

Chapter 1



Traditional Japanese Fermented Foods

Produced by Using Lactic

Acid Bacteria Improve Our Health

I will begin with an explanation of lactic acid bacteria. This might seem a little strange, but these bacteria have a deep relationship with traditional Japanese foods. This will be further explained later on, but let us start with the relationship between yogurt and the scientific basis of longevity.

1.1 Fermented dairy products can contribute to longevity

Residents of Bulgaria, and the Kyrgyz and the Hunza districts of Pakistan have been reported to have long lives. Through epidemiological investigations, it came to be known that their longevity may be related to dairy products such as yogurt, which they eat on a daily basis. Epidemiology is the scientific study which deals with the patterns, causes, and effects of health and disease conditions in certain populations. One hundred years ago, Dr. Ilya Ilyich Mechnikov^{*1}, a Russian biologist, zoologist, proto-zoologist and the director of The Pasteur Institute in Paris, developed a theory that lactic acid bacteria can improve our health and prolong life by eliminating putrefactive bacteria from our intestines.

Races of stock farming have been producing dairy products like yogurt, butter, and cheese for a long time. These dairy products are made by microbial fermentation as follows; milk sugar (lactose) or fats of milk taken from mammals are converted into acidic materials like lactic acid, into sugars like glucose, into amino acids, or vitamins. The microorganism is a kind of bacteria called lactic acid bacteria. In the taxonomy of biology, lactic acid bacteria are also known as gram-positive bacteria.

1.2 Living organisms are classified with evolution as a guide

Biologists have classified the living organisms of the Earth into many groups according to their forms and physiological characteristics. You may think taxonomy to be old-fashioned or the archaic scientific terms to be too difficult. However, through genetic analysis, living organisms came to be classified by their evolutionary process (Fig. 1.1).

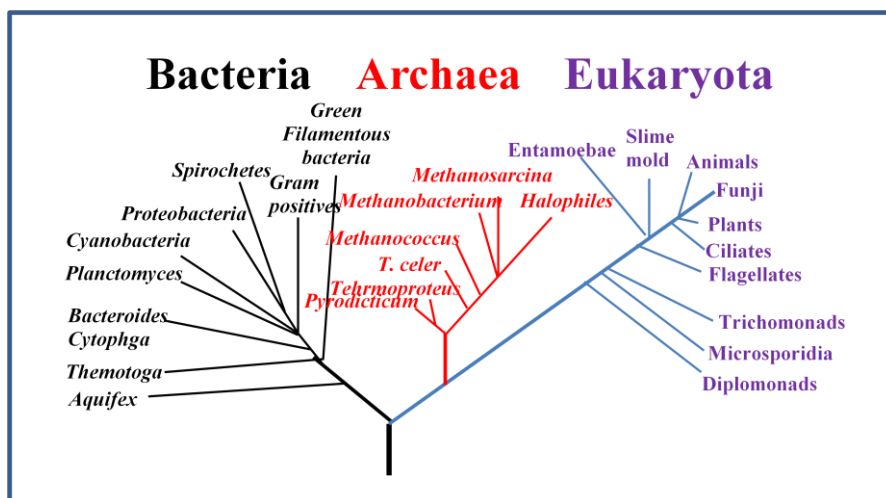


Fig. 1.1 A speculatively rooted tree for rRNA sequences showing major branches Bacteria, Archaea and Eukaryota.

For instance, the 'Archaea' domain encompasses a wide range of bacteria: thermophilus bacteria, which live near chimneys of submarine volcanoes, halophile bacteria live on deserts damaged by salt, and bacteria living in sulfur or iron or methane bacteria or hydrogen bacteria which consume or produce methane or hydrogen. These bacteria are said to have appeared on the Earth just after life began. They are born under extreme circumstances, in temperatures of over 100 °C (212 F) or in water of over the 20% salt concentration. If we look

back at the very origin of evolution for all living organisms, we find our way to these archaea-bacteria. Because some of them are similar to *Eukaryote*, some scholars do not like to refer to them as bacteria and thus propose the name just *Archaea*. I think the latter is more appropriate.

The bacteria found in Japanese fermented soybeans, or *natto*, is called the *natto* bacterium. The *natto* bacterium and the cholera bacterium are classified into 'Gram positive bacteria', and *Escherichia coli* or *Salmonella* species are classified into 'Gram negative bacteria'. Dr. Hans Gram, a Danish bacteriologist, developed a method of staining bacteria, which played a major role in classifying bacteria. For a Gram stain to be positive or negative depends on the chemical structures which form the cell wall of the bacteria.

The composition of the cell membrane is instrumental in determining how an organism protects itself from the environment and receives nutrients. A minor variation in this cell membrane can have a considerable influence on the various attributes of microorganisms. It could control whether antibiotics are effective, or whether antibodies or immunity reaction to an allergy are activated. For instance, an antibiotic, penicillin attacks and kills various pathogenic bacteria by inhibition of the cell wall biosynthesis. These are why taxonomy is essential to understand living organisms. The difference between Gram positive and Gram negative is not related to whether the bacteria are useful to human beings. The immunity reaction of lactic acid bacteria will be explained later.

1.3 The first dairy products traced to cattle depasturing in the Pamir Highland

Some say that yogurt traces its origin back to Turkey. I believe that any people who engaged in cattle breeding in ancient times discovered the method

of lactic acid fermentation on their own. After all, milk sours on its own if you let it sit. Milk becomes sour because lactic acid is made by lactic acid bacteria. We call this 'lactic acid fermentation'. There are other, similar organic acid fermentations called succinic acid and citric acid fermentations with appropriate bacteria or molds.

I spent 3 weeks trekking in Shimshal Pamir of Karakorum in the northern part of Pakistan, near the border between China and Pakistan. As we walked up a narrow path, which wrapped along a steep mountain cliff, a highland plateau came into our sight at around 4500 m (14,763 ft.) above sea level (Fig. 1.2). Truly, the Pamir highland has earned its nickname as the "Roof of the World". A local 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 the 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 the present-day Xinjiang (新疆) 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 from the Hunza Valley around 400-500 years ago, depastures yaks, goats and sheep in those grasslands in summer.

The yak is a long-haired wild cattle living in the high altitudes of the Himalayas or in the Pamir highland, and they are relatively aggressive by nature (Fig. 1.2), whereas 'Dzopkyo', seen in Nepal and the regions near it, are the domesticated offspring of wild yaks and cattle. Yak's milk is processed to handmade yogurt, butter, and cheese. Women make it a rule to milk yaks every day.



Fig. 1.2 Cheese making at Shimshal Pamir highland in Karakorum, Pakistan.

Clockwise from upper left; Shimshal Pamir highland, Yak (*Bos mutus*) groups coming back from the pasture lands of the Pamir highland in the evening, drying cheese on the roof of a stone cottage and cheese-making from yak milk with a Yao tribe family at the summer stone cottage (center in the picture, author).

1.4 The origin of producing yogurt, butter and cheese

The pastoralists of Pamir have continued to practice the traditional method of producing dairy products, and I would like to tell you about the method in more detail. First, they boil 20 liters (5.3 gallon) of milk, 90% of which is yak milk and 10% of which is goat milk, for 3 hours, cool it, then add yogurt. After that, it is fermented for 8 hours, keeping it warm at about 30 °C (86 F). After that, they pour the liquid into a wooden cylinder called *sogo* (Fig. 1.2) and churn it with a wooden stick called a *padaru* (Fig. 1.2). Careful examination of the photo will reveal that it is me, and not a Wakhi man who is churning the liquid. Production of butter and cheese are women's work, but I got tired after 5 minutes of churning as the liquid was very thick. The upper floating liquid,

10% of total milk, becomes butter and the lower remaining liquid is processed to cheese after 8 hours of fermentation and successive boiling. They then dry the cheese on the roof of a stone hut (Fig. 1.2). Nomadic people such as them live in stone huts during the summer. During the 6-month winter they move to lower places, 3,000 m (9843 ft.) above sea level.

I was invited to their stone hut and shared some of their hand-made cheese with a cup of chai mixed with milk and sugar.

Next to the cheese on the roof of the stone hut, yak droppings were drying in the sun. The droppings are used as a valuable source of fuel, because they cannot procure wood on the high treeless plateau. After they dry them, they keep them in their stone huts. The dried yak droppings have no offensive smell and are rather hygienic. They also use the droppings to patch up cracks in walls, which helps keep rooms warm during cold nights, where the temperature falls to -10 °C (14 F).

1.5 Lactic acid bacteria isolated from plants used in traditional Japanese foods

Although lactic acid bacteria don't seem to have anything to do with Japanese foods, there is a deep link between lactic acid bacteria and *washoku* (Japanese cuisine). The flavor of fermented foods like Japanese *tsukemono*, (pickled vegetables), Japanese *sake*, *miso* (soybean-barley paste) and *shoyu* (soy sauce), are explained in detail in later chapters. These flavors are influenced by a fermentation process which uses lactic acid bacteria together with *koji* mold and yeasts.

These lactic acid bacteria found in Japanese foods are different from those in milk products produced by pastoral races. In Japan and other Asian countries,

lactic acid bacteria isolated from plants are able to reproduce on grains or vegetables. They can decompose the starch or cellulose of plants into sugar and use it as the nutrients, whereas lactic acid bacteria isolated from dairy products grow by taking the nutrients of milk and usually cannot decompose starch or cellulose.

1.6 Probiotics can improve our intestinal functions

Probiotics are microorganisms which are believed to improve health by maintaining good intestinal conditions. Lactic acid bacteria, bifidobacteria, and propionic acid bacteria are seen as representative probiotics. Bifidobacteria are present in an infant's intestine and can break down milk, but they decrease as we get older. Propionic acid bacterium is used as a starter (an initiator of fermentation) of Swiss cheese. A kind of propionic acid bacteria is industrial microorganism as it produces vitamin B₁₂.

Asian agrarian races, including the Japanese have been using natural lactic acid bacteria on grains, beans or vegetables for centuries. Application of this process can be seen in traditional fermented products such as soy sauce, *miso*, *sake*, and *tsukemono* in Japan and *kimchi* in Korea.

1.7 Lactic acid bacteria isolated from plants induce cytokines which regulate the immune system

Once, a student from the Philippines in my laboratory isolated a lactic acid bacterium from '*Burong Isda*', a fermented food made from rice and fish (Fig. 1.3), which is a beloved traditional Philippine fermented food. Although lactic acid bacteria very rarely decompose starch of rice, this bacterium named

Lactobacillus plantarum strain L137 (Fig. 1.4) decomposes rice starch and induces cytokines which include interleukin-12, an immune system regulator. We discovered that this lactic acid bacterium is effective for the medical treatment of allergic diseases like house dust or pollen allergies.



Fig. 1.3 Commercially sold Brong-Isda, a traditional fermented product from mudfish and rice in Philippines.

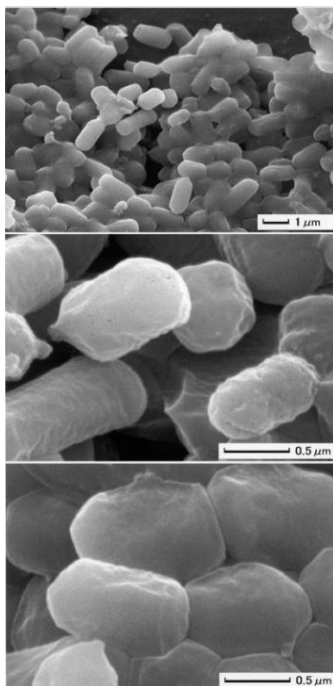


Fig. 1.4 Electron microscope photographs of *Lactobacillus plantarum* L137.

(Photos provided by Dr. Y. Yamamoto, former Takeda Food Industry Co. Ltd, Itami, Hyogo, Japan.)

Cytokines are proteins which are released from immune cells. A cytokine is also a general term for immune cells which are involved in immunity, inflammation, increasing or differentiating cells, cell death, or healing of wounds by transfer of genetic signals to specific cells. Interleukin, a kind of cytokine, is released from white blood corpuscles and controls immunological activities. Various kinds of interleukins are known to us, and each interleukin is assigned its own number.

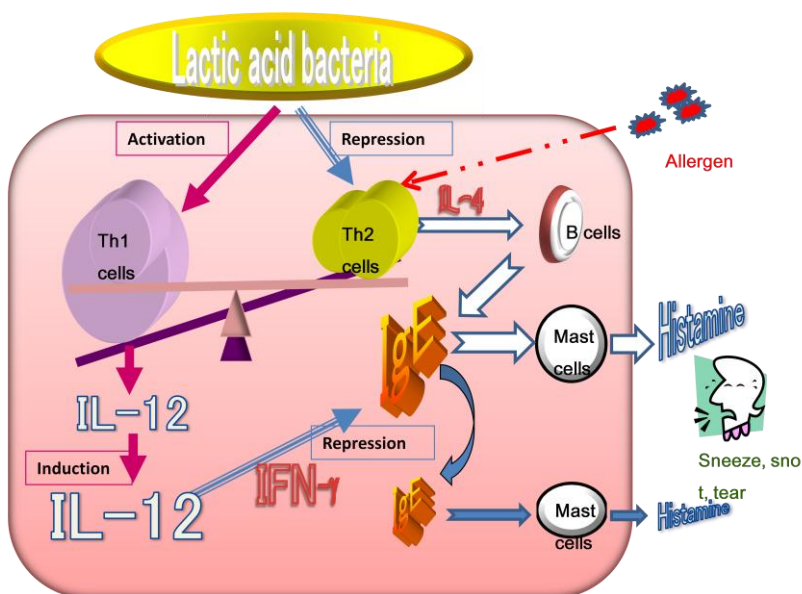


Fig. 1.5 Schematic illustration of allergy reaction and its desensitization mechanism by lactic acid bacteria.

The cells which recognized allergen influence on Th2 cells and produce IL-4. The IL-4 induces the production of IgE by B cells, and then mast cells produce histamine. As a result, symptoms like sneezing, runny nose and teary eyes arise to protect the human body against allergens. Lactic acid bacteria activate Th1 cells that change the balance of Th1 to Th2 cells. This change of the cellular balance promotes the induction of IL-12 and IFN- γ that results in repression of the IgE production. As a result, release of histamine from mast cells is reduced, and allergen reaction is alleviated. This mechanism is called allergenic desensitization. IL, interleukin; IFN, interferon.

We discovered that the lactic acid bacterium screened from fermented foods made from plant materials induce interleukin-12 (IL-12) and interferon gamma (IFN- γ) that resulted in repression of the production of IgE antibody (Fig. 1.5). This discovery was the first report of its kind in the academic world. After that, Japanese milk production companies assumed that plant-sourced lactic acid bacteria could be effective treatment for several allergies, and brought lactic acid bacteria supplements to the market.



Fig. 1.6 Commercial product of heat- killed *Lactobacillus plantarum* L137.

Taxonomically speaking, ‘plant lactic acid bacteria’ are not classified into a definite category. Plant sourced lactic acid bacteria don’t have the same attributes as lactic acid bacteria from milk products or those found in immune systems. The degree of immune system regulation differs according to each strain of lactic acid bacteria. In Japan, *L. plantarum* strain L137 was heat-killed and manufactured into Lacdent, introduce to the market (Fig. 1.6).

1.8 Are lactic acid bacteria effective allergy treatment?

More than 30% of Japanese people suffer from allergies such as pollen or house dust allergies or atopic eczema. This information was reported 15 years ago, so I assume that around half of the Japanese populace now suffers from

some kind of allergic disease. The number of people who suffer from some kind of allergic diseases is increasing in developed countries. Are lactic acid bacteria effective treatments for allergic diseases like hay fever? I can confidently say, "Yes, they are," thinking of our research results and the reports I've read from others^{*2}. Lactic acid bacteria control the balance of Th1/Th2 cells, induce cytokines like interleukin-12, interferons and others, and repress the production of an antibody named IgE. For now, please don't busy yourself with the unfamiliar and complex names of immune cells, as we only need a general idea to continue.

Pollen allergy, its causes and its treatments are complex topics, so I have tried to explain them through the simplified illustration of Figure 1.5. The cells of our bodies recognize pollen as foreign organisms and produce antibodies to remove them. IgE is a kind of antibody which responses to external foreign substances like cedar pollen, or any substance called an allergen. If antibody IgE is produced, mast cells try to cope with foreign substances by releasing histamine. Next, symptoms like redness of the eyes, sneezing, and irritation of the nose develop. It is often these symptoms which we refer to as pollen allergy. Most allergy medicine prescribed by doctors is anti-histamine in function. As I already mentioned, lactic acid bacteria have the ability to reduce the release of histamine by inducing IL-12 and interferon (IFN)- γ production.

The results of our joint research work with a food company indicated the cause of the difference in strength of the cytokine release. This difference is due to the variety of lactic acid bacterial strains, which control the components and cell wall structure. Of all cell walls components, lipoteichoic acid in particular was identified as the primary cause of these differences. It is also thought that in addition to lipoteichoic acid, the degree of cytokine release is controlled by the strain of lactic acid bacteria, which is in turn influenced by the arrangement of

DNA and metabolic substances within cells. Therefore, not only living lactic acid bacteria, but also heat-killed lactic acid bacteria can release cytokines. We developed an oral vaccine against the Japanese cedar pollen allergy using a lactic acid bacterium^{*3}. However, because this is a genetically modified bacterium and requires more various safety tests, we are not allowed to use it at this moments.

1.9 The effectiveness of lactic acid bacterial products on allergy

Then, which lactic acid bacteria are the most effective treatment for allergies? Every company claims that the product we created is the best. I would like to claim to product we created is the best, but in fact our L137 bacterial strain is not known very well and the 'Lacdent' (Fig. 1.6) does not sell well. We had thought it best at the time not to over-advertise, thinking that it would not be effective in the same way as other medicine.

1.10 Double blind tests are necessary to prove the effectiveness of products

Lactic acid bacteria extracted from plants are not necessarily beneficial for humans. Although there has been no direct link proven between cow's milk and BSE (mad cows disease), some consumers may avoid dairy products. However, Japanese dairy products use plant sourced lactic acid bacteria, and there are many people, such as vegetarians, who prefer plant sourced lactic acid bacteria in the world.

Dairy product manufacturers in Europe are applying to the European Union's Health Organization for approval of certain dairy products. Specifically, they would like affirmation that yogurt is effective for the treatment of allergies, but so far they have not been granted this approval, due to lack of data. These review boards require the results of double-blind tests. In a double-blind experiment, a group of people who would drink yogurt and a group of people would not are tested without their knowledge in the hopes that this would reveal the true effects of drinking yogurt. A double blind test requires the elimination of unrecognized biases carried by the subjects as well as the conductors. The more participants there are, the more reliable the result. Participants in this experiment should have identical diets and lifestyles as much as possible. Testing many participants under the same conditions for a long duration requires extensive funds. Further, to isolate only a single produce requires considerable effort.

In conclusion, it is certain that several strains of lactic acid bacteria are effective in the reduction of allergic symptoms. However, it will take a long time to see results and will not completely cure a patient.

1.11 I once had roundworm

Why do you think there are few elderly people who are allergic to pollen? An epidemiological investigation showed that people who had roundworm at one point in time did not have a pollen allergy. Those Japanese who are 65 years of age and older or those who grew up in rural areas often ate vegetables grown at fields where raw human feces were used as organic fertilizer. Roundworm eggs grew on the leaves of vegetables, hatched in human bodies and matured inside their human hosts.

After the Second World War, infected children were treated with drugs for expelling worms, ascaricides, in every school in Japan. Roundworms stunted children's growth, and deprived their bodies of nutrition.

In European countries where raw human feces are not used as fertilizer, I have heard that children who live near cattle rarely develop allergies. Our bodies recognize roundworms, as a foreign substance and protect us by producing IgE antibodies.

It explains that in clean environments like present-day Japan, our bodies are more sensitive to the smallest foreign substance. This sensitivity causes allergic symptoms, as those original protective functions remain in our bodies. People who had roundworm in the past are likely to be insensitive to allergy. I don't know if I should be glad or not, but I can assert that I am insensitive to pollen illness, since I got a roundworm in my childhood. Maybe we shouldn't worry if our young children grow up playing in dirty surroundings.

1.12 Could lactic acid bacteria prevent gastric cancer?

There is a report that lactic acid bacteria are effective in preventing gastric cancer.^{*4} *Helicobacter pylori* is found in the stomach and is concerned with 70% of gastric cancer and gastric ulcers.

Helicobacter pylori is a Gram-negative bacterium and lives in the mucosae of the stomach. It is said that *H. pylori* produces interleukin-8, a kind of inflammatory cytokine that harms the mucosae of stomach. It is thought that some kind of lactic acid bacteria prevent *H. pylori* from increasing. Data indicate that lactic acid bacteria prevent the production of interleukin-8.

The stomach is filled with acid, so the pH level is less than 2. If we were to touch hydrochloric acid or sulfuric acid of pH 1-2, the skin of our hands blackens and peels off. How do *H. pylori* and other kinds of lactic acid bacteria live in such strong acidity? It is thought that *H. pylori* colonize the stomach by degrading the stomach's urea to ammonia and neutralizing the pH of the environment. Some kinds of lactic acid bacteria may neutralize the pH of the stomach by restricting the absorption of hydrogen ions. However, how lactic acid bacteria prevent *H. pylori* from increasing is not yet understood. We tend to say living lactic acid bacteria are effective if the increase of lactic acid bacteria overcomes the growth of *H. pylori*.

There is a lactic acid bacterial strain which produces bacteriocin, a kind of antibiotic. Some strains can kill bacteria, therefore researchers are continuing research activities to try to use bacteriocin produced by lactic acid bacteria as a safe germicide for food preservation.

1.13 Lactic acid bacterial strain determines effect on health

Lactic acid bacteria have more effects on health than one might expect. However, the effects are determined by the bacteria strain. Some effects are listed below;

1. They keep the intestines acidic environments and promote the peristaltic movement of the intestine. 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 amounts of cholesterol in the blood are lowered.

3. They improve the intestinal environments and prevent atopic dermatitis and skin roughening by stimulating immune cells in the stomach.
4. They activate natural killer (NK) cells, a kind of immune cells, and prevent infection by making the immune system strong.
5. They stimulate immune cells in the intestinal tract and prevent pollinosis.
6. They prevent gastric ulcer and gastric cancer by decreasing *H. pylori* bacteria in the stomach.
7. They improve the environments of the mouth and prevent dental caries and pyorrhea.

Although not all the strains of lactic acid bacteria are effective to the above-mentioned symptoms, there are at least some strains of lactic acid bacteria, bifidobacteria, and propionic acid bacteria that are effective to those symptoms. However, a scientist has been arguing that intestinal microorganisms should be kept alive naturally, without being occupied by one species like lactic acid bacteria. However, lactic acid bacteria occupy less than 0.1% of your intestine, even if you eat yogurt every day.

1.14 Summary

Several strains of lactic acid bacteria are effective in the alleviation of allergic symptoms and cancers. However, it will take time to prove these effects and there may be no possibility to completely cure them. How effective these bacteria are differs according to each strain of lactic acid bacteria. Plant sourced lactic acid bacteria taken from fermented foods have traits such as digesting

starch or cellulose and living under the special fermented conditions, and they have the potential for promoting health and longevity.

^{*1} Tauber, AI. "Metchnikov and the phagocytosis theory," Nature Reviews, Molecular Cell Biology, 4:897-901(2003).

^{*2} Hirose, Y., Murosaki, S., Yamamoto, Y., Yoshikai, Y., and Tsuru T., Daily intake of heat-killed *Lactobacillus plantarum* L-137 augments acquired immunity in health adults. Journal of Nutrition, 136:3069-3073(2006).

^{*3} Ohkouchi, K., Kawamoto, S., Tatsugawa, K., Yoshikawa, N., Takaoka, Y., Miyauchi, S., Aki, T., Yamashita, M., Murooka, Y., and Ono, K., Prophylactic effect of *Lactobacillus* oral vaccine expressing a Japanese cedar pollen allergen. Journal of Bioscience and Bioengineering, 113 (4): 536-541 (2011).

^{*4} Murosaki, S., Muroyama, K., Yamamoto, Y. and Yoshikai, Y., Antitumore effect of heat-killed *lactobacillus plantarum* L-137 through restoration of impaired interleukin-12 production in tumor-bearing mice. Cancer Immunology and immunotherapy, 49: 157-164 (2000).

