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Present Status of Fermented Milk Products in Japan

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Ⅰ. Introduction

Although advances in the medical sciences in the last 50 years have significantly increased the human life span, an unfortunate fact is that many of us are now living long enough to experience chronic disorders such as coronary heart disease, hypertension, osteoporosis, diabetes and cancer. In recent years there has been renewed interest in health promotion and disease prevention by incorporating probiotic bacteria into foods to counteract harmful bacteria in the intestinal tract. Therefore, there are now a wide variety of commercial products containing prospective probiotics that claim health-promoting effects, such reductions in large bowel carcinogens and mutagens, antitumor properties, cholesterol-lowering effects, increased lactose digestion, relief from constipation, stimulation of immunocomponent cells and enhancement of phagocytosis.

Traditional probiotic dairy strains of lactic acid bacteria (LAB) and *Bifidobacteria* which have been designated as GRAS (Generally Recognized As Safe) bacteria have a long history of safe use and most strains are considered comestible microorganisms with no pathogenic potential[1~4]. Accordingly, there is considerable interest in extending the range of foods containing probiotic organisms from dairy foods to infant formulas, baby foods, and pharmaceuticals. In addition, the ingestion of probiotics, prebiotics, and symbiotic as well as combinations of pro- and prebiotics has recently aroused renewed interest as enhancing the beneficial relationship between the host and intestinal microflora in both healthy and diseased individuals.

Non-communicable chronic diseases such as cancer, cerebral hemorrhage, coronary heart disease, and diabetes mellitus has recently been recognized as adult diseases in Japan as well as other countries, and are considered to be inevitably associated with aging. These diseases occur as a result of individual life styles. The Japanese Government, Ministry of Health, Labor and Welfare has proposed substituting the term "adult diseases" with "lifestyle-related diseases". It has emphasized the importance of prevention rather than treatment, since the well-known increase in the elderly population in Japan is predicted to result in a variety of socioeconomic problems.

In this short review on the "Present status of fermented milk products in Japan", I will report a strategy for the development of fermented milk products in Japan from the standpoint of view of research in Japan on LAB and *Bifidobacteria*. They could play an important role in preserving human health by controlling intestinal microflora capable of producing toxic effects on the host, and have also now come to be recognized as important in the field of prenventive medicine.

Ⅱ. Role of LAB and *Bifidobacteria* in Maintaining Health

Fig. 1 shows the role of LAB and *Bifidobacteria* in maintaining human health. As we know that human gastrointestinal tract contains a very large and diverse population of microogarnisms. The microflora increases in size and complexity along the length of the gastrointestine, from the stomach which contain only scanty flora to the colon which harbors approximately

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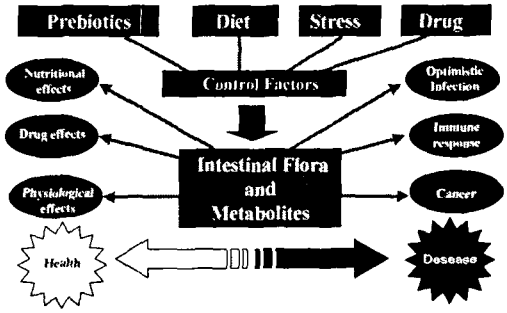

Fig. 1. Role of intestinal flora in maintaining human health.

10^{12} bacteria/gram comprising of at least 500 species. The variation of microflora is controlled by some factors such as prebiotics, diet, stress and drug. In other way we can say that these factors have a very important role in controlling the balance between beneficial microflora and putrefactive/harmful microflora. In case, the harmful or putrefactive microflora, it will cause the incidence of some diseases. Meanwhile if the beneficial flora outnumbers the harmful ones, the body will remain in healthy condition.

Ⅲ. Beneficial Effects of Intestinal Flora

The beneficial effects of intestinal flora usually called "beneficial flora" including LAB and *Bifidobacteria* such as:

- Inhibit the harmful bacteria and other microflora by production of antimicrobial components like organics acids especially lactic acid and acetic acids, hydrogen peroxide and bacteriocin in the gastrointestinal tract[5,6].
- Scavenging function. LAB and *Bifidobacteria* have the ability to scavenge the oxygen radicals because they can produce an enzyme called "superoxide dismutase"[7].
- Stimulate the immune response. So far there is no definite mechanisms explaining how LAB and *Bifidobacteria* improve the immune effects, however, some researchers presumed that LAB and *Bifidobacteria* increase the immune system[8~10].
- Improve the digestion and absorption of food by producing various kind of enzymes to help digest food, and by lowering the pH of gastrointestinal tract and speeding up the transport of gastric contents into the intestinal tract[11].
- Synthesis of vitamins. Some species of LAB have been shown to increase vitamin B complex in fermented foods and others could synthesize vitamin K in the colon[11].

Ⅳ. Harmful Effect of Intestinal Flora

The intestinal flora which is usually known as "harmful or putrefactive flora" is able to synthesize and produce harmful substances during the growth in the gastrointestinal tract[12~14]. The compounds produced are putrefactive products such as NH_3, H_2S, phenols, amines, carcinogens as well as toxins, and they are in good association with the incidence of various diseases like diarrhea, constipation, cancer, hepatic coma, autoimmune disease, hypertension and opportunistic infection.

The putrefactive compounds and other metabolic products formed in the presence of various fecal enzymes of intestinal flora were shown in Fig. 2. These enzymes have an important role in the incidence of diseases. For example, N-nitroreductase and azoreductase could produce nitrosocompounds and N-hydroxy compounds from amines; β-glucuronidase involves in the formation glucuronic acid deconjugates and β-glucuronidase involves in the formation of glucuronic acid deconjgates.

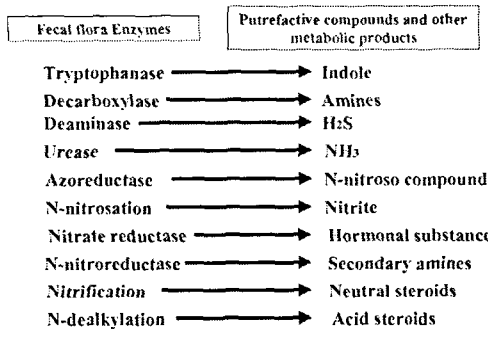

Fig. 2. The putrefactive compounds and other metabolic products formed in the presence of various fecal enzymes.

All these formed compounds show intense carcinogenity and cause cancer in the intestinal tract and in the kidney.

V. Health Effect of Fermented Milk

Health effects of fermented milks are now recognized:

- The nutritious effects of fermented milk components are due to their various nutritional and physiological values such as amino acids and lactose that can promote the production of lactic or acetic acid; and lactic acid that can stimulate gastric secretion and speed up the transport of gastric contents into the intestinal tract as well as lower its pH to inhibit the growth of harmful bacteria, thus preventing constipation[15,16].
- Physiological effects of viable LAB and *Bifidobacteria.*

-(i) The improvement of bowel movements by is ability to produce abundant lactic and acetic acid and to lower the pH of the gastrointestinal tract, thus speeding up the transport of gastric contents into the intestinal tract[17].

(ii) The prevention of lactose intolerance. Most Asian and African populations suffer from lactose intolerance due to a deficiency of enzyme β-galactosidase, resulting in an inability to digest lactose. That undigested lactose is then degraded by intestinal flora, leading to osmotic diarrhea and other symptoms. The consumption of fermented milk containing living LAB and *Bifidobacteria* could alleviate these symptoms by converting lactose to glucose and galactose via the β1,4-galactosidase produced by lactic acid bacteria[18].

- Physiological effects of fermented milk and cells of LAB and *Bifidobacteria.*

(i) Antimutagenicity. We have extensively investigated the antimutagenicity of fermented milk against various mutagens[19~28]. We have found that casein, polysaccharides, and LAB cells were responsible for the antimutagenic activity towards some mutagenic compounds. Other researchers have identified peptides and proteolytic compounds as the components responsible for the antimutagenicity of fermented milk[29~31].

(ii) Antitumor activity. Some researchers suggested that the antitumor action of fermented milk may be attributed to the polysaccharides and slimy substances produced by LAB and *Bifidobacteria.* Others have proposed that such an action is attributable to LAB and *Bifidobacteria* stimulating the immune system. Still others have proposed mechanisms that reduce the activity of these fecal bacterial enzymes involved in procarcinogen activation, and that also reduce the excretion of mutagens in feces and urine[32].

(iii) Activation of the immune system. The colonization of probiotic LAB and *Bifidobacteria* occurs naturally. Their adherence to the epithelial cells of the intestine serves to facilitate the activation of the immune system. Most studies found that LAB and *Bifidobacteria* could improve the immune system by the activation of macrophages. Once activated, macrophages could then stimulate the B cells to secrete antibodies and T cells. LAB and *Bifidobacteria* are also able to modulate the production of cytokines and produce natural killer (NK) cells against cytotoxicity. Other studies reported an immuno-activating activity of LAB by the modulation of interleukins 1, 2 and 6, tumor necrosis factor (TNF) alpha, and interferons (IFNS) alpha, beta and gamma. All of those immunomodulatory effects of probiotic LAB originate from the

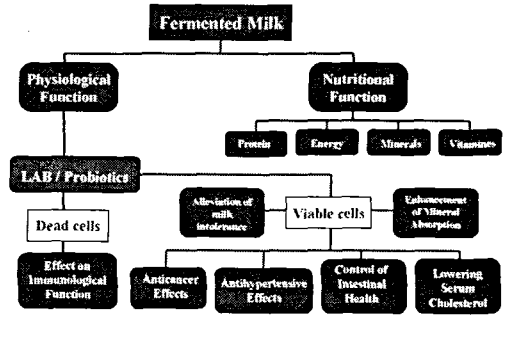

Fig. 3. Health effects of fermented milk.

ability of lactic bacteria to colonize and adhere to the epithelial cells of the intestine[33~35].

(iv) Cholesterol-lowering action. Many reports have been published on the hypocholesterolemic effects of fermented milk[36~38].

(v) Antipathogenic bacteria. The colonization of probiotic LAB and *Bifidobacteria* in the intestine enables it to compete with harmful bacteria[39~41].

Ⅵ. Antimutagenicity of LAB against Various Carciogens

Using streptomycin-dependent SD 510, we evaluated the antimutagenicity of LAB against the mutagenicity of *nitrosocompounds and aflatoxin, and the results in shown* in Fig. 4.[42~45]. The antimutagenicity of nitrosodiethylamine was almost completely inhibited by the cells of LAB isolated from dahih, an Indonesian traditional fermented milk. Among 3 strains of *L. acidophilus,* strain LA-2 showed the highest antimutagencity toward mu*tagenicity induced by N-nitrosoguanidine.* Fig. 4 also shows the significant reduction mutagenicity of aflatoxin B1, G1 by the cells of *L. acidophilus* LA-2 and *L. rhamnosus* LCr 101.

We have also evaluated the effect of feeding human subjects with fermented milk on the fecal mutagenicity [46]. In this experiment, 6 male volunteers of 28 years old in average were selected on the basis of general health, absence of constipation and higher fecal mutagenicity. Each subject was given 200 ml of *Lactobacillus acidophilus* LA-2 fermented milk per day for 7 days. Whole fecal samples were collected before and after the intake of fermented milk and then analyzed by using SD 510 strain. The result showed that the fecal mutagenicity of the six volunteers was significantly reduced after 7 day of fermented milk consumption as indicated by reduction in number of revertant colonies.

Ⅶ. Binding Properties of LAB Bacterial Cells with Mutagens and Carcinogens

One possible mechanism for the antimutagenic activity of LAB is by direct binding of mutagens to cell walls of LAB[47~54]. As shown in Fig. 5 mutagens like Trp-P1, Trp-P2 and Glu-P1; the heterocyclic amines are commonly found and isolated from cooked or heated foods, are completely bound to the cells of *Lactococcus lactis* subsp. *lactis* with rapid reaction[55]. We compared the binding activity of viable and dead cells and it is

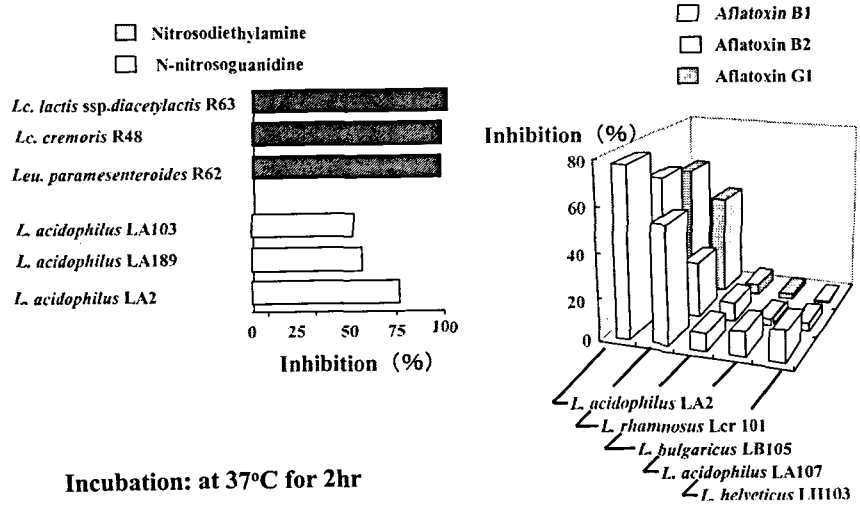

Fig. 4. Antimutagenicity of lactic acid bacteria against various carcinogens.

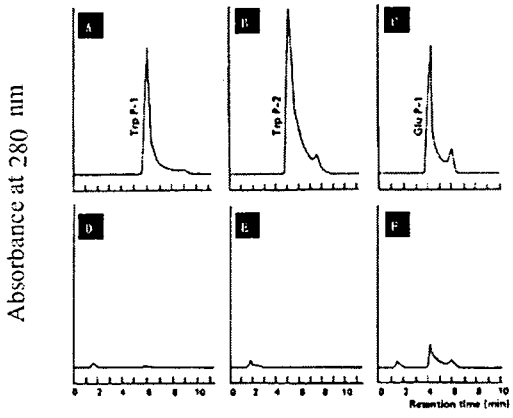

Fig. 5. Binding properties of *Lactococcus lactis* subsp. *lactis* cells towards various mutagenic compound.

found that binding of Trp-P1 by dead cells were slightly lower than that of viable cells.

Ⅷ. Serum Cholesterol Lowering Effect of LAB

Elevated serum cholesterol in humans is generally a risk factor correlated with development of coronary heart disease. Most studies showed that fermented milk products have the potential to reduce serum cholesterol levels and that hypocholesterolemic effect was mainly attributed to LAB. We have proposed two mechanisms for hypocholesterolemic action LAB, the first mechanism is by direct binding of dietary cholesterol with bacterial cells before in the small intestine before cholesterol can be absorbed into the body, and the second mechanism is by deconjugation of bile salts[56~66]. Deconjugated bile acids are excreted more rapid and bound easier to the bacteria and dietary fiber than are conjugated bile acid. As a consequence, more cholesterol is needed to synthesize new bile acids, which in turn reduce the total cholesterol in the body. As it is shown in Fig. 6, various strains of LAB could bind cholesterol and deconjugate bile salt, but the cholesterol-binding and bile salt deconjugating abilities varied among strains.

We investigated the effects of milk products fermented by the probiotic strain *Bifidobacterium longum* BL1 on blood lipids in humans. 32 subjects with total serum cholesterol ranging from 220 to 280 mg/dl were randomly assigned to two treatments: 1) intake of a low-fat yogurt drink prepared with ordinary yogurt starters *S. thermophikus* and *L. delbrueckii* subsp. *bulgaricus* (P-group) and, 2) intake of a low-fat liquid yogurt prepared with those two ordinary yogurt starters plus *B. longum* strain

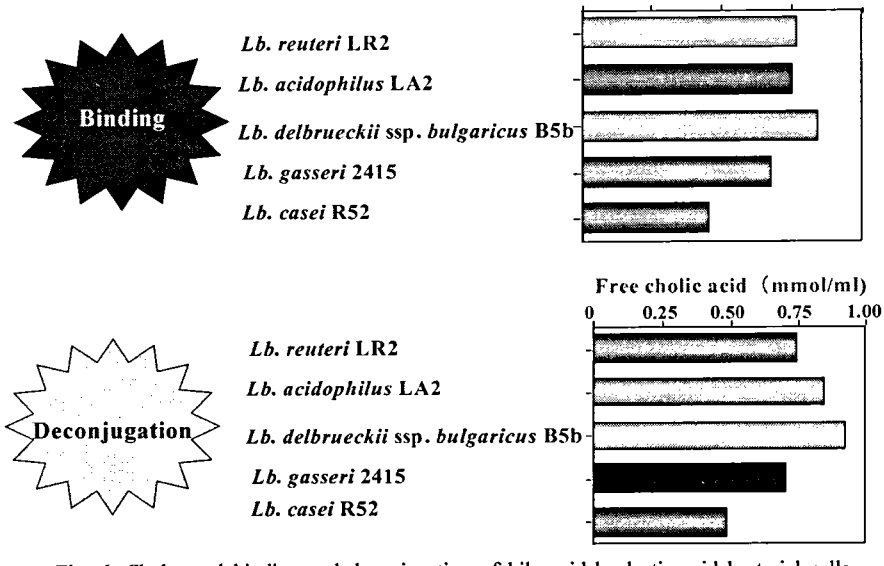

Fig. 6. Cholesterol binding and deconjugation of bile acid by lactic acid bacterial cells.

BL1 (B-group). After an intake for 4 wk at 3 × 100 ml/day, a reduction in total serum cholesterol was observed in approximately half of the B-group subjects, with a particularly significant decrease among subjects with moderate hypercholesterolemia. However, serum lipid concentrations in the P-group subjects remained almost stable during the experimental period[67].

Ⅸ. Antidiabetic Effect

There have been many studies on the effects of LAB on hypertension, cholesterolemia and cancer. However, their antidiabetic effects have hardly been investigated. We have recently studied the antidiabetic effects of *Lactobacillus* GG on streptozotecin-induced diabetic rats [68]. Neonatally streptozotecin-induced diabetic (n-STZ) rats were given food containing *Lactobacillus* GG cells or a control diet from 9 to 18 wks of age. The GG cells significantly lowered the blood hemoglobin (HbA1C) level and improved glucose tolerance in those n-STZ rats ($p<0.05$) (Fig. 7).

Ⅹ. Antibiotics vs probiotics

Prof. Fuller, a British microbiologist, proposed the term "probiotics", which may be defined as deliberately digested, health-promoting live bacteria that beneficially affect the microbial intestinal balance of the host.

Antibiotics play an important role in treating various infections and diseases caused by viruses, bacteria and other microorganisms. However, we have recently faced serious problems concerning the resistance of certain bacteria and other pathogens, and the risk to patients from a change in the balance of intestinal flora, resulting in diarrhea[69].

The application of probiotics is the answer to such problems. Probiotic causes no resistance to certain micro-organism in the host gastrointestinal tract. In fact, when administered to the host, they will colonize, grow and quickly exert their beneficial effects. Probiotics also improve balance of intestinal flora by increasing the number of beneficial microflora while reducing the number of harmful ones.

Ⅺ. Development of Functional Foods in Japan

Recently in Japan, definite concepts have been established as to the tertiary function of food, i.e., the fortification and modulation of physiological systems, in

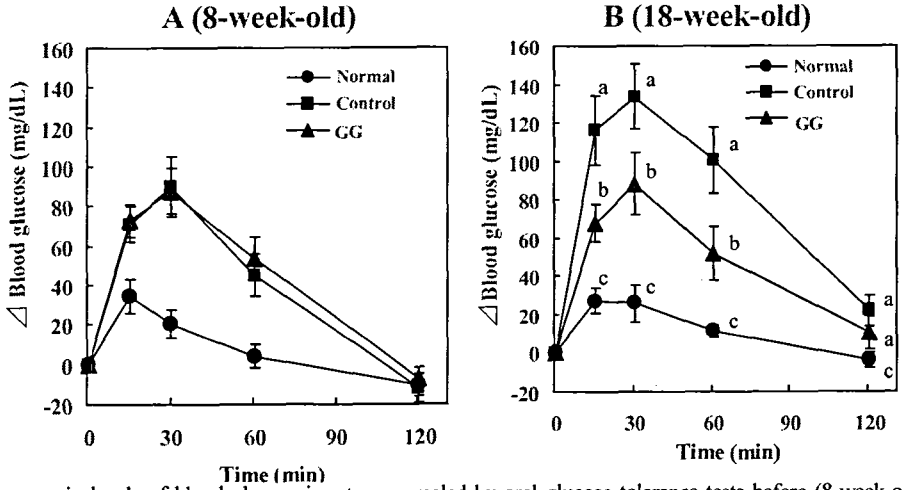

Fig. 7. Increase in levels of blood glucose in rats as revealed by oral glucose tolerance tests before (8-week-old) and after 9-week experimental diet (18-week-old).

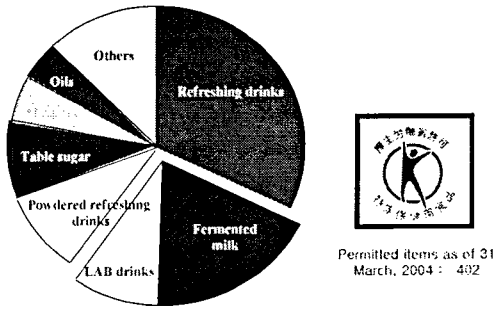

Fig. 8. Foods for Specialized Health Use (FOSHU).

addition to the primary function (nutrition) and the secondary function (sensory satisfaction) that were already recognized. The term "functional food" was created to express these idea. The Ministry of Health, Labor and Welfare (MHLW) of Japan also took up functional food from the standpoint of preventive medicine, and it is a well-known fact that in 1991 it introduced the concepts of functional food in the form of for specified health use (FOSHU).

The MHLW adopted legislation concerning FOSHU. Now more than 400 products have received approval to be labeled FOSHU foods. More than 30% of such foods are LAB drinks and fermented milk products. Consumers are demanding foods that provide specific health benefits. Food, pharmaceutical, and biotech companies are spending billions of dollars in research and development to take advantage of those demands (Fig. 8).

Reflecting these situations in recent years, people's interest in the relations between food and health is increasing further, and food industries are positive toward the development of functional foods. In this sense, the role played by milk and milk products is very important. The production of fermented milk in Japan will tend to increase overtime. In fact, fermented milk products are becoming more and more popular in Japan. The production of fermented milk has currently reached more than 91 hundred thousand liters.

XII. Synbiotics

Food is no longer considered as merely providing sufficient nutrients, but also as giving the consumer a feeling of satisfaction and well being. Diet also controls and modulates various functions in the body, thus contributing to maintaining good health and reducing the risk of some diseases. Probiotics (viable beneficial bacteria, mainly LAB) and health-promoting non- digestible (prebiotics) ingredients are being commercially marketed, especially in developed countries. Currently, research and development are focused on both a foods probiotics and prebiotics properties. Moreover, the synergistic effect of the combination of probiotics and prebiotics ingredients in improving as well as maintaining human health has been given top priority by research workers in the field of nutrition 70).

A further potential development is the use of "symbiotics", where probitics and prebiotics are combined, thus the live microorganisms would be used in conjunction with a specific substrate for growth. Even though the feasibility of this concept has not yet been intensively tested and validated so far, but it does offer increased efficacy and synergetic effect between probiotics and prebiotics.

XIII. Acknowledgement

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XIV. References

1. Sanders, M. E. and Klaenhammer, T. R. 2001. The scientific basis of *Lactobacillus acidophilus* NCFM functionality as a probiotic. J. Dairy Sci., 84:319.
2. Piano, M. D., Ballare, M., Montino, F., Orsello, M., Garello, E., Ferrari, P., Masini, C., Strozzi, G. P. and Sfprza, F. 2004. Clinical Experience with probiotics in the elderly on total enteral nutrition., J. Clin Gas-

troenterol. 38:S111.
3. Reid, G. and Devillard, E. 2004. Probiotics for mother and child., J. Clin. Gastroenterol. 38:S94.
4. Prevot, M. B. 2004. New regulatory trends for probiotics. J. Clin. Gastroenterol. 38:S61.
5. Hosono, A., Yatsuki, K. and Tokita, F. 1977. Isolation and characterization of an inhibitory substance against *Escherichia coli* produced by *Lactobacillus acidophilus*. Milchwissenschaft, 32:727.
6. Ross, R. P., Galvin, M. and McAuliffe, O. 1999. Developing application for lactococcal bacteriocins. Antonie van Leeuwenhoek, 76, 337.
7. Hosono, A. and Satake, N. 1993. Isolation and characterization of superoxide dismutase from *Lactococcus lactis* subsp. *cremoris* R-48. Animal Sci. Tech., 64: 599.
8. Clancy, R. 2003. Immunobiotics and the probiotic evolution., FEMS Immunology and Medical Microbiol., 38:9.
9. Kitazawa, H., Watanabe, H., Shimosato, T., Kawai, Y., Itoh, T. and Saito, T. 2003. Immunostimulatory oligonucleotide, CpG-like motif exists in *Lactobacillus delbrueckii* ssp. *bulgaricus* NIAI B6. Int. J. Food Microbiol. 85:1.
10. Jijon, H., Backer, J., Diaz, H., Yeung, H., Thiel, D., McKaigney, C., Imone, C. De and Mdsen, K. 2004. DNA from probiotic bacteria modulated murine and human epithelial and immune function. Gastroenterology, 126:1358.
11. Dave, R. I. and Shah, N. P. 1998. Ingredient supplementation effects on viability of probiotic bacteria in yogurt. J. Dairy Sci., 81:2804.
12. Holdeman, L. V., Good, I. J. and Moore, W. E. C. 1976. Human faecal flora: variation in bacterial composition within individuals and a possible effect of emotional stress., Appl. Environ. Microbiol. 31: 359.
13. Hanson, L. A., Adlerberth, I., Carlsson, B., Castrignano, S. B., Dahlgren, U., Jalil, F., Khan, S. R., Mellander, L., Eden, C. S. and Svennerholm, A. M. 1989. Host defecse of the neonate and intestinal flora., Acta Paediatr. Scand. Suppl. 351:122.
14. Mikelsaar, M. E., Siigur, U. H. and Tamm, A. O. 1989. Microecological aspects of investigation of influence of antibacterial preparations in intestinal microflora. Antibiotiki, 6:420.
15. Kroger, M., Kurmann, J. A. and Rasic, J. L. 1989. Fermented milks: past, present, and future. Food Technology, 43, 92, 94, 99.
16. Oberman, H. and Libudzisz, Z. 1998. Fermented milks. In: Wood BJB (ed.) Microbiology of Fermented Foods, 2nd ed., pp 308-350. London: Blackie Academic and Professional.
17. Robinson, R. K. and Tamime, A. Y. 1990. Microbiology of fermented milks. In: Robinson R. K. (ed.) Dairy Microbiology, vol. 2, The Microbiology of Milk Products, 2nd ed., pp 291-343. London: Elsevier Applied Science.
18. Takano, T. and Yamamoto, N. 2002. Health effects of fermented milks. In: Roginski, H. (ed.) Encyclopedia of Dairy Sciences, pp. 1063-1069. Amsterdam: Academic Press.
19. Hosono, A., Yoshimura, A. and Otani, H. 1987. Antimutagenic activity of cellular components of *Streptococcus faecalis* IFO 12965. Netherland Milk and Dairy J., 41:239.
20. Hosono, A., Shashikanth, K. N. and Otani, H. 1988. Antimutagenic activity of whole casein on pepper induced mutagenicity of streptomycin-dependent strain SD 510 of *Salmonella typhimurium* TA98. J. Dairy Res., 55:435.
21. Hosono, A., Yoshimura, A. and Otani, H. 1988. Desmutagenic property of cell walls of *Streptococcus faecalis* on the mutagenicities induced by amino acid pyrolyzates. Milchwissenschaft, 43:168.
22. Hosoda, M., Hashimoto, H., Morita, M., Chiba, M. and Hosono, A. 1992. Studies on antimutagenic effect of milk cultured with lactic acid bacteria on the Trp-P2 induced mutagenicity to TA98 strain of *Salmonella typhimurium*. J. Dairy Res., 59:543.
23. Thyagaraja, N. and Hosono, A. 1993. Antimutagenicity of lactic acid bacteria from Idly against food related mutagens. J. Food Protect., 56:1061.
24. Hosono, A. and Shirai, H. 1996. Antimutagenicity of

milk cultured with lactic acid bacteria against heated solution of cysteine-glucose. Animal Sci. Tech. (Jpn), 67:1076.

25. Hosono, A., Usman, P. and Ohba, R. 1997. Inhibitory activity of Maillard reaction products against Trp P1-induced mutagenicity of the *Salmonella typhimurium* TA 98 streptomycin dependent strain assayed in the absence of S9 mix., Biosci. Biotech. Biochem., 61:424.
26. Usman, P. and Hosono, A. 1997. Antimutagenic activity of Maillard reaction products against mutagenic heated tauco. Italian J. Food Sci., 9:267.
27. Yamad, M., Nakazawa, Y., Tsukasaki, F. and Hosono, A. 1997. Antimutagenic activity of Camembert cheese on the Trp-P1-induced mutagenicity to streptomycin-dependent strain SD 510 of *Salmonella typhimurium* TA98. Int. Dairy J., 7:795.
28. Hosoda, M., Hashimoto, H., He, F., Yamaguchi, K. and Hosono, A. 1998. Inhibitory effects of fecal lactobacilli and bifidobacteria on the mutagenicities to Prp-P2 and IQ. Milchwissenschaft, 53:309.
29. Roginski, H. 2002. Fermented milks, Northern Europe. In: Roginski, H. (ed.) Encyclopedia of Dairy Sciences, pp 1034-1041. Amsterdam: Academic Press.
30. Hosono, A. 1992. Diet and mutagenic toxicity. In: Nakazawa, Y. and Hosono, A. (ed.) Challenges for the Science of Health and Function of Fermented Milk, pp 103-126, London: Elsevier Applied Science.
31. Hosono, A. 1997. Desmutagenicity of lactic acid bacteria. In: Pandalai, S. G.. (ed.) Recent Research Development in Agaricultural & Biological Chemistry, pp 173-191, Trivandrum: Research Signpost.
32. Kitazawa, H., Toba, T., Itoh, T., Kumano, N. and Adachi, S. 1990. Antitumor activity of ropy sour milks in murine solid tumor. Jpn. J. Zootech. Sci., 61:1033.
33. Kalliomaki, M., Salminen, S. and Arvilommi, H.: Probiotics in primary prevention of atopic desease: a randomized placebo-conrolled traial. Lancet, 357, 1076 (2001).
34. Pelto, L., Isolauri, E., Lilius, E-M., Nuutila, J. and Salminen, S. 1998. Probiotic bacteria down-regulate the milk induced inflammatory response in milk hypersensitive subjects but have an immunostimulatory effect in healthy subjects. Clinical and Experimental Allergy, 28:1471.
35. Giudice, M. M. and de Luca, M. G. 2004. The role of probiotics in the clinical management of food allergy and atopic dermatitis. J. Clin. Gastroenterol., 38:S121.
36. Usman, P. and Hosono, A. 2000. Cholesterol- lowring acitivity of *Lactobacillus gasseri*: *In vitro* and *in vivo* results. In: Pandalai, S. G.. (ed.) Recent Research Development in Agaricultural & Biological Chemistry, pp 1-34, Trivandrum: Research Signpost.
37. Lin, S. Y., Ayres, J. W., Winkler, W. and Sandine, W. 1989. Lactobacillus effects on cholesterol: *in vitro* and *in vivo* results. J. Dairy Sci., 72:2885.
38. Gilliland, S. E. and Walker, D. K. 1990. Factors to consider when selection a culture of *Lactobacillus acidophilus* as a dietary adjunct to produce a hypocholesterolemic effect in humans. J. Dairy Sci., 73:905.
39. DSouza, A. L., Rajkumar, C., Cooke, J. and Bulpitt, C. J. 2002. Probiotics in prevention of antibiotic associated diarrea: meta-analysis. BMJ, 324:1361.
40. Carrillo, M., Estrada, E. and Hazen, T. C. 1985. Survival and enumeration of fecal indicators *Bifdobacterium adolescentis* and *Escherichia coli* in a tropical rain forest watershed. Appl. Environ. Microbiol., 50:468.
41. Gibbs, R. S., Blanco, J. D., St. Clair, P. J. and Castaneda, I. S. 1982. Quantitative bacteriology of amniotic fluid from women with clinical intraamniotic infection. Am. J. Obstet. Gynecol. 69:208.
42. Hosono, A., Kashina, T. and Kada, T. 1986. Antimutagenic properties of lactic acid cultured milk on chemical and fecal mutagens. J. Dairy Sci., 69, 2237.
43. Hosono, A., Wardojo, R. and Otani, H. 1990. Inhibitory effect of lactic acid bacteria from fermented milk on the mutagenicities of volatile nitrosamines. Agric. Biol. Chem., 54:1639.

44. Hosoda, M., Hashimoto, H., Morita, H., Chiba, M. and Hosono, A. 1992. Antimutagenicity of milk cultured with lactic acid bacteria against N-methyl-N-nitro-N-nitrosaguanidine. J. Dairy Sci., 75: 976.
45. Hosoda, M., Hashimoto, H., He, F., Yamazaki, K. and Hosono, A. 1997. Inhibitory effect of milk cultured with *Lactobacillus* strains on the aflatoxin mutagenicity. Animal Sci. Tech. (Jpn), 68:555.
46. Hosoda, M., Hashimoto, H., He, F., Morita, H. and Hosono, A. 1996. Effect of administration of milk fermented with *Lactobacillus acidophilus* LA-2 on fecal mutagenicity and microflora in the human intestine. J. Dairy Sci., 79:745.
47. Hosono, A., Wardojo, R. and Otani, H. 1990. Binding of amino acid pyrolyzates by lactic acid bacteria isolated from dadih. Lebensmitt.-Wiss. Und Tech., 23: 149.
48. Zhang, X. B., Ohta, Y. and Hosono, A. 1990. Antimutagenicity and binding of lactic acid bacteria from Chinese cheese to mutagenic pyrolyzates. J. Dairy Sci., 73:2702.
49. Tanabe, T., Otani, H. and Hosono, A. 1991. binding of mutagens with cell wall peptidoglycan of *Leuconostoc mesenteroides* subsp. *dextranicum* T-180. Milchwissenschaft., 46:622.
50. Tanabe, T., Suyama, K. and Hosono, A. 1994. Effect of pepsin, trypsin or bile acid on the binding of tryptophane pyrolysates by *Lactococcus lactis* subsp. *lactis* T-80. Milachwissenschaft, 49:438.
51. Tanabe, T., Suyama, K. and Hosono, A. 1994. Effect of sodium dodecylsulphate on the binding of *Lactococcus lactis* subsp. *lactis* T-80 cells with Trp-P1. J. Dairy Res., 61:311.
52. Thyagaraja, N. and Hosono, A. 1994. Binding properties of lactic acid bacteria from Idly toward food borne mutagens. Food and Chem. Toxicol., 32: 805.
53. Sreekumal, O. and Hosono, A. 1998. Antimutagenicity and the influence of physical factors in binding *Lactobacillus gasseri* and *Bifidobacterium longum* cells to amino acid pyrolysates. J. Dairy Sci., 81: 1508.
54. Sreekumal O. and Hosono, A. 1998. The heterocyclic amine binding receptors of *Lactobacillus gaseri* are the carbohydrate moieties of the cell wall. Mutation Res., 421:65.
55. Hosono, A., Tanabe, T. and Otani, H. 1990. Binding properties of lactic acid bacteria isolated from kefir milk with mutagenic amino acid pyrolyzates. Milchwissenschaft, 45:647.
56. Usman, P. and Hosono, A. 1999. Bile tolerance, taurocholate deconjugation and binding of choresterol by *Lactobacillus gasseri* strain. J. Dairy Sci., 82:243.
57. Hahimoto, H., Yamazaki, K., He, F., Kawase, M., Hosoda, M. and Hosono, A. 1999. Hypocholesterolemic effect of *Lactobacillus casei* subsp. *casei* TMC 0409 strain observed in rats fed cholesterol contained diets. Animal Sci. J., 70:90.
58. Usman, P. and Hosono, A. 1999. Binding of cholesterol to the cells and peptideglycan of *Lactobacillus gasseri.* Milchwissenschaft, 541-495.
59. Usman, P. and Hosono, A. 1999. Viability of *Lactobacillus gasseri* and its cholesterol binding and antimutagenic activities during subsequent refrigerated storage in nonfermented milk. J. Dairy Sci., 82:2536.
60. Kawase, M., Hashimoto, H., Hosoda, M., Morita, H. and Hosono, A. 2000. Effect of administration of fermented milk containing whey protein concentrate to rats and healthy men on serum lipids and blood pressure. J. Dairy Sci., 83:255.
61. Hashimoto, H., Kawase, M., Hosoda, M., He, F., Morita, H. and Hosono, A. 2000. Binding deconjugation and oxidation of taurocholic acid with lactobacilli cells. Milchwissenschaft, 55:316.
62. Hosono, A. and Tono-oka, T. 1995. Binding of cholesterol with lactic acid bacterial cells. Milchwissenschaft, 50:556.
63. Usman, P. and Hosono, A. 2000. Effect of administration of *Lactobacillus gasseri* on serum lipids and fecal steroids in hypercholesterolemic rats. J. Dairy Sci., 83:1705.

64. Kawase, M., Hashimoto, H., Hosoda, M., Morita, H. and Hosono, H. 2001. Serum cholesterol lowering effect of fermented milk with *Streptococcus thermophilus* TMC 1543. Animal Sci. J., 72:54-62.
65. Usman, P. and Hosono, A. 2001. Hypocholesterolemic effect of *Lactobacillus gasseri* SBT 0270 in rats fed a cholesterol enriched diet. J. Dairy Res., 68:61.
66. Tabuchi, M., Tamura, A., Yamada, N., Ishida, T., Hosoda, M. and Hosono, A. 2004. Hypocholesterolemic effects of viable and heat-sterilized cells of *Lactobacillus* GG in rats fed a high-cholesterol diet. Milchwissenschaft, 59:249.
67. Xiao, J. Z., Kondo, S., Takahashi, N., Miyaji, K., Hiramatsu, A., Iwasaki, K., Kokubo, S. and Hosono, A. 2003. Effects of milk product, fermented by *Bifidobacterium longum*, on blood lipids in rats and healthy adult male volunteers., J. Dairy Sci., 86:2452.
68. Tabuchi, M., Ozaki, M., Tamura, A., Yamada, N., Ishida, T., Hosoda, M. and Hosono, A. 2003. Antidiabetic effect of *Lactobacillus* GG in streptozotocin induced diabetic rats. Biosci. Biotechnol. Biochem., 67:1421.
69. DSouza, A. L. 2002. Probiotics in prevention of antibiotic associated diarrhoea: meta-analysis. BMJ, 324:1361.
70. Marteau P. and Seksik, P. 2004. Tolerance of probiotics and prebiotics. J. Clin. Gastriebterol., 38: S67.