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ANTIMUTAGENIC AND ANTICANCER EFFECTS OF LACTIC ACID BACTERIA ISOLATED FROM KIMCHI

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Lactic acid bacteria(LAB) from kimchi(*Leu. mesenteroides, Lac. brevis, Lac. fermentum, Lac. plantarum* and *Ped. acidilactici*) revealed high preventive capability of the mutation induced by 4-NQO, MeIQ and Trp-p2 as much as (or higher than) *Lac. acidophilus* from yogurt, regardless of their viability. The cell wall fraction, rather than in the cytosol fraction was responsible for the antimutagenic activity of the lactic acid bacteria.

The cell lysate of *Lac. plantarum* from kimchi markedly inhibited ascites tumor induced by sarcoma-180 cells, and prolonged the life span of the Balb/c mice. The rats fed with the *Lac. plantarum* cell lysate also showed a remarkable inhibition of lung metastasis. Macrophage of the rats fed on broth culture of *Lac. plantarum* was activated and the activity of phagocytosis against *Staphylococcus* was significantly increased.

During the administration of kimchi in humans, the cell counts of *Lactobacillus* and *Leuconostoc* increased ($p<0.05$), but other intestinal microflora were not changed in the fecal samples. The enzyme levels of β-glucosidase, β-glucuronidase, and nitroreductase in the colon decreased during the kimchi intake ($p<0.05$). The fecal pH was also significantly reduced when the kimchi was administered, suggesting that kimchi and kimchi lactic acid bacteria play an important role to prevent colon cancer in humans.

Introduction

Lactic acid bacteria are commonly found in the gastrointestinal tract of humans and animals, in dairy products, in plants and fermented vegetables such as kimchi. Kimchi is a Korean traditional vegetable fermented food. Park (1) demonstrated that kimchi extracts revealed antimutagenic and anticancer effect *in vitro* and *in vivo*. The antimutagenic compounds of kimchi were suggested that vitamin C, β-carotene, phenolic compounds, isothiocyanate, indole compound, β-sitosterol, diallylsulfide, and dietary fiber, unknown fermented products, lactic acid bacteria, etc. Lactic acid bacteria(LAB) are the main natural starter for kimchi fermentation. The LAB counts of optimally ripened kimchi (pH 4.3) are about 10^8CFU/ml. The major LAB for kimchi fermentation are *Leu. mesenteroides, Lac. plantarum, Lac. brevis, Lac. ferementum, Ped. acidilactic,* etc. *Leu. mesenteroides* initiates the fermentation of kimchi and is the predominant LAB in the early fermentation stages. As the pH drops to 4.6-4.9, *Leu. mesenteroides* is relatively inhibited, but other LAB such as *St. faecalis, Lac. brevis, Ped. cerevisiae* and *Lac. plantarum* continue the fermentation process (2).

LAB from yogurt and other dairy products are usually believed as probiotics and a lot of studies were carried out on the fuctionality of the LAB such as antimutagenic and antitumor effects, immunomodulatory proporties, etc(3). *Lac. acidophilus, Lac. bulgaricus*, and *Lac. casei* from dairy products were reported to exhibit anticarcinogenic effects and activate the immune system in mice treated with sarcoma 180 and Ehrlich carcinoma 57 (3,4). Dietary supplementation of *L. acidophilus* exhibited the significant reduction of fecal enzymes associated with colon carcinogenesis in human subjects (5) and experimental animals (6).

There are a few studies on kimchi lactic acid bacteria and their functionality related to cancer preventive effects. In this review, the antimutgenic effect of LAB from kimchi, antitumor effect of the LAB and prevention of colon cancer by the LAB and kimchi that were reported in Korea are introduced and possible researches related on this area will be discussed.

Antimutagenic effect of kimchi LAB

The antimutagenic activities of cell body of several LAB isolated from kimchi were compared in Ames test and SOS chromotest (7,8). The mutagenicities mediated by 4-NQO, MeIQ, and Trp-P2 were effectively suppressed by the LAB in the tests. Cell body of *Leu. mesenteroides* exhibited higher antimutagenic activity on 4-NQO, MeIQ and MNNG than any other LABs tested. As shown in Fig. 1. *Leu. mesenteroides* showed the highest antimutagenic activity among the LAB. However, *Ped. acidilactici* did not show antimutagenic effect against 4-NQO. From their studies, whether the LAB from kimchi are viable or nonviable, the antimutagenic activity was still effective. The antimutagenic activity of LAB was found in the cell wall fraction, rather than in the cytosol fraction. It was reported that glycopeptide cell wall fragments are responsible for the antitumor activity (3). However, Pool-Zobel *et al.* (9) demonstrated that most, but not all, LAB tested could strongly inhibit genotoxicity in the GI tract of the rat, and in this case viable organisms were required for the protective effect *in vivo*.

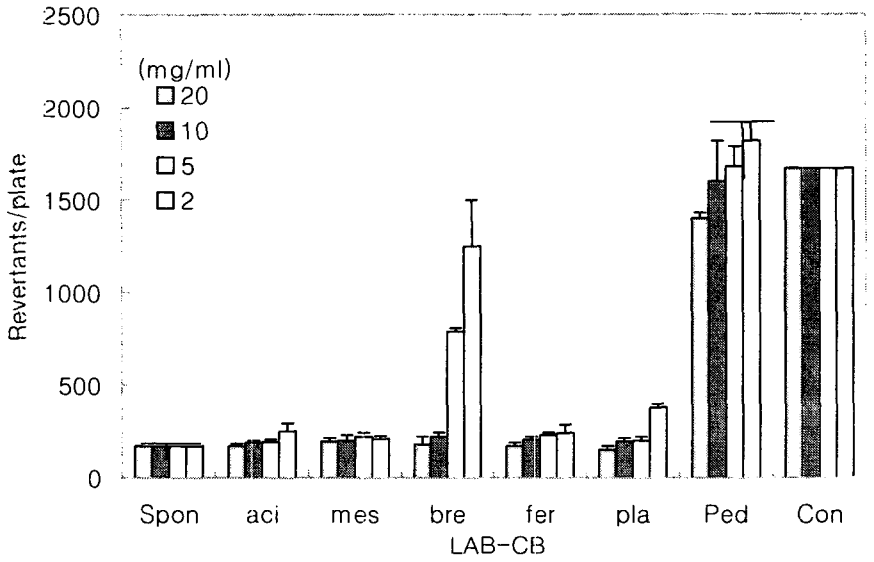

Fig. 1. Antimutagenic activity of lactic acid bacteria-cell body (LAB-CB) against 4-NQO(0.15μg/plate) on *Salmonella typhimurium* TA100(7)
Spon: spontaneous, aci: *Lactobacillus acidophilus*, mes:*Leuconostoc mesenteroides*, bre: *Lactobacillus brevis*, fer: *Lactobacillus fermentum*, pla:*Lactobacillus plantarum*, Ped: *Pediococcus acidilactici*, Con: control

Antitumor and immunostimulant effects of kimchi LAB

The administration of kimchi LAB reduced the tumor formation in animals. The kimchi LAB of *Lac. plantarum* and *Leu. mesenteroides* significantly reduced the tumor formation rate in ICR mice treated with sarcoma 180 cells (4). The inhibition rate was 57% and 39%, respectively, while *Lac. casei* was the most effective, showing 88% inhibition rate in this study. The tumor formations also decreased when the kimchi LAB, *Lac. plantarum* and *Leu. mesenteroides* were administered using lewis lung carcinoma in C57BL/6 mice, the inhibition rates were 42% and 44%, respectively. However , *Lac. acidophilus*, a dairy LAB inhibited the tumor formation rate to be 28%, and *Lac. casei* exhibited 78% inhibition rate. It seems that the antitumor activity was somewhat different depending on the LAB strains. The activity was not different whether the LAB are from kimchi or dairy foods.

Shin *et al.* (10) reported that antitumor effects of kimchi LAB, using mice fed with cell lysate of *Lac. plantarum* from kimchi. The ascites tumor induced by sarcoma-180 was markedly

inhibited and the expected life span was extended to 60% in the Balb/c mice fed with *Lac. plantarum* cell lysate for two weeks. As lung was the metastasis site of SOS, the weight of lung was measured to determine the degree of metastasis inhibition by the *Lac. plantarum* the cell lysate feeding. The rats fed with the cell lysate for one week showed a remarkable inhibition of lung metastasis by 63%(before) and 46%(after), respectively. These results suggested that the feeding of *Lac. plantarum* cell lysate can induce a stimulation of immune system and these effects result in an antitumor activity. Fig. 2 shows that effect of feeding with kimchi components on the prolongation of expected life span of Balb/c mice inoculated with sarcoma-180. When *Lac. plantarum* fed to the mice the life span was markedly prolonged.

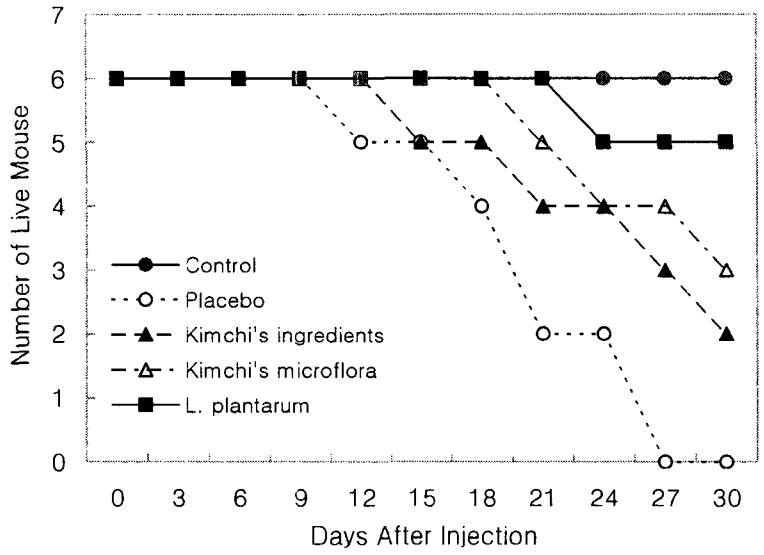

Fig. 2. Effect of feeding with kimchi components on the prolongation of expected life span of Balb/c mice inoculated with S-180 (10) Control; healthy mice, Placebo; 0.75% saline, Kimchi's ingredients; mixture of garlic, stone-leek and powdered red pepper, Kimchi's microflora; mixture of microorganisms isolated from kimchi, *L. plantarum*; 40mg/kg/day

Chae *et al.* (11) reported that immunostimulation effects of the mice fed with the cell lysate of *Lac. plantarum* isolated from kimchi. They examined 1) prolongation of splenocytes and peyer's patch cells 2) production of nitric oxide by peritoneal macrophages 3) production of intestinal secretory Ig A 4) increase on the TNF-α and IL-2 concentration in blood and 5) production of specific Ig G against sheep red blood cells. They observed that the general enhancement in enteric and systemic immune response with a simple oral administration of the cell lysate of the *Lac. plantarum*. Park (12) also reported that the administration of culture broth of *Lac. plantarum* isolated from kimchi to mice increased phagocytosis of the *Staph. aureus*. Perdigon *et al.* (13), studied that the effects of an orally-administered mixture of *Lac. casei* and *Lac. acidophilus* on the immune system in Swiss albino mice. They observed that the enhanced macophage and lymphocytic activity by administering cultures via the oral route, suggesting the synergistic effect of using the mixture of bacteria for more efficient stimulation of the host immune response.

Friend and Shahani (3) reviewed the antitumor properties of *Lactobacilli* and suggested that the *Lactobacilli* inhibit carcinogenesis by a) inactivating or inhibiting formation of carcinogenic compounds in the GI tract or b) suppressing promotion of cancer through stimulation or enhancement of the immune functions of the host.

Decreases in the fecal pH and activities of microflora enzymes related to colon cancer by kimchi LAB

It was curious whether kimchi intake changes the composition of human fecal bacteria. Lee *et al.* (14) examined that the viable cells of *Lactobacillus* and *Leuconostoc* delivered to the colon. The kimchi LAB counts increased significantly ($p<0.05$) during the administration of kimchi, however, other intestinal microflora such as *Bacteroides*, *Bifidobacterium*, *E. coli*, *Streptococcus*, *Staphylococcus* and *Cl. perfringens* did not change significantly, indicating that a portion of lactic acid bacteria present in kimchi can pass human stomach and reside in the large intestinal tract. Especially the enzyme levels of β-glucosidase and β-glucuronidase during kimchi intake in humans significantly decreased ($p<0.05$). As shown in Fig. 4, the enzymes which mediate the conversion of procarcinogens to proximal carcinogens involved in colon cancer decreased when kimchi were administered.

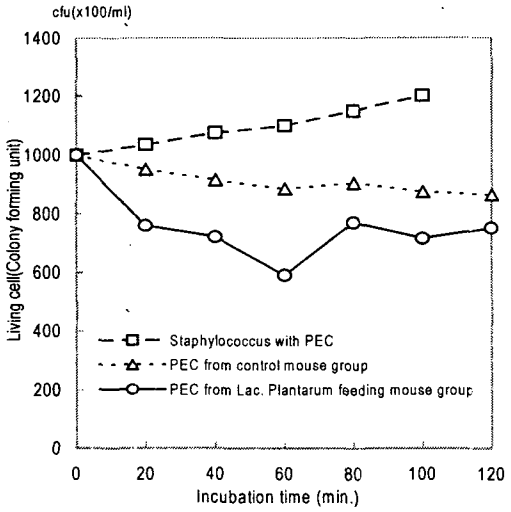

Fig. 3. Changes of *Staphylococcus* cells during phagocytosis test (Ten days feeding of *Lac. plantarum*) (12)

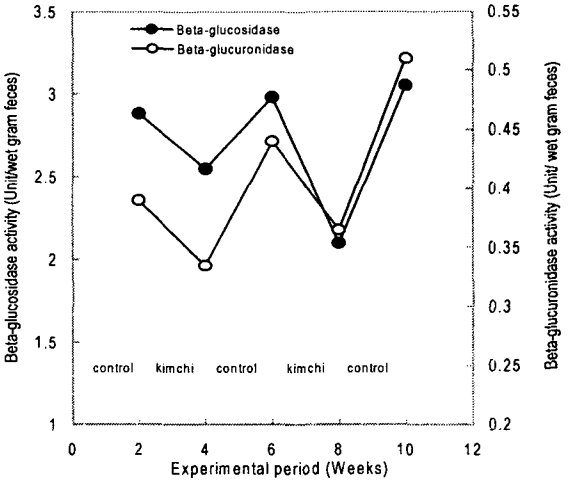

Fig. 4. Effect of kimchi intake on the fecal beta-glucosidase and beta-glucuronidase during experimental period (14)

Oh *et al.* (15) also reported that β-glucuronidase and nitroreductase activities in colon were significantly reduced for Koreans and Germans during the kimchi phase. Especially in their experiment, a significant decline in fecal pH was found for both groups during the kimchi phase. These results partly confirmed by the experiment the epidemiological hypothesis that kimchi consumption correlated with a low incidence of colon cancer in Korean.

Future research emphasis on kimchi LAB

LAB are now generally regarded as probiotics and many health related researches have been reported on LAB from yogurt and dairy products (16). LAB used as probiotics are as follows (17) ; *Lactococcus cremoris, Lactococcus lactis, Strep. thermophilus, Enterococcus faecium, Lactobacillus rhamnosus, Lac. acidophilus, Lac. casei, Lac. bulgaricus, Lac. gasseri*, etc. However, kimchi LAB could also be regarded as probiotic by the studies from the Korean researchers for kimchi. Kimchi has been eating for centuries like yogurt. Thus the safety has been already proved. From the previous studies using kimchi LAB the functions are almost same as the yogurt LAB. Kimchi is more valuable anticancer food than yogurt since kimchi carries LAB along with other functional phytochemicals that yogurt does not have.

Recently, a curious report was reported (18) that *Lac. salivarius* inhibited both the attachment and release of IL-8 *in vitro*. *Helicobacter pylori* could not colonise the stomach of *Lac. salivarius* infected gnotobiotic Balb/c mice, but colonised in large numbers and subsequently caused active gastritis in germ free mice. In addition, *Lac. salivarius* given after *H. pylori* implantation could eleminate colonisation by *H. pylori*. These results suggested the possibility of *Lactobacilli* being used as probiotic agents against *H. pylori*. Koreans and Japanese have higher incidence rates of stomach cancer. The kimchi LAB also have the possibility to show this kind of the activity. If the kimchi LAB reduce *H. pylori* proliferation, the LAB might be used as preventive agent for stomach cancer.

A number of species, within the LAB genera have been shown to exhibit antitumor properties. *Lac. acidophilus* and *Lac. casei* have been the most commonly reported to inhibit tumorigenesis. However, the kimchi LAB were also warranted to have antitumor activity along with other phytochemicals found in the kimchi for further study. It is widely opened for kimchi LAB researches on the probiotic activities, possible mechanisms and identification of the active compounds from the LAB.

References

1. Park, K. Y. 1995. The nutritional evaluation and antimutagenic and anticancer effects of kimchi. *J. Korean Soc. Food Nutr.* 24:169-182.
2. Lee, C. W., C. Y. Ko, and D. M. Ha. 1992. Microfloral changes of the lactic acid bacteria during kimchi fermentation and identification of the isolates. *Korean J. Appl. Microbiol. Biotechnol.* 20:102-109.
3. Friend, B. A. and K. M. Shahani. 1984. Antitumor properties of Lactobacilli and dairy products fermented by Lactobacilli. *J. Food Prot.* 47:717-723.
4. Kim, H. Y., H. S. Bae, and Y. J. Baek. 1991. *In vivo* antitumor effects of lactic acid bacteria on sarcoma 180 and mouse Lewis lung carcinoma. *J. Korean Cancer Assoc.* 23:188-196.
5. Goldin, B. R., L. Swenson, J. Dwyer, M. Sexton, and S. L. Gorbach. 1980. Effect of diet and *Lactobacillus acidophilus* supplements on human fecal bacterial enzymes. *J. Natl. Cancer Inst.* 64:255-261.
6. Goldin, B. R. and S. L. Gorbach. 1977. Alterations in fecal microflora enzymes related to diet, age, Lactobacilli supplements, and dimethylhydrazine. *Cancer*, 40:2421-2426 .
7. Son, T. J., S. H. Kim, and K. Y. Park. 1998. Antimutagenic activities of lactic acid bacteria isolated from kimchi. *J. Korean Assoc. Cancer Prev.* 3:65-74.
8. Park, K. Y., S. H. Kim, and T. J. Son. 1998. Antimutagenic activities of cell wall and cytosol fractions of lactic acid bacteria isolated from kimchi. *J. Food Sci. Nutr.* 3:329-333
9. Pool-Zobel, B. L., C. Neudecker, I. Domizlaff, S. Ji, U. Schillinger, C. Rumney, M. Moretti, I. Vilarini, R. Scassellati-Sforzolini, and I. Rowland. 1996. Lactobacillus- and Bifidobacterium-mediated antigenotoxicity in the colon of rats. *Nutr. Cancer* 26:365-380.
10. Shin, K. S., O. W. Chae, I. C. Park, S. K. Hong, and T. B. Choe. 1998. Antitumor effects of mice fed with cell lysate of *Lactobacillus plantarum* isolated from kimchi. *Korean J. Biotechnol. Bioeng.* 13:357-363.
11. Chae, O. W., K. S. Shin, H. W. Chung, and T. B. Choe. 1998. Immunostimulation effects of mice fed with cell lysate of *Lactobacillus plantarum* isolated from kimchi. *Korean J. Biotechnol. Bioeng.* 13:424-430.
12. Park, I. S. 1992. Function and physiological characteristics of lactic acid bacteria isolated from kicmhi. *Ph.D. Thesis*, Chung-Ang Univ., Korea
13. Perdigon, G., M. E. N. de Macias, S. Alvarez, M. Medici, G. Oliver and A. P. R. Holgado. 1986. Effect of a mixture of *Lactobacillus casei* and *Lactobacillus acidophilus* administered orally on the immune system in mice. *J. Food Prot.* 49:986-989.

14. Lee, K. E., U.H. Choi, and G. E. Ji. 1996. Effect of kimchi intake on the composition of human large intestinal bacteria. *Korean J. Food Sci. Technol.* 28: 981-986
15. Oh, Y. J., I. J. Hwang, and C. Leitzmann. 1993. Regular intake of kimchi prevent colon cancer. *Kimchi Sci. Ind.* 2:9-22.
16. Salminen, S., M. A. Deighton, Y. Benno, and S. L. Gorbach. 1998. Lactic acid bacteria in health and disease. In "Lactic acid bacteria", Ed Salminen, S. and A. Wright, p211-253, Marcel Dekker Inc., New York.
17. Macfarlane, G. T. and J. H. Gummings. 1999. Probiotics and prebiotics: can regulating the activities of intestinal bacteria benefit health? *Brit. Med. J.* 318:999-1003.
18. Kabir, A. M. A., Y. Aiba, A. Takagi, S. Kamiya, T. Miwa, and Y. Koga. 1997. Prevention of *Helicobacter pylori* infection by lactobacilli in a gnotobiotic murine model. *Gut.* 41:49-55