promoted energy consumption in living systems, while contrarily controlling a ketone body β-hydroxybutyrate, which is synthesized in the liver. In addition, they found that those fatty acids suppressed insulin signals through GPR43 expressed in adipose cells and played a role in inhibiting lipid accumulation. Thus, SCFAs generated as by-products when intestinal bacteria convert a sugar source into adenosine triphosphate (ATP) are not only used as a nutrient source for intestinal epithelial cells but also have systemic effects; this suggests that intestinal bacteria are important partners for living systems.

The part played by gut microbiota in maintaining human health has not been defined. Intestinal bacteria were categorised as either beneficial or harmful. But the view now is that the diversity and balance of microbiota are tightly linked to human health. Loss of diversity and an imbalance of gut microbiota are deeply associated with obesity, allergies and autoimmune diseases. A well-balanced existence of diverse intestinal microbes is more important than a dominance of specific ones, just as a good diet includes eating a broad, well-balanced range of foods.

Intestinal functions and microbes

Probiotics and the immune system

Living systems have evolved sophisticated immune systems to protect against disease and maintain homeostasis. Mucosal surfaces that border the external environment, particularly the intestinal tract, deploy immune cells in response to a variety of risks. Living systems also take advantage of stimuli from the external environment along the intestinal tract to ensure sustained function. We are researching how the immune system within the intestinal tract is crucial for health maintenance, and are working on the challenges of using the activities of intestinal bacteria and probiotics to modulate immune functions and ensure good health.

The relationship between gut microbiota and health

The part played by gut microbiota in the development and function of the immune system is a field of study to which we have made a significant contribution. We realized that to analyse the functions of certain types of intestinal bacteria, molecular biological approaches do not work and instead the bacteria must be isolated and cultivated. Using the intestinal tracts of mice and rats we succeeded in separately propagating and maintaining segmented filamentous bacteria (SFB), which cannot be cultivated *in vitro*, and found that they are important in IgA production and in the development of intestinal intraepithelial lymphocytes[6]. SFB are involved in diverse types of important immune responses through inducing Th17 cells, a subset of CD4-positive T cells secreting IL-17. We have provided SFB to more than 40 research institutes in Europe, the United States and Asia, as well as within Japan.

Research on the relationship between gut microbiota, immune function and health maintenance is now one of the most important subjects in the field of life sciences. In this context, much attention has also been paid to probiotics — specific bacteria that are proactively delivered into the intestines. Researchers across the world use various strains of probiotics to conduct studies aimed at the prevention and control of immunity-associated diseases. *Lactobacillus casei* strain Shirota (LcS) is a probiotic strain that has immunomodulatory functions and has been consumed as a fermented milk drink since 1935.

Probiotics could assist the immune system

Studies investigating the effects of LcS on the immune system began with those on cancer prevention and infection protection through activation of biophylactic functions, whose impact was described in a research article[7] in 1981. Research on activation mechanisms of the innate immune system mediated by LcS went further, and the strain has been identified not only as an activator of biophylactic functions, but also as a potential suppressor of excessive immune reactions that may also be effective against inflammatory bowel disease, allergy and autoimmune disease. Research on the immunomodulatory effects of LcS is unusual in that its efficacy is not limited to specific diseases, but that it exerts diverse effects by restoring decreased immune functions and suppressing excessive immune reactions[8]. Ongoing research into the immunomodulatory functions of many other probiotics is conducted in discrete fields, such as inflammatory bowel disease, allergy and infection protection. Primarily, we should not expect efficacy on particular diseases from probiotics consumed as food. Probiotics will have crucial roles in helping living systems to cope with varied health risks by ensuring maintenance and recovery of normal immune functions (Figure 2). The flexible effects of LcS on the immune system should be consistent with those views above.

The extent to which LcS affects the immune system is not understood in detail, but the effects are thought to be attributed to their specific cell wall structures. Phagocytes deployed throughout every tissue in the body, including the intestinal tract (which have pattern-recognition receptors such as Toll-like receptors (TLRs)), rapidly capture microbes invading the living system, recognize constructs presented on the microbes or newly exposed peptidoglycan digests and nucleic acids that are digested within the phagocytes, and start adequate immune reactions. However, LcS has two types of neutral polysaccharides densely covering the cell wall surface that are not recognized by TLRs[9]. These polysaccharides may not only conceal the potential TLR ligands of LcS, but may also prevent their exposure by conferring tolerance to enzymatic digestion that lyse cell wall peptidoglycans in the phagosome in which it was captured[10]. At the same time, LcS keeps its three-dimensional cellular structures within a phagocyte for some time; LcS stimulates the phagocyte by using its structure and the stimuli may, rather than induce a specific unidirectional response to the phagocyte, enhance immune reactions to maintain homeostasis.

We expect that efforts to explore the immunomodulatory functions of LcS will enable us to determine the ideal probiotics to help the immune system.

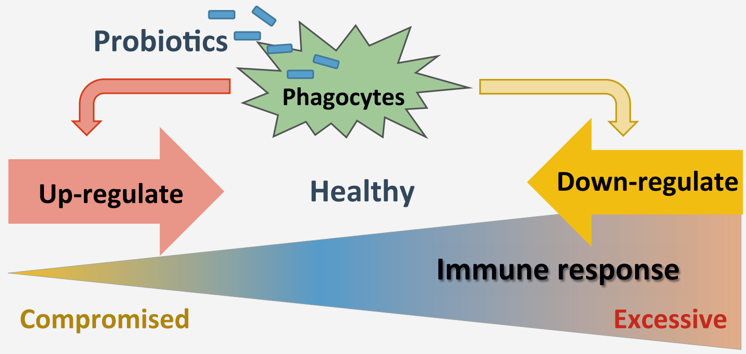

Figure 2 | Immunomodulatory functions expected from probiotics. Probiotics can help to modify the immune system's homeostatic functions by either upregulating or downregulating reactions to keep the immune system healthy.

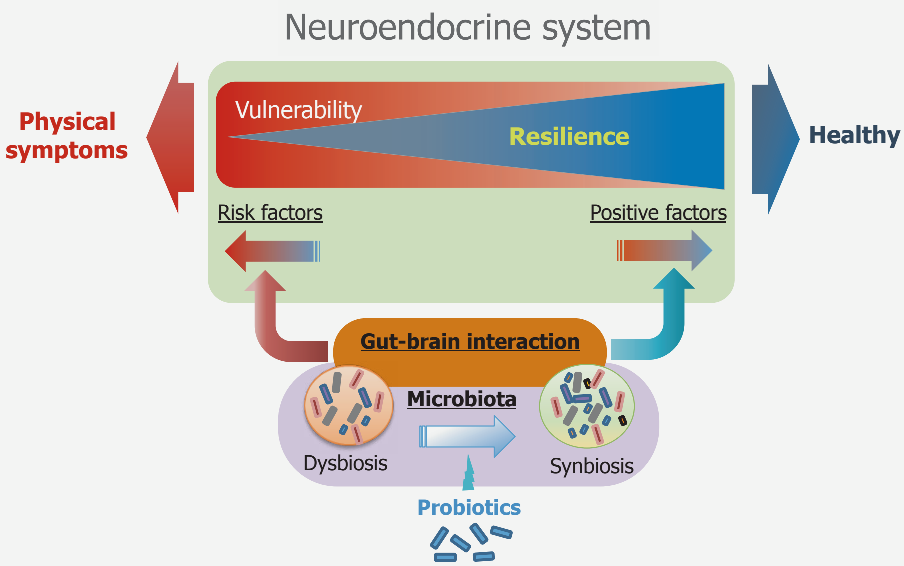

Figure 3 | Neuroendocrine-modulatory functions expected from probiotics. Probiotics directly or indirectly act on the brain through gut–brain interaction and maintain function of the neuroendocrine system, thereby enhancing resilience and enabling responses to multiple risks (physical or psychological stressors).

Introducing the microbiota–gut–brain interaction

The nervous, endocrine and immune systems maintain homeostasis in complex living systems. The concept of 'gut–brain interaction', which refers to the two-way communication between the brain and the intestinal tract, was proposed in a primitive way in 1880s and has evolved to become the 'microbiota–gut–brain interaction'. We assume that normalisation of gut microbiota might positively influence the development and maturation of the nervous and endocrine systems, as well as those of the immune system, and are conducting research on the relationship between the intestine and human health.

The effect of gut microbiota on the neuroendocrine system

Over the past ten years, research on the effects of gut microbiota on human physiological functions has moved on from establishing links with physiological homeostasis, including immunomodulation, to looking at psychological connections involved in anxiety or depression.

Notions that gut microbiota have effects on the neuroendocrine system have been derived from basic research using germ-free mice. For example, R. D. Heijtz and colleagues at the Karolinska Institute in Stockholm, Sweden[11], showed that commensal microbiota were important for the development of the cranial

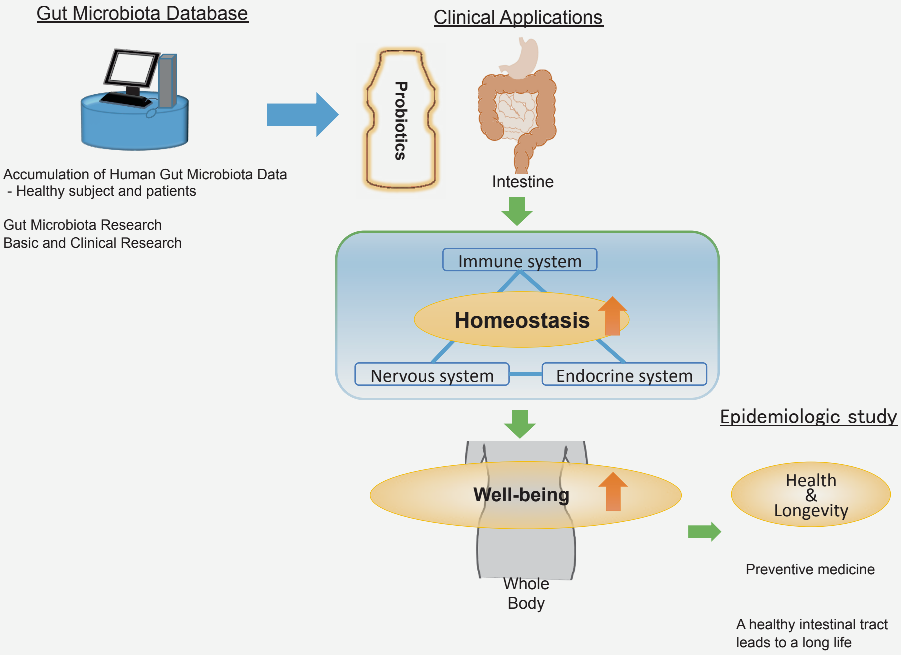

Figure 4 | Probiotic research to which the Yakult Central Institute is oriented. Daily intake of probiotics enhances the body's natural healing ability and resilience, recovery from disturbed homeostasis, and helps to maintain health.

nervous system associated with motor control and anxiety behaviour. In another study, N. Sudo and colleagues at Kyushu University in Fukuoka, Japan[12], worked with germ-free mice to show that commensal microbiota were involved in the development and maturation of the hypothalamic–pituitary–adrenal axis. These studies suggest that gut microbiota provide stimuli directly or indirectly from the intestinal tract, and are deeply involved in the development and maturation of the cranial nervous and endocrine systems. Using model mice that display features of autism spectrum disorder, E. Y. Hsiao of California Institute of Technology in the United States and colleagues[13] found that disturbances in gut microbiota were related to psychological and physical symptoms. They reported that the model mice showed characteristic dysbioses, and that treatment with *Bacteroides fragilis* altered their gut microbiota and ameliorated autistic spectrum-like symptoms.

Gut microbiota seem to be important in the development and maturation of the cranial nerves and the endocrine system, and disturbances may have negative psychological effects. Research on the ways in which microbiota can affect neurodevelopmental disorders in humans is in its early stages. At Yakult Central Institute we analyse gut microbiota data from people with and without neurodevelopmental disorders.

Imbalanced gut microbiota

We are currently undertaking research into whether imbalanced gut microbiota affects the health of the neuroendocrine system. We have conducted a large number of analytical studies on gut microbiota and confirmed that LcS is a beneficial probiotic that recovers disturbed gut microbiota. D. Benton and colleagues at Swansea University in Wales[14] asked healthy individuals to consume LcS-containing milk drinks for three weeks. The study reported that the individuals who had impaired moods or emotions at the start of the study reported an improvement in mood by the end of the study. In another study, A. V. Rao of the University of Toronto, Canada, and colleagues[15] asked people with chronic fatigue syndrome to consume LcS-containing milk drinks for eight weeks and found that the number of faecal lactic acid bacteria and bifidobacteria increased and the participants' anxiety symptoms were alleviated. Several studies using stress animal models have suggested that gut microbiota in the intestines would become imbalanced if placed under stress. In collaboration with researchers at Tokushima University in Japan we conducted a placebo-controlled, double-blind study in which we asked healthy undergraduate medical students who were revising for examinations to consume LcS-containing milk drinks for eight weeks prior to their exams. We found that the placebo group showed development and exacerbation of physical symptoms (abdominal and cold symptoms) accompanied by increase of stress markers, but the LcS-consuming group showed suppression of the onset of these symptoms and had significantly suppressed salivary cortisol levels that otherwise elevated immediately before examinations[16]. A basic study with stress model animals confirmed that intake of LcS reduced hypersecretion of a stress hormone under stressful conditions, and reduced stress reactivity in the brain[16].

Thus, probiotics beneficial for humans should, by preventing disturbances of gut microbiota, maintain homeostasis of the neuroendocrine and immune systems, enhance resilience inherent in living systems, and sustain healthy minds and bodies (Figure 3). However, the relationship between gut microbiota or probiotics and the neuroendocrine system is still not fully understood and further research is recommended.

Healthy intestines lead to longevity

Research on intestinal bacteria for health

Research into gut microbiota and probiotics has helped us to understand the close relationship between healthy infants and bifidobacteria, and has led to the idea of harnessing bifidobacteria to maintain intestinal health in infants and children. In addition, the development of techniques such as the culture method and gnotobiotic animal technology (which can produce animals containing a known strain of bacteria from germ-free animals) to analyse gut microbiota has helped to advance research into the effects of probiotics on the intestinal environment. The generation of strain-specific primers and monoclonal antibodies has enabled analysis of intestinal and faecal probiotics at a strain level, which has helped understand the mechanisms of probiotic survival and *in vivo* dynamics.

An important topic in basic gut microbiota research is the advance of 16S rRNA gene segments analysis techniques using next-generation sequencers. Technical advances in 16S rRNA gene segments analysis targeting specific regions of 16S rRNA genes in all faecal microbes have markedly developed gut-microbiota research and helped to evolve what was once a niche field of research into a major one. However, the analysis is suitable for major microbes but not for minor microbes in faeces. Therefore, the combination of 16S rRNA gene segments analysis for all major microbes and YIF-SCAN® analysis for specific minor microbes will help to interpret data from research into gut microbiota and further our understanding of the mechanisms of probiotics.

One subject that is dominating gut microbiota clinical research is the development of faecal microbiota transplantation (FMT), a treatment in which faeces taken from healthy individuals are transplanted into patients who have gastrointestinal diseases. At the University of Amsterdam, E. van Nood and colleagues[17] found that FMT was more effective against recurrent diarrhoea associated with *Clostridium difficile* than traditional treatments with antibacterial drugs. P. Moayyedi of McMaster University in Hamilton, Canada, and colleagues[18] reported that a placebo-controlled study in people with ulcerative colitis showed that FMT had a high remission rate. None of the studies reported side effects caused by FMT. Although implementing FMT as a therapy faces challenges, including those related to safety, it is developing as a treatment for intractable gastrointestinal diseases because of its high efficacy rates when compared to conventional treatment.

In conclusion, we can say that basic and clinical research into gut microbiota has made a huge contribution to human health maintenance through probiotics.

The future of probiotics research

The history of probiotics research began with studies considering phylactic effects and how to improve the intestinal environment and includes work on LcS and on ameliorating symptoms of intestinal diseases, and extended to research investigating properties such as cancer-prevention, anti-allergy, anti-inflammatory and effects linked to mental health.

Why do probiotics such as LcS exert such a variety of physiological effects? The answer lies in the intestine. The intestine functions to digest and absorb nutrients from food, absorb water and generate faeces. It is also a protective barrier against bacteria and foreign bodies that have entered the body through the digestive tract, and functions to exchange information with the brain (gut–brain interaction) — these perhaps surprising functions of the intestine could exert a variety of physiological functions when combined with beneficial probiotics.

Probiotics could help to improve the environment within the intestines and enhance the activities of other organic acid-producing bacteria. Furthermore, they are not only directly taken in by intestinal antigen-presenting cells to stimulate the immune system, but also act on the centre of a living system, the brain, through the neuroendocrine system using unknown receptors in intestinal cells. The brain uses the neuroendocrine and immune systems to help maintain homeostasis. In other words, probiotics act not on single sites, as drugs do, but on the whole body through the immune and neuroendocrine systems from multiple intestinal sites in a direct or indirect way. We suggest a new hypothesis that daily intake of probiotics enhances the body's natural healing ability and resilience, recovery from disturbed homeostasis and helps to maintain health (Figure 4).

To prove the hypothesis above, we need to accumulate additional evidence on the function of probiotics and compile a database of gut microbiota. To do so will involve analysing the relationship between gut microbiota and probiotics with various clinical symptoms, short-term intervention studies and long-term intervention studies. Retrospective epidemiological studies have already reported the relationships between risk reduction of bladder[19] or breast[20] cancers and probiotic intake.

One final thought

We have worked on the relationship between the maintenance of human health, intestinal bacteria and probiotics. Our dedication to research will never change as long as Dr Minoru Shirota's health-related principles are handed down, just as genes are passed to offspring.

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