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Antimutagenic Activities of Cell Wall and Cytosol Fractions of Lactic Acid Bacteria Isolated from *Kimchi*

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Abstract

Cell wall(lactic acid bacteria-sonicated precipitate: LAB-SP) and cytosol (lactic acid bacteria-sonicated supernatant: LAB-SS) fractions were prepared from *kimchi* fermenting lactic acid bacteria such as *Leuconostoc mesenteroides, Lactobacillus brevis, Lactobacillus fermentum, Lactobacillus plantarum* and *Pediococcus acidilactici,* with *Lactobacillus acidophillus* isolated from yogurt. Using the Ames mutagenicity test and SOS chromotest system, the antimutagenic activity of those cell fractions was studied. One hundred eighty μl of LAB-SP from lactic acid bacteria isolated from *kimchi,* excepting *Pediococcus acidilactici,* supressed the mutagenicity of 4-nitroquinoline-1-oxide (4-NQO) in Ames mutagenicity test and SOS chromotest system, by above 90% and 60%, respectively. LAB-SP from lactic acid bacteria exhibited the antimutagenic activity aganist 2-amino-3,4-dimethyl-imidazo (4,5-f) quinoline (MeIQ) in Ames mutagenicity test, depending on the concentration. Especially, *Lactobacillus plantarum* which were isolated from *kimchi* had the strongest antimutagenicity on MeIQ. LAB-SP from lactic acid bacteria also inhibited the mutagenicity mediated by 3-amino-1-methyl-5H-pyrido [4,3-b]indole (Trp-P-2). *Lactobacillus fermentum, Lactobacillus plantarum,* and *Lactobacillus acidphillus* had higher antimutagenicity against Trp-P-2 than the other lactic acid bacteria. However, LAB-SS of lactic acid bacteria did not show any mutagenic activity against 4-NQO in Ames mutagenicity test and SOS chromotest systems. On the mutagenicity of MeIQ and Trp-P-2, LAB-SS of lactic acid bacteria from *kimchi* or dairy products exhibited a weaker inhibitory effect than LAB-SP of those bacteria. These results represent that, whether the lactic acid bacteria from *kimchi* are viable or nonviable, antimutagenic activity was still effective. We suggest that the strong, antimutaganic activity of lactic acid bacteria might be found in the cell wall fraction, rather than in the cytosol fraction.

Key words: lactic acid bacteria, *kimchi*, antimutagenicity, Ames mutagenicity test, SOS chromotest

INTRODUCTION

Lactic acid bacteria are commonly found in the gastrointestinal tract of humans and animals, in dairy products and naturally on some plant surfaces. Recently, the role of lactic acid bacteria in the etiology of cancer has received much attention (1-4). Several studies have investigated that the antitumor effects were shown by lactic acid bacteria or dairy products in the animal system. Reddy et al. observed that feeding of yogurt or yogurt components containing *Streptococcus thermophilus* and *Lactobacillus bulgaricus* inhibited cell counts and DNA synthesis in mice implanted with Ehrlich ascites tumor cells (1-3). Epidemiological studies (5) have indicated that consumption of high levels of cultured dairy products may reduce the risk of colon cancer. Dietary supplementation of *Lactobacillus acidophilus* exhibited the significant reduction of fecal enzymes associated with colon carcinogenesis in human subjects (6,7) and experimental animals (8,9).

It was reported that *Lactobacillus acidophilus* and *Lactobacillus bulgaricus* from dairy products had anticarcinogenic effects and activated the immune system in mice treated with sarcoma 180 and Ehrlich carcinoma 57 (10). Mitogenic and immunoadjuvant activity of lactic acid bacteria with respect to cellular, humoral immunity has also been reported (11). But there is no clear answer to the actions of lactic acid bacteria on tumor and immunity.

So far, many studies have mainly reported the physiological effects of lactic acid bacteria which were from dairy products. But there have been little reports on the antimutagenicity of lactic acid bacteria from *kimchi.* Lactic acid bacteria are also the main microorganism responsible for ripening of *kimchi,* a Korean traditional food. *Leuconostoc mesentroides* initiates the fermentation of *kimchi* and is the predominant lactic acid bacteria in the early fermentation stages (12,13). As the pH drops to 4.6-4.9, *Leuconostoc mesentroides* is relatively inhibited, but other lactic acid bacteria such as *Streptococcus faecalis, Lactobacillus brevis, Pediococcus cerevisiae, and Lactobacillus plantarum* continue the fermentation process (12-14).

Previously (15), we reported that cell bodies of lactic acid bacteria isolated from *kimchi* had antimutagenic effects. This study focused on the finding of a cell fraction from *kimchi* fermenting lactic acid bacteria, which exhibited a strong, antimutagenic activity. We prepared cell wall and cytosol fractions of lactic acid bacteria from *kimchi.* Using the Ames mutagenicity test and the SOS chromotest system, the inhibitory

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activity of those fractions on mutagenicity mediated by several mutagens were investigated.

MATERIALS AND METHODS

Preparation of cell wall and cytosol fraction from lactic acid bacteria

Leuconostoc mesenteroides, Lactobacillus brevis, Lactobacillus fermentum, Lactobacillus plantarum, and *Pediococcus acidilactici,* which were isolated from *kimchi,* were obtained from Korean Collection for Type Culture. *Lactobacillus acidophillus* isolated from yogurt was also used. Lactic acid bacteria (LAB) were cultured in Difco lactobacilli MRS medium at 37°C for 24 hours. After centrifugation, LAB were washed with saline solution 2 times and freeze-dried. 20 mg/ml of LAB were sonicated for 15 minutes (sonicator: ARTEK sonic dismembrator model 300), 500 μl of sonicated LAB samples were dispensed to an *eppendorf* tube and added to 500 μl of distilled water. After centrifugation at 12,200×g for 30 minutes(4°C), 800 μl of supernatant was taken as LAB-SS (lactic acid bacteria-sonicated supernatant, cytosol fraction). 800 μl of distilled water was added to the residues(200 μl) and then used as LAB-SP (lactic acid bacteria-sonicated precipitate, cell wall fraction). 45, 90 or 180 μl of LAB-SP, and 180 μl of LAB-SS were added to an antimutagenicity test.

Antimutagenicity test

Chemicals

4-nitroquinoline-1-oxide (4-NQO) were obtained from Aldrich Chemical Co. (USA) and dissolved in distilled water. 2-amino-3,4-dimethyl-imidazo (4,5-f)quinoline (MeIQ) and 3-amino-1-methyl-5H-pyrido[4,3-b]indole(Trp-P-2) were purchased from Wako Chemical Co. (Japan) and dissolved in methanol. O-nitrophenyl-β-D-galactopyranoside (ONPG) and P-nitrophenyl phosphate disodium (PNPP) were also obtained from Sigma Chemical Co. (USA). All chemicals used in the present experiment were sterilized through millipore membrane filtration or were autoclaved.

Treatment of LAB fractions with mutagens

LAB fractions (LAB-SP, LAB-SS) were mixed with direct mutagens (4-NQO) in a ratio of 9 to 1 (V/V) and reacted at 37°C for 1 hour. 50 μl of reacted LAB fractions with direct mutagens were added to the Ames mutagenicity test system. Treated LAB fractions were diluted 3 times and 20 μl of those samples were added to the SOS chromotest system.

LAB fractions were mixed with indirect mutagens (MeIQ, Trp-2-P) in a ratio of 9 to 1 (V/V) and the same volume of S9 mix was also mixed. After vortexing, those mixed samples were incubated at 37°C for 20 minutes. 100 μl of treated samples were added to the Ames mutagenicity test system.

Bacterial strain

Salmonella typhimurium strains which were histidine-requiring strains, were kindly presented by Professor B. N. Ames, University of Califonia, Berkeley, CA, USA. The genotypes of *Salmonella typhimurium* strains were confirmed routinely for their histidine mutation, deep rough (*rfa*) mutation, *uvrB* mutation and for the presence of the R factor. *Escherichia coli* PQ37/plasmid pKM101 (PQ37) was from the parental strain GC4436 which carried a *sfiA*::*lacZ* opern fusion due to Mud (Ap *lac*) *Cis* insertion. *Escherichia coli* PQ37 was also confirmed routinely for their histidine mutation, deep rough (*rfa*) mutation, *uvrB* mutation and for the constitution of *PHO*^{c} gene and the inducibility of *sfiA*::*lacZ* fusion.

Ames mutagenicity test

Sprague-Dawely male rats weighing around 200 g were used for the preparation of the metabolic activation mixtures. To induce the rat liver enzymes, aroclor 1254 was diluted in corn oil to a concentration of 200 mg/ml. On the 5 th day, the removed livers of the rats were minced in 0.05 M of KCl, homogenized and centrifuged at 9,000 g. Then the supernatant (S9 fraction) was saved and distributed by 2 ml portions into plastic Nunc tubes, frozen quickly and stored immediately at -80 until used. The S9 mixes were prepared from the S9 fraction according to the method by Maron and Ames (16). In all assays, the components of the S9 mix were 8 mM $MgCl_2$, 33 mM sodium phosphate, pH 7.4 with the S9 fraction in a concentration of 10%. To test antimutagenicity, a modified method of Matsushima et al. (17) was used. 0.1 ml of a test strain from an overnight culture ($1-2\times10^9$ cells/ml) and 50 (direct mutagen) or 100 (indirect mutagen) μl of test samples treated with mutagen as above were added to 2 ml of the top agar, kept at 45°C, and vortexed for 3 seconds. The entire mixture was poured on the minimal agar plate. After incubating for 48 hrs at 37°C, the revertant bacterial colonies on each plate were counted.

SOS chromotest

The antimutagenicity test was performed by a modified method of Quillardet and Hofnung (18). Cultured *Escherichia coli* PQ37 (2×10^8 bacteria/ml) in L medium at 37°C were diluted with L medium 10 times. 100 μl of diluted *Escherichia coli* PQ37 were disposed to 2 series of 96 well microplate containg 20 μl of test samples treated with mutagens and then cultured at 37°C for 90 minutes. For β-galactosidase assay, 100 μl of ONPG was added in one series. 100 μl of PNPP was disposed to the other series for alkaline phosphotase assay. After 10 minutes, the color developments were terminated by the addition of 1.5 M Na_2CO_3 (100 μl) and 1M HCl (50 μl), for β-galactosidase and alkaline phosphotase assay, respectively. Following 5 minutes, the pH in alkaline phosphotase assay system was changed again by adding 50 μl of 2 M Tris buffer. The absorbance of each plate were read at 420 nm. The units of enzyme activites were calculated according to the following formula: $Eu=(1000\times A_{420})/t$ (min)

Statistical analysis

Data were presented in means±SD after one-way ANOVA analysis. Significant differences of treatment from the control were determined by using the Student's *t* test (19).

RESULTS AND DISCUSSION

Previously, it was observed in our laboratory that hole cells of lactic acid bacteria isolated from *kimchi* have antimutagenic effects. In this study, we prepared cell wall (LAB-SP) and cytosol (LAB-SS) fractions of lactic acid bacteria. The antimutagenic activities of those fractions of lactic acid bacteria from *kimchi* were tested in the Ames mutagenicity test and the SOS chromotest systems. These results provide additional information regarding the antimutagenic properties of lactic acid bacteria.

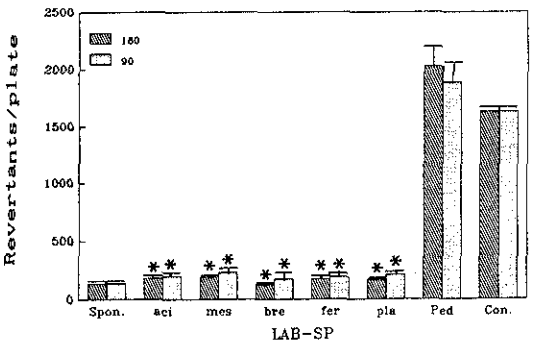

Fig. 1. Antimutagenic activity of lactic acid bacteria-sonicated precipitate (LAB-SP, μl/200 μl) against 4-nitroquinoline-1-oxide (4-NQO, 0.15 μg/plate) on *Salmonella typhimurium* TA100. Spon.: spontaneous, aci: *Lactobacillus acidophillus*, mes: *Leuconostoc mesenteroides*, bre: *Lactobacillus brevis*, fer: *Lactobacillus fermentum*, pla: *Lactobacillus plantarum*, Ped: *Pediococcus acidilactici*, Con.: control *Significantly different from control by student's *t* test ($p<0.05$).

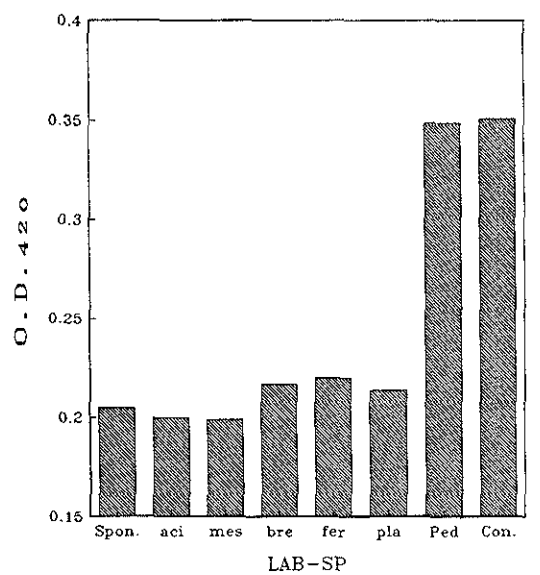

Fig. 2. SOS response of 4-nitroquinoline-1-oxide (4-NQO, 0.02 μg/assay) treated with lactic acid bacteria-sonicated precipitate (LAB-SP, μl/200 μl). The abbreviations are the same as shown in Fig. 1.

Antimutagenic effects of cell wall fractions (LAB-SP) from lactic acid bacteria

Fig. 1 shows that cell wall fractions (LAB-SP) from lactic acid bacteria supressed the mutagenicity of 4-NQO. Except for *Pediococcus acidilactici*, *Leuconostoc mesenteroides*, *Lactobacillus brevis*, *Lactobacillus fermentum*, *Lactobacillus plantarum* which were isolated from *kimchi*, had strong, antimutagenic activity as well as *Lactobacillus acidophillus* isolated from yogurt. 180 μl of LAB-SP from those lactic acid bacteria inhibited the mutagenicity of 4-NQO by above 90%.

In the SOS chromotest system, LAB-SP from lactic acid bacteria decreased the SOS response of 4-NQO by above 60% compared to control (Fig. 2). But *Pediococcus acidilactici* did not exhibit any antimutagenicity. These results were the same as those in the Ames mutagenicity test.

LAB-SP from lactic acid bacteria exhibited antimutagenic activity aganist MeIQ (Fig. 3), depending on the concentration. *Lactobacillus plantarum* which was isolated from *kimchi*, especially, had the strongest antimutagenicity aganist MeIQ even with the addition of 45 μl.

As shown in Fig. 4, LAB-SP from lactic acid bacteria also inhibited the mutagenicity mediated by Trp-P-2. *Lactobacillus fermentum*, *Lactobacillus plantarum* and *Lactobacillus acidphillus* had higher inhibitory effects on the mutagenicity of Trp-P-2 than the other lactic acid bacteria.

Antimutagenic effects of cytosol fractions (LAB-SS) from lactic acid bacteria

As shown in Table 1 and 2, three kinds of lactic acid bacteria from *kimchi* did not exhibit any mutagenic activity in the Ames mutagenicity test and the SOS chromotest systems. Therefore, it was proposed that the antimutagenic effects of lactic acid bacteria from *kimchi* aganist 4-NQO as described in previous study were in part due to the antimutagenic activity of cell wall, not cytosol.

On the mutagenicity of MeIQ, LAB-SS from *kimchi* exhibited weaker, inhibitory effects than LAB-SP of those bacteria (Table 3). The antimutagenicity of lactic acid bacteria might be due mainly to the action of cell wall. But it seems that the antimutagenic components aganist MeIQ is also pres-

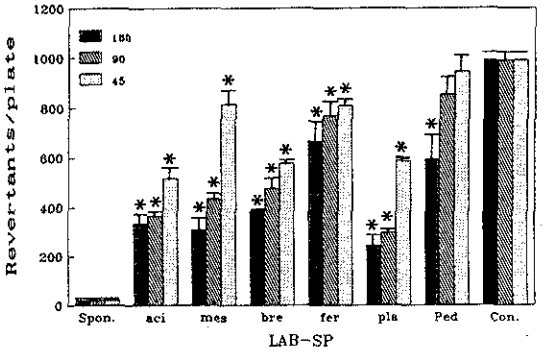

Fig. 3. Antimutagenic activity of lactic acid bacteria-sonicated precipitate (LAB-SP, μl/200 μl) against S9 activated 2-amino-3,4-dimethyl-imidazo (4,5-f) quinoline (MeIQ, 0.01 μg/plate) on *Salmonella typhimurium* TA98. The abbreviations are the same as shown in Fig. 1. *Significantly different from control by student's *t* test ($p<0.05$).

ent in cytosol of those bacteria.

Table 4 shows the antimutagenic activity of LAB-SS aganist Trp-P-2 in the Ames mutagenicity test. LAB-SS of lactic acid bacteria from *kimchi* or dairy products inhibited the mutagenicity of Trp-P-2 by above 40%. But their antimutagenic activities of cytosol were also less than those of LAB-SP. *Pediococcus acidilactici* suppressed the mutagenicity of Trp-P-2 by only 10%.

In previous reports, lactic acid bacteria from dairy products

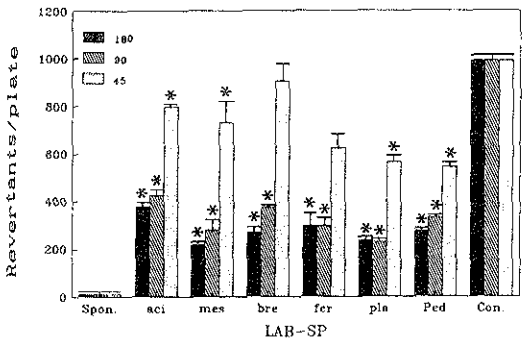

Fig. 4. Antimutagenic activity of lactic acid bacteria-sonicated precipitate (LAB-SP, μl/200 μl) against S9 activated 3-amino-1-methyl-5H-pyrido[4,3-b]indole (Trp-P2, 0.05 μg/plate) on *Salmonella typhimurium* TA98. The abbreviations are the same as shown in Fig. 1. *Significantly different from control by student's *t* test ($p<0.05$).

Table 1. Effect of lactic acid bacteria-sonicated supernatant (LAB-SS) on the mutagenicity induced by 4-nitroquinoline-1-oxide (4-NQO, 0.15 μg/plate) in *Salmonella typhimurium* TA100

Samples (μl/200 μl)		Revertants/plate	Inhibition ratio (%)
Spontaneous		139±20[1)]	100
Control		1109±181	0
Lactobacillus acidophillus	(180)	1163±33	-6
	(90)	1232±63	-13
Leuconostoc mesenteroides	(180)	1296±95	-19
	(90)	945±74	17
Lactobacillus plantarum	(180)	1124±81	-2
	(90)	1192±11	-9

[1)]Values are means±SD

Table 2. SOS response of 4-nitroquinoline-1-oxide (4-NQO, 0.02 μg/assay) treated with lactic acid bacteria-sonicated supernatant (LAB- SS, 180 μl/200 μl)

Sample	A_{420}[1)]	Eu[2)]	Inhibition ratio (%)
Spontaneous	0.172	17.2	100
Control	0.334	33.4	0
Lactobacillus acidophillus	0.333	33.3	1
Leuconostoc mesenteroides	0.329	32.9	3
Lactobacillus brevis	0.340	34.0	-4
Lactobacillus fermentum	0.335	33.5	-1
Lactobacillus plantarum	0.328	32.8	4
Pediococcus acidilactici	0.329	32.9	3

[1)]A_{420} is the optical density at 420 nm
[2)]EU means enzyme unit. $Eu=(1000\times A_{420})/10$ min

exhibited antimutagenic activity aganist N-nitrosodiethylamine (20). Bogdanov et al. (21) reported that the peptidoglycan of the cell wall in *Lactobacillus delbrueckii* subsp. *bulgaricus* was the major compound that suppressed tumor formation. This peptidoglycan was a compositional compound in the cell wall, and this compound was combined with muramyl peptide. Muramyl dipeptide and its derivatives stimulated superoxide anion and H_2O_2 production by the macrophage. Chung (22) also reported that the feeding of a cell wall fraction of *Lactobacillus plantarum* activated the phagocytic activity of macrophage in mice. The polysaccharides binding by phosphodiester to the muramic acid in the cell wall exhibited the immune activity. This kind of binding showed the strong, antitumor activity to ascite and solid tumors in mice.

β-Gluronidase, nitroreductase and azoreductase are the main human intestinal bacterial enzyme that stimulate the conversion of precarcinogen to carcinogen (23). It was reported that the feeding of *Lactobacillus* decreased the activites of these enzymes (24,25). Oh et al. (26) indicated that low incidence of colon cancer in Korea is due to the consumption of *kimchi*, which reduced the activities of β-glucuronidase and nitroreductase.

These results as above in this study represent that, whether *kimchi* fermenting lactic acid bacteria were viable or not, their

Table 3. Effect of lactic acid bacteria-sonicated supernatant (LAB-SS) on the mutagenicity induced by S9 activated 2-amino-3,4-dimethyl-imidazo (4,5-f) quinoline (MeIQ, 0.01 μg/plate) on *Salmonella typhimurium* TA98

Samples (180 μl/200 μl)	Revertants/plate	Inhibition ratio (%)
Spontaneous	18±6[1)]	100
Control	991±62	0
Lactobacillus acidophillus	781±44*	22
Leuconostoc mesenteroides	923±142	7
Lactobacillus brevis	1100±123	-11
Lactobacillus fermentum	976±10	2
Lactobacillus plantarum	830±32*	17
Pediococcus acidilactici	905±92	9

[1)]Values are means±SD
*Significantly different from control by student's *t* test ($p<0.05$)

Table 4. Effect of lactic acid bacteria-sonicated supernatant (LAB-SS) on the mutagenicity induced by S9 activated 3-amino-1-methyl-5H-pyrido[4,3-b]indole (Trp-P2, 0.05μg/plate) on *Salmonella typhimurium* TA98

Samples (180 μl/200 μl)	Revertants/plate	Inhibition ratio (%)
Spontaneous	18±6[1)]	100
Control	1513±8	0
Lactobacillus acidophillus	808±32*	47
Leuconostoc mesenteroides	784±26*	49
Lactobacillus brevis	644±8*	58
Lactobacillus fermentum	841±69*	45
Lactobacillus plantarum	886±26*	42
Pediococcus acidilactici	1358±11*	10

[1),*]The explanation is the same as shown in Table 3.

antimutagenic activities were still effective at least as much as those of yogurt fermenting bacteria. It was thought that the antimutagenic activity of the cell wall fraction was stronger than that of the cytosol fraction in lactic acid bacteria from *kimchi*. We suggest that the antimutagenic components of those bacteria might be mainly present in the cell wall fraction rather than the cytosol fraction.

But, the question of how the cell wall fraction of lactic acid bacteria from *kimchi* or dairy products exhibited the antimutagenic activity remained to be investigated. Further research regarding the antimutagenic compounds and their mechanism of lactic acid bacteria will have to be continued.

REFERENCES

1. Reddy, G. V., Shahani, K. M. and Banerjee, M. R. : Inhibitory effect of yogurt on Ehrlich ascites tumor cell proliferation. *J. Natl. Cancer Inst.*, **50**, 815 (1973)
2. Farmer, R. E., Shahani, K. M. and Reddy, G. V. : Inhibitory effect of yogurt components upon proliferation of the ascites tumor cells. *J. Dairy Sci.*, **58**, 787 (1975)
3. Reddy, G. V., Friend, B. A., Shahani, K. M. and Farmer, R. E. : Antitumor activity of yogurt components. *J. Food Prot.*, **46**, 8 (1983)
4. Ayebo, A. D., Shahani, K. M. and Dam, R. : Antitumor components of yogurt: fractionation. *J. Dairy Sci.*, **64**, 2318 (1981)
5. IARC Intestinal Microecology Group : Dietary fiber, transittime, fecal bacteria, steroids, and colon cancer in two Scandanavian populations. *Lancet*, **2**, 207 (1977)
6. Ayebo, A. D., Angelo, I. A. and Shahani, K. M. : Effect of ingesting *Lactobacillus acidophilus* milk upon fecal flora and enzyme activity in humans. *Milchwissenschaft.*, **35**, 730 (1980)
7. Goldin, B. R., Swenson, L., Dwyer, J., Sexton, M. and Gorbach, S. L. : Effect of diet and *Lactobacillus acidophilus* supplements on human fecal bacterial enzymes. *J. Natl. Cancer Inst.*, **64**, 255 (1980)
8. Goldin, B. R. and Gorbach, S. L. : Alterations in fecal microflora enzymes related to diet, age, *Lactobacillus* supplements, and dimethylhydrazine. *Cancer*, **40**, 2421 (1977)
9. Lee, H. : Effect of *Lactobacillus acidophilus* on colon carcinogenesis. *Ph. D. Thesis*, The Univ. of Nebraska-Lincoln, USA (1989)
10. Kim, H. Y., Bae, H. S. and Baek, Y. J. : *In vivo* antitumor effects of lactic acid bacteria on sarcoma 180 and mouse Lewis lung carcinoma. *J. Korean Cancer Assoc.*, **23**, 188 (1991)
11. Fernandes, C. F. and Shahani, K. M. : Anticarcinogenic and immunological properties of dietary *Lactobacilli*. *J. Food Prot.*, **53**, 704 (1990)
12. Kim, H. S. and Chun, J. K. : Studies on the dynamic changes of bacteria during the *kimchi* fermentation. *J. Nucl. Sci.*, **6**, 112 (1966)
13. Kim, H. S. and Whang, K. C. : Microbiological studies on *kimchi*. I. Isolation and identification of anaerobic bacteria. *Bull. Sci. Res. Inst.*, **4**, 56 (1959)
14. Lee, C. W., Ko, C. Y. and Ha, D. M. : Microfloral changes of the lactic acid bacteria during *kimchi* fermentation and identification of the isolates. *Korean J. Appl. Microbiol. Biotechnol.*, **20**, 102 (1992)
15. Son, T. J., Kim, S. H. and Park, K. Y. : Antimutagenic activities of lactic acid bacteria isolated from *kimchi*. *J. Korean Asso. Cancer Prev.*, **3**, 65 (1998)
16. Maron, D. M. and Ames, B. N. : Revised methods for the *Salmonella* mutagenicity test. *Mutat. Res.*, **113**, 173 (1983)
17. Matsushima, T., Sugimura, T., Nagao, M., Yahagi, T., Shirai, A. and Sawamura, M. : Factors modulating mutagenicity in microbial test. In "*Short term test systems for detecting carcinogens*" North, K. H. and Garner, R. C. (eds.), Springer, Beling, p. 273 (1980)
18. Quillardet, P. and Hofnung, M. : The SOS chromotest, a colorimetric bacterial assay for genotoxins: Procedures. *Mutat. Res.*, **147**, 65 (1985)
19. Steel, R. G. and Torrie, J. H. : *Principles and procedures of statistics*. McGraw-Hill Kogakusha, Ltd., Tokyo, p. 187 (1980)
20. Hosono, A., Wardojo, R. and Otani, H. : Inhibitory effects of lactic acid bacteria from fermented milk on the mutagenicities of volitile nitrosamines. *Agric. Bio. Chem.*, **54**, 1639 (1990)
21. Bogdanov, I. G., Dalev, P. G., Gurevich, A. I., Kolosov, M. N., Mal'kova, V. P., Plemynnikova, L. A. and Sorokina, I. B. : Antitumor glycopeptides from *Lactobacillus bulgaricus* cell wall. *FEBS Lett.*, **57**, 259 (1975)
22. Chung, H. K. : The physiological characteristic and immunological functions of lactic acid bacteria from *kimchi*. *Kimchi Sci. Ind.*, **2**, 23 (1993)
23. Goldin, B. R. and Gorbach, S. L. : The relationship between diet and rat fecal bacterial enzymes implicated in colon cancer. *J. Natl. Cancer Inst.*, **64**, 263 (1976)
24. Goldin, B. R. and Gorbach, S. L. : The effect of milk and *Lactobacillus* feeding on human intestinal bacterial enzyme activity. *Am. J. Clin. Nutr.*, **39**, 756 (1984)
25. Perosa, M. C., Golner, B., Goldin, B., Baraket, S., Dallal, G. and Russell, R. M. : Effect of *Lactobacillus acidophilus* or yogurt feeding on bacterial fecal enzymes in the elderly. *Gastroenterology*, **98**, A425 (1990)
26. Oh, Y. J., Hwang, I. J. and Leitzmann, C. : Regular intake of *kimchi* prevents colon cancer. *Kimchi Sci. Ind.*, **2**, 9 (1993)

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