乳酸菌と酵母による豆乳発酵産物に関する研究

(Studies on soybean milk fermented with lactic acid bacteria and yeast)

学位論文の内容の要約（英語）

新　良一

Summary

Recently, soymilk products fermented by lactobacilli and bifidobacteria have become widely adopted for promoting and maintaining health. Fermented soy milk is known to alleviate intestinal disorders, suppress breast and colon cancer, regulate blood pressure, and improve diabetes. Soybeans contain substances beneficial for human health, including soy protein, peptides, oligosaccharides, phospholipids, isoflavones, saponins, minerals, and vitamins. The fermentation of soybeans by different kinds of micro-organisms results in various compositional and functional changes, such as the production of a large variety of peptides that stimulate immunomodulation and regulation of blood pressure. Isoflavones and saponins exist in glucoside form in the soybean, but are degraded by the β-glucosidase of microorganisms into aglycons that are easily absorbed in the intestine. Soybean milk-fermented product (SFP) used in this study was the product which was fermented with a symbiotic combination of several species of lactic acid bacteria (*Lactobacillus plantarum*, *L. casei*, *Lactococcus lactis*, etc.) and yeast (*Saccharomyces cerevisiae*).

Intestinal microbiota and flora play numerous symbiotic physiological roles in the host, including the production of energy, promotion of intestinal peristaltic motion, digestion and absorption, metabolic regulation of bile acids and cholesterol, protection from infection, immunostimulation, and prevention of cancer. From recent research, intestinal flora has also been implicated in obesity, diabetes, cancer, allergies, autoimmune diseases, and aging in humans. Aging, stress, diet, drugs, and pathogens all influence intestinal flora. Diet in particular greatly impacts the intestinal microenvironment, for which recent advances in flora research have identified functional foods that contribute to human health. Functional foods can be classified into 3 groups based on their mechanism of action: probiotics, prebiotics, and biogenics. Probiotics are viable

microorganisms, such as lactobacilli, bifidobacteria, and yeasts, that benefit the host by improving intestinal bacteria balance. Prebiotics are nondigestible food ingredients, including oligosaccharides and dietary fiber, which selectively stimulate the growth or activities of beneficial intestinal bacteria in the colon to improve host health. Biogenics are biologically active peptides, immunopotentiators (i.e., biological response modifiers), and plant flavonoids that ameliorate health directly or indirectly through the modulation of intestinal microbiota. SFP is also considered to be a biogenic, however, the precise function and mechanisms of SFP remain unclear. This study evaluated the effects of SFP on the intestinal environment and immunity in mucous membranes (1) and on the suppression of colon cancer in an animal model (2). *Lactobacillus plantarum* BF-LP284 (LP284) was selected for SFP fermentation based on its high cytokine inducibility properties for investigation of anti-tumor activity and mechanisms (3). Lastly, the impact of soluble fraction SFP (SFP-s) on hypertension (4), liver and kidney dysfunction (5), and arthritis (6) were assessed.

1) Improvement of the intestinal environment and immunological enhancement of mucous membranes in humans by SFP

The effect of SFP (450 mg/day) on human fecal flora was determined by comparing the changes in intestinal flora between human volunteers consuming SFP and those receiving a placebo. An occupation rate of *Bifidobacterium* of more than 25% was significantly greater in the SFP group than in the placebo group. The concentration of secretory IgA in the saliva which enhance immunity in mucous membranes was also significantly higher in the SFP group than in the placebo group. The occupation rate of *Clostridium* in the fecal flora of volunteers increased after shifting from a traditional Japanese diet (TJD) to a Western diet (WD), in which a total daily amount of 300 g of meat (900 kcal) was eaten at lunch for 3 days. The occupation rate of *Clostridium* in the

WD group was reduced by SFP ingestion (900 mg/day) to a level similar to that in the TJD group, while the occupation rate of *Bifidobacterium* was higher in the WD + SFP ingestion group than in the WD group. Moreover, β-glucuronidase activity in the feces was up to 5 times higher after conversion from the TJD to the WD but returned to normal levels by SFP inclusion in the WD. The above findings suggested that SFP could reduce the risk of colon cancer by improving the intestinal environment, enhancing immunity in mucous membranes, and accelerating the excretion of carcinogenesis-induced agents.

2) Suppressive effect of SFP on colon cancer in an animal model

The incidence of 1, 2-Dimethylhydrazine-induced colon cancer was significantly lower in CF#1 mice given SFP as compared with controls. Winn assays were performed using spleen cells to examine the mechanism of SFP tumor suppression. SFP administered for more than 6 days inhibited tumor growth relative to tumor cells inoculated with Meth-A alone, which indicated an induction of anti-tumor immune cells in SFP-treated spleen cells. Whereas the number of spleen cells increased to a greater degree in gnotobiotic BALB/c mice in association with *Bifidobacterium* than in saline-treated germ-free mice, that in mice given SFP or soybean milk for 4 weeks was comparable. Our results implied that the suppressive effect of SFP on tumorigenesis involved intestinal bacteria modulation of host immunity.

3) Anti-tumor activity of LP284

Heat-killed LP284 (H-Lp) was selected as the most potent immunomodulator from among 7 strains of lactobacilli during fermentation of SFP in terms of TNF-α induction ability in peritoneal macrophages. *In vitro* TNF-α and IFN-γ induction in Peyer's patch (PP) cells was significantly higher when incubated with H-Lp than with live LP284 (L-Lp). Suppression of syngeneic Meth-A tumors in a murine model by oral

administration of H-Lp was greater than that of L-Lp and of controls. Thus, H-Lp was considered to contribute to the anti-tumor activity of SFP by stimulating IFN-γ production in spleen cells, which displayed inhibited tumor growth in Winn assays under H-Lp treatment. Moreover, H-Lp increased the ratio of CD3+ cells among peripheral blood mononuclear cells in Meth-A tumor-bearing mice, suggesting an H-Lp-mediated anti-tumor mechanism whereby immune cells that are activated by H-Lp in PP and acquire anti-tumor activity in the spleen migrate to tumor sites through cytotoxic lymphocyte homing to inhibit tumor growth.

4) Anti-hypertensive effects of SFP and SFP-s

The anti-hypertensive effects of orally administered SFP and SFP-s were investigated in spontaneously hypertensive rats. Systolic blood pressure was reduced significantly by a single dose of SFP or SFP-s compared with controls. However, soy milk administration alone did not produce this result. The sugar fraction of SFP-s separated by Sephadex-G25 column chromatography exhibited a lowering effect on systolic blood pressure and inhibited the activity of angiotensin I-converting enzyme. Accordingly, SFP-s may play a role in hypertension management and have utility in maintaining health.

5) Improvement of liver and kidney dysfunction by SFP-s

In rat hepatic disorders induced by oral administration of deoxycholic acid or intraperitoneal injection of D-Galactosamine, the increase in serum L-Aspartate aminotransferase level was significantly inhibited by a diet containing dried SFP-s. Moreover, the SFP-s rat group displayed a lower concentration of blood urea nitrogen and greater urinary output as compared with a control group. Pretreatment of primary rat hepatic and renal cell cultures with SFP-s prior to exposure to dichromate resulted in a marked decrease in dichromate-induced cytotoxicity as evaluated by the leakage of

lactate dehydrogenase. The levels of dichromate-induced lipid peroxidation, as evidenced by malondialdehyde formation, were also reduced by SFP-s pretreatment of hepatocytes. These collective findings indicated that the anti-oxidative activity of SFP-s might improve hepatic and renal disorders by suppressing membrane peroxidation.

6) Anti-arthritic effect of SFP-s

The anti-arthritic effects of SFP-s were investigated in bovine type II collagen (bCII)-induced arthritis (CIA) in mice. The disease incidence and mean clinical score of CIA were significantly suppressed in an SFP-s + glucosamine (GM) group, while hind foot pad thickness was significantly reduced. Histopathological scores of the severity of lesions in arthritic hind paw joints were significantly ameliorated in the SFP-s and SFP-s + GM groups. Serum bCII-specific IgG antibody production was markedly down-regulated in SFP-s and SFP-s + GM groups, and IL-6 level in the homogenate supernatant of diseased hind foot pad tissue was significantly lower in the SFP-s + GM group. Taken together, a combination of SFP-s and GM is considered to be additively effective in reducing the severity of CIA, presumably due to the abilities of SFP-s to modulate the immune response to bCII and of GM to reduce CIA-associated inflammation.

In conclusion, there is a considerable body of evidence demonstrating SFP to be a clinically meaningful fermented biogenic that acts directly or indirectly through the modulation of intestinal microflora. SFP may alleviate intestinal disorders, enhance immune function, suppress cancer, regulate blood pressure, and improve hepatic and renal disorders and arthritis, and is thus potentially useful for maintaining health in humans.