Japanese Food for Health and Longevity

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The Science behind a Great Culinary Tradition

By

Yoshikatsu Murooka

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PREFACE

We often hear that Japanese food is good for health. But how popular is Japanese food, *Washoku*, with people from foreign countries? Why are the local foods of few countries popular with travelers all around the world? A survey by Hotel.com ranked people's favorite cuisines: the most popular country is Italy, the second is France and, the third is Japan, then China is next, followed by Spain, the USA, Mexico, Thailand, and India at tenth place. Surprising that Japanese food beat Chinese food. This might be due to its increasing popularity across the world. According to another survey, Japanese food is the most preferable (report from the Japan External Trade Organization 2013). The inclination towards Japanese food seems to be because it is good for health and longevity without compromising on delicious taste.

Japanese women and men have had the longest longevity in the world since 1990 (report from the World Health Organization 2015). It is thought that the reason for the longevity is their dietary habits and lifestyle, including medical care, the high quality of hygiene, and a rather mild climate all year round. However, although many Japanese cooking books exist, books about Japanese foods from the scientific aspects of the health and longevity were rarely published. Once I was asked to write about the scientific evidence of Japanese traditional fermented food as a source of health and longevity in a review paper for a scientific journal (Murooka and Yamashita 2008). Although I am not completely satisfied now, this paper is the most read and cited among many of my original papers and reviews concerned with bioscience and biotechnology.

When I spent my younger days in foreign countries, I could do without Japanese food for a couple of years. But now that I am getting old I long for Japanese food. I particularly prefer simple foods like *Udon* (noodles made of wheat flour), *Soba* (noodles made from buckwheat), and *Ochazuke* (steamed rice with hot tea poured on it), as these foods are non-fatty and non-heavy. Since I am not a foodie, I cannot introduce you to the charms of Japanese cuisine.

Therefore, in this book, I will explain the background of the Japanese food boom from a scientist's point of view. Also, for people who are not familiar with Japanese foods or Japanese culture, I will introduce typical dishes and traditional lifestyles; this will be based on my previous book, written both in Japanese (Murooka 2013) and English (Murooka and Saeki 2015). Furthermore, I will shed some light upon biochemistry, life sciences, molecular biology, and genetics, in simple terms for those who are not familiar with these topics.

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CHAPTER ONE

LACTIC ACID BACTERIA USED IN TRADITIONAL JAPANESE FERMENTED FOODS

I will begin with an explanation of lactic acid bacteria used in Japan. However, you may find it a little strange because dairy foods are associated with central Asian and Western countries. However, lactic acid bacteria have a deep relationship with the production of traditional Japanese fermented foods. I will discuss this deep relation later in this chapter, but before that, I would like to explain probiotics, which you may find somewhat confusing. The subject is concerned with, for example, the relationship between yogurt and the scientific basis of longevity.

1.1 Probiotics are microorganisms that benefit health

Once I heard that people who live longer are mostly from the Caspian Sea area, Bulgaria, Kyrgyz-Kazakhstan, and the Humza district of Pakistan. The epidemiological investigations suggested that this might be related to their daily consumption of fermented dairy products. According to the World Health Organization, "Epidemiology is the study of the distribution and determinants of health-related states or events (including disease), and the application of this study to control the diseases and other health problems. Various methods can be used to carry out epidemiological investigations: surveillance and descriptive studies can be used to study distribution; analytical studies are used to study determinants" (https://www.who.int/topics/epidemiology/en/).

More than a hundred years ago, Dr. Ilya Ilyich Mechnikov (Tauber 2003), a Russian biologist, zoologist, proto-zoologist, and the director of the Pasteur Institute at Paris, proposed a theory that yogurt-consuming people lived longer lives because microbes in the daily product can replace the putrefactive microbes with useful microbes in their intestines. These microorganisms that benefit our health are now called "probiotics" (Hill et al. 2014).

Pastoral farmers have been producing dairy products such as yogurt, butter, and cheese for a long time. These dairy products are made by microbial fermentation as follows: milk sugar (lactose) and fats of milk are converted into acidic materials like lactic acid, sugars like glucose, amino acids, and vitamins, etc. The representative of these microorganisms is called lactic acid bacteria. Lactic acid bacteria belong to “Gram-positive bacteria” in the taxonomy of biology.

1.2 Taxonomy is essential to understand microorganisms

Biologists have been classifying living organisms into several groups according to their forms or physiological characteristics. However, recently it became clear through the technique of genetic analysis that the classification of living organisms is closely related to their evolution process. For instance, there are different categories of bacteria, such as thermophilic bacteria, which live near chimneys of submarine volcanoes; halophilic bacteria, which live in high salt containing deserts; extreme sulfate or iron loving bacteria and many more. The other type of single-cell organisms includes organisms that generate methane or hydrogen or live by using them as an energy source, are classified into the “*Archaea*” domain. These archaea appeared on the earth just after the origin of life. They are born under peculiar circumstances: when the temperature is over 100°C or when the salt concentration is over 20%. When we look at the evolution of all living organisms, we trace the origin of these archaea.

Bacteria also identified as Gram-positive or Gram-negative bacteria. For example, *Natto* bacterium and cholera bacterium are classified into “Gram-positive bacteria,” and *Escherichia* or *Salmonella* species are classified into “Gram-negative bacteria.” What does Gram-positive or -negative bacteria mean? Dr. Hans Gram (1884), a Danish bacteriologist, developed a method of staining bacteria, which played a major role in classifying them. The Gram stain, whether positive or negative, depends on the chemical compositions or structures of the bacterial cell wall or cell membrane.

The cell walls or the cell membranes are significant for the living organisms in terms of deciding the level of protection from the environment or receiving nourishment. The difference in the surface of bacterial cells defines various phenomena, for example, whether antibiotics will be effective or not, on the activation of antibodies, or an immune reaction to an allergy when we get infected with bacteria. For instance, penicillin (β-lactam group antibiotics) attacks and kills various pathogenic bacteria by preventing the synthesis of the cell wall (Blumberg 1974). This is why taxonomy is essential to understand living organisms. The difference between Gram-positive and Gram-negative bacteria does not necessarily lead to the bacteria being useful or not to human beings. I will explain about the immune reaction of lactic acid bacteria later.

1.3 Primitive experiments at making yogurt

Some time ago, I heard that yogurt might have its origins in Turkey. However, milk becomes sour after a while if we leave it at room temperature, and so, it would be better for us to think that people engaged in cattle breeding in old times discovered the method of lactic acid fermentation naturally. The reason why milk becomes sour is that lactic acid bacteria make lactic acid. We call this "lactic acid fermentation." Other similar organic acid fermentations are called "acetic acid, succinic acid, and citric acid fermentations." Acetic acid fermentation is explained in detail later in Chapter Five.

As a matter of fact, I spent three weeks trekking in Shimshal Pamir of Karakoram in the northern part of Pakistan near the border between China and Pakistan. After we continued to walk up along a narrow path made on the mountain cliff surface, a highland plateau came into our sight at the altitude of 4,000 m (13,120 ft.) to 5,000 m (16,400 ft.) above sea level (Fig. 1-1 upper). Pamir's high land is known as the roof of the earth. A local person told me that Pamir means fertile grasslands. Xuanzang (玄奘) (AD 602–664), a famous Chinese Buddhist monk, crossed this region, the Pamir, at the beginning of the Tang (唐) Dynasty of China. He crossed over Pedal Pass of the Tian Shan (天山) Mountains into Lake Issyk-Kul of Kyrgyzstan and reached India by way of Afghanistan. On his way back to China, he crossed the WA Khan Corridor of Afghanistan and came back to Tashkurgan of Xinjiang (新疆) in the Uyghur Autonomous Region. Therefore, Shimshal Pamir, which I visited, is at the eastern side of the Pamir over which Xuanzang crossed. The Wakhi tribe, whose ancestors moved there 400–500 years ago from Kyrgyz-Kazakhstan, sent yaks, goats, and sheep out to pasture in those grasslands in summer.

Yaks are long-haired wild cattle living at high altitudes, like in the Himalayan region or by Pamir, and they are quite fierce by nature (Fig. 1-1 upper). Yak's milk can be used to make yogurt, butter, and cheese. People living by Pamir use a traditional method of producing dairy products, and I would like to tell you about their method in a little more detail. First, they boil 20 liters of (5.3 gallons) milk, 90% of which is from yaks and 10% from goats, for three hours, and then they cool it and add preserved yogurt. After this, it is fermented for 8 hours and kept warm at about 30 °C (86 F). Then they pour the liquid into a wooden cylinder called "sogo" and churn it using a wooden stick called "padaru" (Fig. 1-1 lower right). There it is mainly women's work to produce butter and cheese. When I tried my hands on it, I became groggy soon after churning because the liquid was too thick and sticky. The upper floating liquid, 10% of which is milk, becomes butter, and the lower remaining liquid is processed into cheese by fermenting it for 8 hours and boiling it. They dry the cheese on the roof of a stone hut (Fig. 1-1 lower left). I think this method may be called "fundamental cheese production."

Figure 1-1 Cheesemaking at Shimshal Pamir highland in Karakorum, Pakistan.

Clockwise from upper left: Yaks (Bos mutus) in the Pamir highlands, cheese making using yak milk with a family from the Wakhi tribe at the summer stone cottage (the author is in the center of the picture), and drying cheese on the roof of a stone cottage. (Photos, taken by the author).

Nomadic people live in stone huts in summer. During winter, they move to lower places, the altitude of which is 3,000 m (9843 ft.) above sea level. I was invited to their stone hut and was served very delicious "chai," which is a tea-based beverage mixed with milk and sugar, and their handmade cheese.

1.4 Lactic acid bacteria used in Japanese fermented foods are of plant origin

Although it appears that lactic acid bacteria have a connection with the production of Japanese foods, but the fact is, there is a deep link between lactic acid bacteria and traditional fermented products in Japan. The deliciousness and flavors of fermented foods, such as Japanese *tsuke-mono* (pickled vegetables), *sake* (Japanese rice wine), *miso* (soybean-barley paste) and *shoyu* (soy sauce), are influenced by fermentation, which uses lactic acid bacteria together with *koji* mold and yeasts (Fig. 1-2).

The lactic acid bacteria contained in Japanese foods are different from those in milk products produced by cattle breeding races. In Japan and some Asian countries, lactic acid bacteria isolated from plants live on grains or vegetables; therefore, they can decompose plant starch or cellulose into sugar. They use this for their nutrients and energy. A lactic acid bacterium, which has been isolated from dairy products, grows by taking the nutrients from the milk and cannot usually have the ability to decompose starch or cellulose. Lactic acid bacteria used in traditional plant-derived fermented foods, such as *sake*, *shochu* (Japanese spirit), rice vinegar including *kurosu*, *miso*, soy sauce, and *tsuke-mono* or *kimchi* in Korea, usually live in brewing rooms or instruments used for brewing. Therefore, cultivated lactic acid bacteria are not cultured in these traditional fermentations. Naturally occurring lactic acid bacteria acquire unique characteristics to adapt to their circumstances. Fermented products, such as *sake*, *kurosu*, *miso*, soy sauce, *tsuke-mono*, *shochu*, and *awamori* are explained in the following chapters.

Lactic acid bacteria isolated from a *moromi* mash of *sake* were *Lactobacillus plantarum*, *Leuconostoc mesenteroides*, and *Lactobacillus sakei* (Katagiri et al. 1934). Lactic acid bacteria require nicotinic acid, which is coagulated with *sake* yeast to decrease its respiration. Furthermore, *Leuconostoc citreum*, which ferments at 4°C, was isolated from the rice *koji* and used for brewing *sake* (Kurose et al. 2004). In *moromi* mashes of *shochu* and *awamori*, lactic acid bacteria contribute to the production of vanillin, which is a component of vanilla flavor (Furukawa et al. 2014). "*Moromi*" means softening ingredients through enzyme reactions during the fermentation of *sake* and soy sauce.

Figure 1-2 Making traditional Japanese foods with lactic acid bacteria.

Clockwise from upper left: *Sake* brewing by "*kimoto*" method with naturally habited lactic acid bacteria, Taketsuru-shuzo (竹鶴酒造) Co., Takehara, Hiroshima; making black vinegar (*Kurosu*) in pots, Sakamoto Kurozu (坂本醸造) Inc., Fukuyama, Kagoshima; making soy sauce (*Shoyu*), Kanro-shoyu (甘露醤油), Yanai, Yamaguchi; making *miso*, Masuyamiso (ますや味噌) Co., Kure, Hiroshima; center is a sample of *Tsuke-mono* taken from the Creative Commons. Lactic acid bacteria used in soy sauce, *miso*, and *tsuke-mono* are salt-tolerant strains (photos, obtained from the respective companies).

Lactic acid bacteria in soy sauce, *miso*, and *tsuke-mono*, which are produced in the presence of high concentrations of salt, are salt-tolerant and a halophilic species. A lactic acid bacterium isolated from soy sauce mash has salt-tolerant characteristics and was initially named *Pediococcus sojae* (Sakaguchi 1958), but it is now called as *Tetragenococcus halophilus*. Recently, the whole genomic sequence of this bacterium was completed, and the genes involved in the maintenance of osmotic balance that explain its high-salt resistance were found (Nishimura et al. 2017). As a lactic acid bacterium from *tsuke-mono*, *Lactobacillus brevis* was isolated from a traditional pickle made from cress (*suguki*), which is cultivated in the Kyoto area, and the bacterium is commercialized as probiotics (Kishi et al. 1996). This strain induces the production of interferon-α and stimulates natural killer cells that help to prevent infectious diseases and tumors (Yajima et al. 2007).

During *miso* fermentation, the halophilic bacterium, *Tetragenococcus halophilus*, increases moderately (Onda et al. 2003). "The non-halophilic strains showed a complex growth pattern in the *miso* fermentation process and were identified as *Enterococcus faecium, Enterococcus durans, Enterococcus faecalis, Pediococcus acidilactici, Pediococcus pentosaceus, Lactobacillus plantarum,* and *Weissella confusa.* The predominant species throughout the fermentation process were *T. halophilus, E. faecium*, and *E. durans*." Onda et al. (2003) further found that "among them, only the strains of *E. faecalis* and *E. durans* produced bacteriocin that had an antibacterial effect on *Bacillus subtilis.* The bacteriocin producers seem to play an important role in maintaining normal bacterial flora during *miso* fermentation."

1.5 Lactic acid bacteria are effective for allergic episodes

Microorganisms, which are believed to improve health by maintaining good intestinal conditions, are called "probiotics." Lactic acid bacteria, bifidobacteria, propionic acid bacteria, and *natto* bacteria are known as probiotics. Bifidobacteria are found in infants' intestines and can break down mother's milk, but they get decreased as we become an adult. A propionic acid bacterium is used as a starter (an initiator of fermentation) for foods, such as Swiss cheese. A species of propionic acid bacteria, *Propionibacterium spp.*, is an industrial microorganism since it can produce vitamin B_{12} (Murooka et al. 2005).

Although lactic acid bacteria usually decompose rice starch, this bacterium also decomposes lipids. We found a gene for a starch degrading enzyme called amylopullulanase, which was located on one of the 15 plasmids in *L. plantarum* L-137 (Kim et al. 2008).

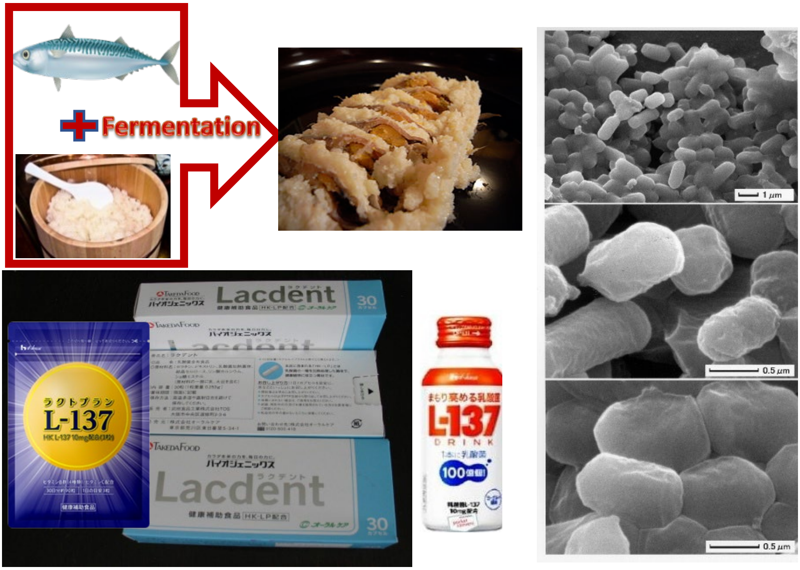


Figure 1-3 Lactic acid bacteria isolated from *Narezushi*, traditional fermented food from fish and rice.

Upper left, *narezushi*, which is made by fermenting fish and steamed rice as a preservative food in Japan and Philippines; right, electron microscope photographs of *Lactobacillus plantarum* L-137 isolated from *narezushi*; lower left, supplements with "heat-killed" *Lactobacillus plantarum* L-137. (Photos provided by Dr. Y. Yamamoto, House Wellness Foods Co. Ltd, Itami, Hyogo, Japan.)

Most probiotics bacteria, which have been studied, are isolated from dairy products in Western countries (Fuller 1989; Reid et al. 2003). We have isolated several strains of lactic acid bacteria from non-dairy fermented foods produced in Asia. The *Lactobacillus* species are the most common bacteria isolated from traditional Asian fermented foods, which are made from rice, beans, or vegetables. One such strain, *Lactobacillus plantarum* L-137, was isolated from a kind of *narezushi* called *brong isuda*, which is a fermented food made from rice and fish (Fig. 1-3 upper left); this is a much-loved traditional Philippine fermented food (Olympia et al. 1992). We also use *narezushi* as preservative food in Japan.

L. plantarum L-137 has a strong promotive effect on the immune response. Both the live cells and heat-killed strain L-137 (HKL-137) cells at 100°C induced cytokines. Cytokine includes interleukin-12 (IL-12) and interferon-γ (IFN-γ), which regulate the immune system (Murosaki et al. 1998). Cytokines are proteins that are released from immune cells, and they are involved in immunity, inflammation, cell growth, killing cells, and healing wounds by transferring genetic signals. Interleukin, a kind of cytokine, is released from white blood corpuscles and controls immunity activities. There are various types of interleukins, and each one has been given a number.

"The *L. plantarum* L-137 cells suppressed the production of IgE against a naturally fed antigen via stimulation of the production of IL-12 in mice" (Murosaki et al. 1998). I will explain this later but, as a result, it proved that this lactic acid bacterium was effective at managing allergic diseases, such as house dust and pollen allergies (Fig. 1-4). Both living cells and "heat-killed" cells exhibit the same impact on the immune response. Thus, by using the "heat-killed" *L. plantarum* strain L-137, the "Lacdent, Lactoplan L-137, or L-137 beverage" came into the market (Fig. 1-3). The Japanese dairy industry thought that plant-sourced lactic acid bacteria would be effective at managing several allergic diseases. Taxonomically speaking, "plant lactic acid bacteria" are not classified into a definite category.

More than 30% of Japanese people suffer from allergic diseases, such as atopic eczema, house dust allergy, and pollen allergy, which are mostly due to Japanese cedar (Allergen Research Report of the Advisory Panel to the Ministry of Health and Welfare, Japan 1996). This information was reported 20 years ago, supposedly, about half of Japanese people are still suffering from an allergic disease. Similarly, the number of people who suffer from allergies in developed countries is also increasing. Are lactic acid bacteria effective as a treatment for allergic diseases, such as pollen allergies? I can say, "Yes, they are" based on the result of our research (Hirose et al. 2006). Since the mechanisms of allergy and its desensitization by lactic acid bacteria are quite complicated, their explanation is rather difficult. I have tried to explain using the simplified illustration in Figure 1-4.

Our body's cells recognize allergens, such as cedar pollen and house dust, as foreign particles (non-self) and produce antibodies to remove them from the body. Our immune system activates allergen-influenced Th2 cells, which in turn produce interleukin- 4 (IL-4). IL-4 induces the production of IgE, a type of antibody, using B cells, and then mast cells release histamine (Lund 2008). As a result, symptoms like sneezing, running nose, and tears arise to protect the human body against allergens. Most of the medicines for allergies that are prescribed by doctors are anti-histamines. When we orally consume lactic acid bacteria, it is recognized by Tol-like receptors, such as TLR2 and TLR4, and induces cytokines, such as IL-12 and IFN-γ, that result in changing the cell balance of Th1 to Th2. This change results in the repression of IgE production (Pochard et al. 2005). As a result, the release of histamine from mast cells is reduced, and the allergen reaction is alleviated; this is called "desensitization."

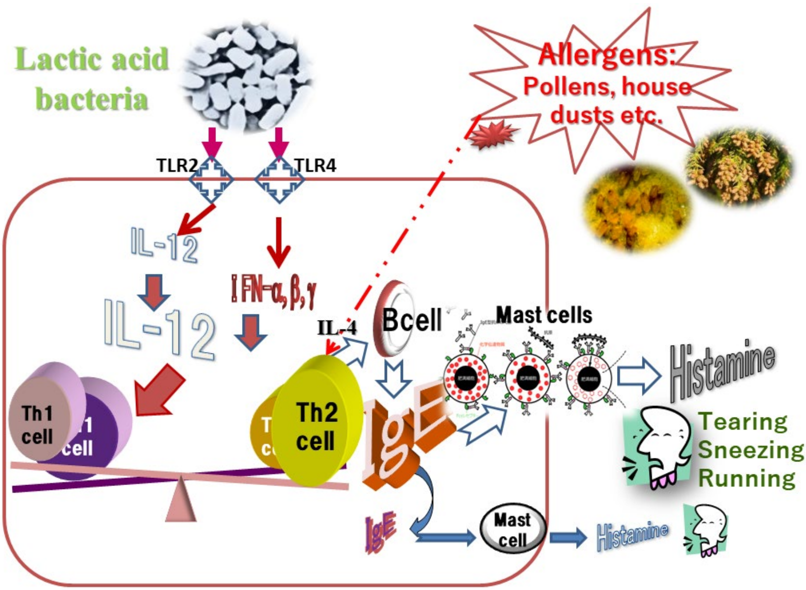
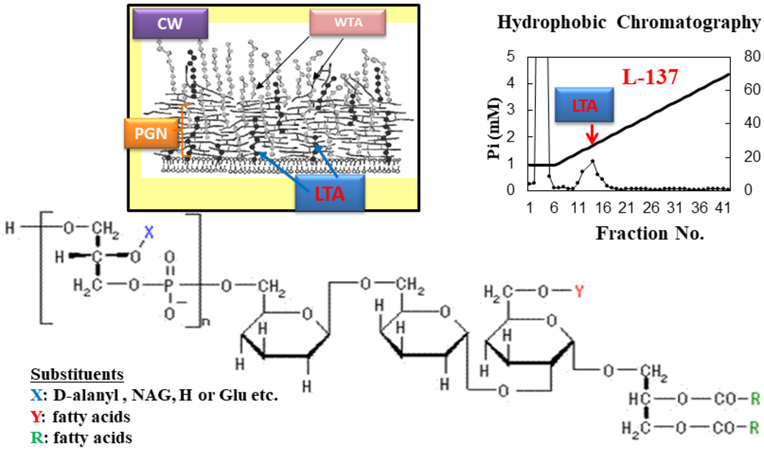


Figure 1-4 Schematic illustration of an allergy reaction and its desensitization mechanism using lactic acid bacteria

The immune cells recognize the allergen, and activation of Th2 cells leads to the production of IL-4. IL-4 induces the production of IgE by B cell activation, and then mast cells release histamine in response. As a result, symptoms like sneezing, running nose, and tears arise to protect the human body against allergens. Lactic acid bacteria are recognized by Tol-like receptors on the surface of immune cells, such as TLR2 and TLR4, and induce cytokines, such as IL-12 and IFN–γ, that result in changing the balance of Th1 to Th2 cells. This change of cellular balance results in the repression of IgE production. As a result, the release of histamine from mast cells is reduced, and the allergen reaction is alleviated.



	Substituent	L-137	JCM1149
X	Alanine	> 90	30
	Glucose or H	< 10	70
n	Chain length	41	18

Figure 1-5 Purification of lipoteichoic acid for strains L-137 and JCM1149 with hydrophobic ion chromatography and analysis by NMR spectroscopy.

The cell wall component (upper left) of *L. plantarum* L-137 and JCM1149, a standard strain, was separated by flow cytometry and purified with hydrophobic ion chromatography (upper right, Hirose et al. 2010). The structures of lipoteichoic acids (LTA) were analyzed by NMR spectroscopy. The differences in the structures of LTAs between L-137 and the standard strain JCM1149 are shown in the Table (bottom): WTA, wall teichoic acid; LTA, lipoteichoic acid; PGN, peptide glycan. Figures and Table provided by Drs. Hirose and Yamamoto, House Wellness Foods Co. Ltd. Itami, Japan.

It became clear from the result of our research work with a food company that the main reason for the difference of strength and weakness in the release of cytokines was attributable to the cell components and cell wall structure. It also suggested that differences in a component of the cell wall, known as lipoteichoic acid, were the leading cause (Fig. 1-5) (Hirose et al. 2010). It is also thought that, in addition to lipoteichoic acid, the difference in releasing cytokines arises due to the lactic acid bacteria, which are influenced by the differences in their DNA and metabolic substances.

Therefore, not only living lactic acid bacteria but also heat-killed lactic acid bacteria, which have been sterilized by boiling, can release cytokines.

To enhance the function of a probiotic strain, we developed an expression vector (a plasmid carrying target gene) for *L. plantarum* and succeeded in the expression of the cholesterol oxidase (*choA*) gene, and produced a cholesterol-degrading enzyme in both *L. plantarum* and *Propionibacterium freudenreichii* (Kiatpapan et al. 2001). By using this expression vector, we developed an oral vaccine against Japanese cedar pollen allergy (Ohkouchi et al. 2011). Since this is a genetically modified bacterium, it is still on its way to being approved. In another group's experiment (Ishida et al. 2005), the oral administration of *Lactobacillus acidophilus* strain L-92 was isolated from fermented milk called Calpis to alleviate the symptoms of perennial allergic rhinitis; however, statistically, significant changes were not shown in blood parameters.

Figure 1-6 shows a clinical test of "heat-killed" L-137 (HKL-137) cells using oral administration in humans (Hirose et al. 2006). The HKL-137 cells enhanced natural killer (NK) cells and T cell proliferation, and also increased Th1/Th2 cell ratios and quality of life (QOL). These results suggest that HKL-137 cells have the potential to protect against infectious diseases and tumors in addition to their anti-allergy effect through the stimulation of immune functions.

Plant-sourced lactic acid bacteria are not always superior to lactic acid bacteria from milk products, although the plant origin bacteria are usually stronger because they live in a rough environment. The strength of immune system regulation differs according to each strain of lactic acid bacteria. *Lactobacillus paracasei* isolated from a daily product stimulated the production of IL-12 and repressed that of IL-4, production of IgE was also suppressed against naturally fed antigens, which resulted in desensitization of mice against the antigens (Fujiwara 2004).

Dairy products manufacturers in Europe are applying to the EU's Health Organization to get approval for the effectiveness of dairy products, insisting that yogurt is successful in treating allergic illnesses, but this has not yet been approved due to a lack of data. It requires double-blind tests. In a double-blind experiment, a group of people who drink yogurt and a group of people who do not are tested and the result of whether it is valid or not is found. Elimination of unrecognized biases carried by the experiment's subjects and conductors is required, which is why it is called "a double-blind test." The more participants there are, the more reliable the result of the experiment. Participants have to eat the same food and live under the same conditions every day. It costs a lot because we have to test many participants under the same conditions for a long time.

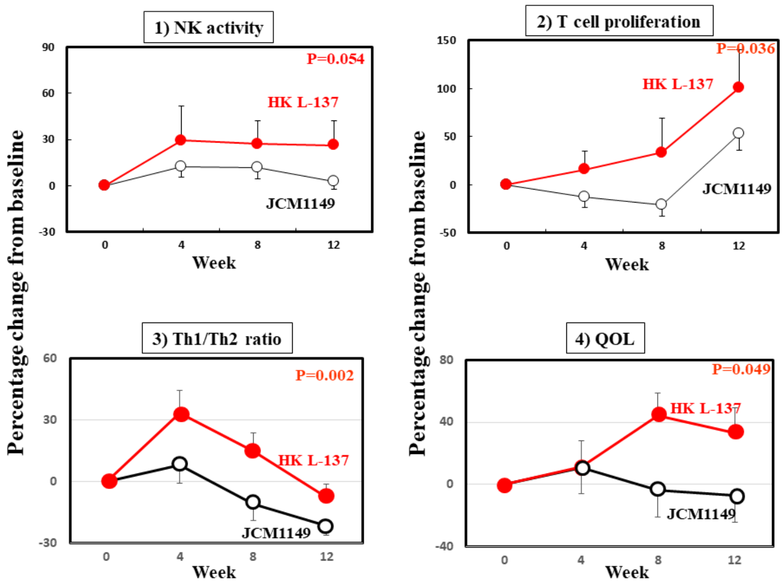


Figure 1-6 Clinical trials: Dietary L-137 stimulates immune functions and health-related QOL

60 (30 men and 30 women) healthy subjects over 40 years old consumed *Lactobacillus plantarum* "heat-killed" L-137 cells (10mg) and the same amount of *L. plantarum* JCM1139 cells for 12 weeks. From upper left, clockwise: activities of natural killer (NK) cells from L-137 and JCM1139 control cells; T cell proliferation; quality of life (QOL); and the Th1/Th2 cell ratio. Figures provided by Drs. Hirose and Yamamoto, House Wellness Foods Co. Ltd., Itami, Japan.

In conclusion, several strains of lactic acid bacteria certainly have some positive effects on reducing allergic symptoms, although they cannot cure them completely. However, it will take a long time to clear clinical trials and bring them to the market.

For example, what do you think could be a reason that few older people are allergic to a pollen disease? An epidemiological investigation showed that people who had roundworms in their bodies are not allergic to pollen (Fujita K, https://www.athome-academy.jp/). People who are over 70 years old at present in Japan and those who grew up in farmers' homes after the 2nd World War ate vegetables grown in fields where raw human feces were used as an organic fertilizer. Roundworm eggs, which were found on vegetables, hatched in human bodies and grew. For a while, after the 2nd World War, infections were treated with the drug, santonin, to expel worms. Roundworms removed the nutrition from children's bodies and made them thin. I hear that allergic reactions rarely occur in children living with cattle in European countries. Our bodies recognize roundworms, cattle dung, and so on as foreign substances and protect us by making an antibody named IgE. Scientists have explained that in today's clean environments in developed countries, our bodies react to a little foreign substance sensitively, and this causes allergic symptoms. People who had roundworms in the past can be said to be "insensitive to allergies." I can assert that I am insensitive to pollen illness since I had roundworm in my childhood. Maybe we do not have to worry whether our young children are playing in dirty surroundings.

1.6 Lactic acid bacteria prevent cancer and infectious diseases

There is a report that lactic acid bacteria are effective in preventing gastric cancer. It became known that *Helicobacter pylori*, which is found in the stomach, is concerned with 70% of gastric cancer and gastric ulcers. *Helicobacter pylori* are a Gram-negative bacterium and live in the mucosae of the stomach. *H. pylori* produce interleukin-8, a kind of inflammatory cytokine, and harm the mucosae carcinoma in the stomach (O'Hara et al. 2006). It is found that some types of lactic acid bacteria prevent *H. pylori* from increasing. There is a report that suggests that lactic acid bacteria prevent the production of interleukin-8 (Kim et al. 2014). However, how these lactic acid bacteria prevent *H. pylori* from increasing is not yet known. If the increase in the growth of lactic acid bacteria overcomes the growth of *H. pylori*, we can say lactic acid bacteria are effective at protecting us.

To return to the story of *Lactobacillus plantarum* L-137, Yamamoto et al. found the antitumor effect of "heat-killed" L-137 through the restoration of impaired interleukin-12 production in tumor-bearing mice (Murosaki et al. 2010). The same group also demonstrated that "heat-killed" L-137 cells provided enhanced protection against the influenza virus by stimulating type I interferon production in mice (Maeda et al. 2009). As mentioned before, they examined the effects of the oral administration of L-137 and JCM1139 control cells on human subjects' immune functions. Natural killer (NK) activity, T cell proliferation, and their ratio of Th1/Th2 cells were all increased by a diet of L-137 cells (Fig. 1-6) (Hirose et al. 2006). The immune response from L-137 cells was significantly higher than that of the JCM1139 control cells. In a survey of health-related questions, the Quality of Life (QOL) factor was also supplemented by a diet of HKL-137 cells.

It was thought that intestinal bacteria, which are estimated to be around 10^{14}, have beneficial effects for health. Intestinal bacteria in mice do not produce an IgA antibody (Kikuchi et al. 2014). They found that orally administered lactic acid bacterium induced IL-6 production in dendritic cells, which stimulated IgA production. The administration of this lactic acid bacterium protected mice from infection of influenza (Kikuchi et al. 2014). "In humans, consumption of yogurt fermented with *Lactobacillus bulgaricus* OLL1073R-1 augmented natural killer cell activity and reduced the risk of catching the common cold in elderly individuals" (Makino et al. 2010). *Lactobacillus brevis* isolated from a traditional *tsuke-mono* (*suguki*) prevents infectious diseases and tumors (Yajima et al. 2007). These results suggest that specific strains of lactic acid bacteria could prevent some cancers and infectious diseases, in addition to allergies.

There are more effects of lactic acid bacteria on health than we expect. However, the effects depend on the kinds of strains of bacteria. For example, *Lactobacillus herveticus* isolated from Calpis (fermented milk) reduced blood pressure by the inhibition of an angiotensin-converting enzyme. Two peptides were identified as the main effector (Nakamura et al. 1995). *Lactobacillus gasseri* isolated from *tsuke-mono* usually shows high resistance to gastric acid and bile acid. A strain of *L. gasseri* PA-3 showed high decomposing activity to purine nucleoside, and commercial goods have been developed with this strain to reduce ureic acid through decreasing purine nucleoside in the human intestine (Yamada et al. 2016). Recently, two novel strains of lactic acid bacteria were isolated from *Funazushi*, which is the same as *Narezushi*. "These strains showed strong anti-inflammatory effects on DSS-induced colitis through induction of β8 integrin expression on dendritic cells" (Okada et al. 2018).

1.7 Bifidobacteria live in the large gut

Bifidobacteria are also known as one of the important probiotics. "Naturally occurring *Bifidobacterium* species are Gram-positive bacteria that colonize the human gastrointestinal tract, vagina, and mouth. Although the bacteria are not numerically dominant in the complex intestinal microflora, they are considered as key commensals that promote a healthy gastrointestinal tract" (Schell et al. 2002). *Bifidobacterium longum* subsp. *longum* is the main species since it is found in all infants and adults' guts. The addition of bifidobacteria as a probiotic for the treatment of ulcerative colitis was shown to be associated with both improved rates of remission and maintenance of remission (Ghouri et al. 2014). Bifidobacteria have many health effects; these are as follows: "the regulation of intestinal microbial homeostasis, the inhibition of pathogens and harmful bacteria that colonize and infect the gut mucosa, the modulation of local and systemic immune responses, the repression of procarcinogenic activities within the microbiota, the production of vitamins, and the bioconversion of dietary compounds into bioactive molecules" (Odamaki et al. 2011). Some *Bifidobacterium* strains are considered to be essential probiotics and are used in the food industry (Mayo and van Sinderen 2010).

Asia differs substantially within its regions, populated by diverse ethnic groups that maintain their own respective cultures and dietary habits. To address the diversity in their gut microbiota, Nakayama and more than 30 Asian researchers (2015) studied and characterized the bacterial community in fecal samples obtained from 303 school-age children living in both urban and rural regions in 5 countries spanning both temperate and tropical areas of Asia. They classified the microbiota profiled from the 303 subjects into two enterotype-like clusters, each driven by *Prevotella* (P-type) or *Bifidobacterium/Bacteroides* (BB-type), respectively. The majority of subjects from China, Japan, and Taiwan harbored the BB-type, whereas those from Indonesia and Khon Kaen in Thailand mainly harbored the P-type. In particular, children living in Japan harbored a less diversified microbiota with a high abundance of *Bifidobacterium* and a smaller number of potentially pathogenic bacteria. They suggest that the microbiota may reflect their living environment and unique diet (Nakayama et al. 2015).

A group from the Nestlé Research Center (Schell et al. 2002) determined the 2.26-Mb genome sequence of an infant-derived strain of *Bifidobacterium longum*, and identified 1,730 possible coding sequences. They found that "a large number of the predicted proteins appeared to be specialized for catabolism of a variety of oligosaccharides, some possibly released by rare or novel glycosylic hydrolases acting on 'non-digestible' plant polymers or host-derived glycoproteins and glycoconjugates. This ability to scavenge from a large variety of nutrients likely contributes to the competitiveness and persistence of bifidobacteria in the colon" (Schell et al. 2002). They also identified complete pathways for amino acids, nucleotides, and some key vitamins. Furthermore, they identified polypeptides "that showed homology to most major proteins needed for the production of glycoprotein-binding fimbriae, structures that could be important for adhesion and persistence in the human gastrointestinal tract" (Schell et al. 2002). Moreover, they found that a eukaryotic-type serine protease inhibitor (serpin) was possibly involved in the immunomodulatory activity of bifidobacteria (Schell et al. 2002).

Mother's milk contains high concentrations of lactose and lower quantities of phosphate (pH buffer). When lactic acid bacteria ferment the mother's milk, and bifidobacteria forms lactic acid and acetic acid in the infant's gastrointestinal tract, the pH is reduced, making it more difficult for Gram-negative bacteria to grow. Acetic acid produced by bifidobacteria was found to play an essential role in protecting us from *Escherichia coli* O157 infections (Yoshimura et al. 2010).

Genetically engineered *Bifidobacterium longum* was examined as a tumor-targeting enzyme-prodrug therapy for autochthonous mammary tumors in rats (Sasaki et al. 2006).

1.8 Further effects of probiotics on our health

The further impact of probiotics on our health are expected as follows:

1. They keep the intestine acidic and promote its peristaltic movement. They also stimulate the cells in the intestine and produce water-soluble mucin, which facilitates regular defecation.
2. They absorb cholesterol in the intestine and remove it from the body. As a result, the amount of cholesterol in the blood is lowered.
3. They improve the intestinal environment, as well as prevent atopic dermatitis and skin roughening, by stimulating the stomach's immune cells.
4. They activate natural killer (NK) cells and prevent viral infections by making the immune system reliable.
5. They stimulate immune cells in the intestinal tract and prevent allergies.
6. They prevent gastric ulcers and gastric cancer by decreasing *H. pylori* bacteria in the stomach.
7. They help lower high blood pressure.
8. They improve the environments in the mouth and prevent dental caries and pyorrhea.
9. They stabilize moods and alleviate stress by activating the autonomic nervous system.

Some lactic acid bacteria have a strain which produces bacteriocin, a type of antibiotic, and some strains can kill bacteria; therefore researchers are continuing their research activities to try to use bacteriocin produced by lactic acid bacteria as safe germicides for food preservation (Delves-Broughton et al. 1996; De Vuyst and Leroy 2007). However, lactic acid bacteria are known as food spoilage bacteria. In most cases, these spoilage bacteria originate on the production line or in raw materials and are resistant to environmental stresses due to their biofilms (Kubota et al. 2008).

A scientist has argued that intestinal microorganisms should be kept alive naturally, without being occupied by one species like lactic acid bacteria (Benno 2009). However, we do not need to worry about this, since the lactic acid bacteria will be less than 0.1%, even if you drink a lot of yogurts every day. Thus, manipulation of the gut microbiota holds great promise for the treatment of inflammatory and allergic diseases. Atarashi et al. (2013) argue the following: "although numerous probiotic microorganisms have been identified, there remains a compelling need to discover organisms that elicit more robust therapeutic responses, are compatible with the host, and can affect a specific arm of the host immune system in a well-controlled, physiological manner." They tried to isolate bacterial strains from the human indigenous microbiota, which induce CD4(+) FOXP3(+) regulatory T (Treg) cell. Dr. Sakaguchi (2008) of Osaka University found the Treg cell, which is a regulatory T cell. Atarashi et al. (2013) isolated 17 strains of bacteria that enhanced Treg cell abundance. The 17 strains were identified as Clostridia, which lacks prominent toxins and virulence factors. The 17 strains of *Clostridium* species in the gut "act as a community to provide bacterial antigens and a TGF-β-rich environment to help expansion and differentiation of Treg cells. Oral administration of the combination of 17 strains to adult mice attenuated disease in models of colitis and allergic diarrhea. Use of the isolated strains may allow for tailored therapeutic manipulation of human immune disorders."

The relationship between gut microbial metabolism and mental health is one of the most intriguing and controversial topics in microbiome research. Valles-Colomer et al. (2019) comment on the relationship as follows, "A study of two large groups of Europeans has found that several species of gut bacteria are missing in people with depression. The researchers cannot say whether the absence is a cause or an effect of the illness, but they showed that many gut bacteria could make substances that affect nerve cell function and maybe mood." Meanwhile, a Swiss team is testing the effectiveness of fecal transplants in relieving depression (Pennisi 2019). Two gut bacteria, *Faecalibacterium* and *Coprococcus*, were found to be consistently