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RESEARCH NOTE

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Antimutagenic Effects on Aflatoxin B_1 of Soybean Pastes Fermented by *Bacillus* Strains

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Abstract Antimutagenic effects of methanol extracts of various soybean pastes against aflatoxin B1 were examined using *Salmonella typhimurium* TA98 and TA100. Antimutagenic activities of boiled soybeans, Japanese Miso, traditional Korean soybean pastes, soybean pastes fermented by wild type strains, and soybean pastes fermented by mutants, transformants, and cell fusants were 53.6 to 54.6%, 73 to 79.7%, 78.3 to 95.7%, 85 to 97.1%, 71.9 to 78.3%, 65.5 to 77.7%, and 73.4 to 79.0%, respectively. Soybean pastes fermented by wild type strains showed higher activities than those fermented by mutant, transformant, and cell fusant strains.

Keywords: antimutagenicity, soybean paste, *Bacillus* species, aflatoxin B_1

Intoduction

Recently, research has focused on the functional and physiological activities of traditional Korean soybean paste. Soybean paste is reported to have superior effects of recovering liver function and neutralizing poisons in the liver, as well as displaying superior anticancer effects (1-3). The causative ingredient for these functions has not yet been proved definitely. In addition, antioxidative (4-7) and antimutagenic effects (8-10) of soybean paste have been reported. The main strain that produces the unique taste and flavor of soybean paste during fermentation is known to be *Bacillus subtilis*. Although studies have been performed on the quality and characteristics of soybean paste fermented by a single ferment strain (11-13) no reports are yet available on the antimutagenic effect of soybean paste made by a single ferment strain. In this study, the antimutagenic activities of soybean paste fermented using *B. subtilis* wild type strains and those bred by mutation, cell fusion, and gene manipulation were evaluated. In addition, antimutagenic activities of boiled soybeans, traditional Korean soybean pastes, and Japanese miso were investigated and compared.

Materials and Methods

Materials Soybean paste (200 g) was extracted twice with 600 mL methanol for 12 hr, filtered with a 0.45-μm membrane filter, concentrated, and freeze-dried to obtain the hydrophobic materials. The obtained hydrophobic materials were then dissolved in 200 mg dimethyl sulfoxide (DMSO), filtered through a 0.22-μm membrane filter, and used for the antimutagenic test.

Antimutagenic activity test The antimutagenic activity test was conducted according to Yahagi's preincubation method (Fig. 1), a modification of Ames' method (14-16) using *Salmonella typhimurium* TA98 (frame shift and histidine auxotrophic mutants) and *S. typhimurium* TA100 (basepair substitution and histidine auxotrophic mutants) in S9 mix (Aroclor-1254-induced rat liver S9 20 μL, $MgCl_2$-KCl salts 10 μL, 0.1 M NADP 20 μL, 1 M glucose-6-phosphate 2.5 μL, 0.2 M phosphate buffer 250 μL, sterile distilled water 197.5 μL).

Test strains were obtained from KCTC as histidine auxotrophic mutants. The strains were used for antimutagenic activity test after confirming the genotype histidine requirements, deep rough character, UV sensitivity, and R factor existence prior to the experiment. Five hundred microliters of S9 mix and 100 μL of cultivated strain (1~2 $\times 10^9$ cell/mL) for 24 hr at 37°C in the nutrient broth, and 50 μL of the soybean paste extract dissolved in DMSO was mixed in a cap tube, lightly vortexed, and pre-cultured at 37°C for 20 min. Two mL of top agar (agar 0.6%, NaCl 0.5%) kept at 45°C exposed 0.5 mM L-histidine/biotin

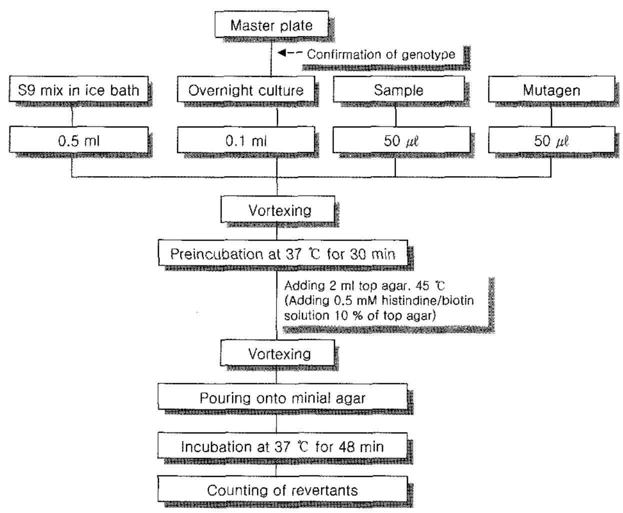

Fig. 1. A scheme of antimutagenicity test by using Ames preincubation method.

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solution beforehand were poured into each tube, vortexed for 3 s., poured into a minimal glucose agar plate (distilled water 1 L, $MgSO_4 \cdot 7H_2O$ 0.2 g, citric acid monohydrate 2 g, K_2HPO_4 10 g, $NaHNH_4(PO_4) \cdot H_2O$ 3.5 g, agar 15 g, 50% glucose 40 μL), and incubated for 48 hr at 37°C. The inhibition rate yielded a reverse mutation number as follows.

$$\text{Inhibition rate (\%)} = \frac{a-b}{a-c} \times 100$$

a : The number of reverse mutations in each case that exists with only a single mutagen
b : The number of reverse mutations when mutagens and samples are added
c : The number of spontaneous reverse mutations

Results and Discussion

The weights of the hydrophobic materials abstracted from soybean pastes are shown in Table 1.

Antimutagenic activities of 200 μg methanol extracts of the soybean pastes on AFB1 in *Salmonella typhimurium* TA98 and TA100 are shown in Fig. 2. (redundant-all shown in figure) Antimutagenic activities of soybean pastes made by *Bacillus* wild type strains were the highest, among which SSA3 was highest at 97.1%, followed by traditional Korean soybean paste, Japanese Miso, soybean pastes made by gene-manipulated strains, and boiled soybean. These results are similar to those of Park *et al.* (17), who reported that antimutagenic activities on AFB1 of 50% soybean paste extract were 100%, increasing in the following order: boiled soybeans, unboiled soybeans, and soybean paste. Hong *et al.* (18) reported that antimutagenic activity of *Doenjang* was 65%, higher than those of *Kochujang and Ganjang.* Among the substances in soybean paste, the antimutagenic activity of linoleic acid was confirmed by an Ames test, SOS chromotest, and a rec assay system (17, 19). The antimutagenic activity of traditional Korean soybean paste fermented by mold and

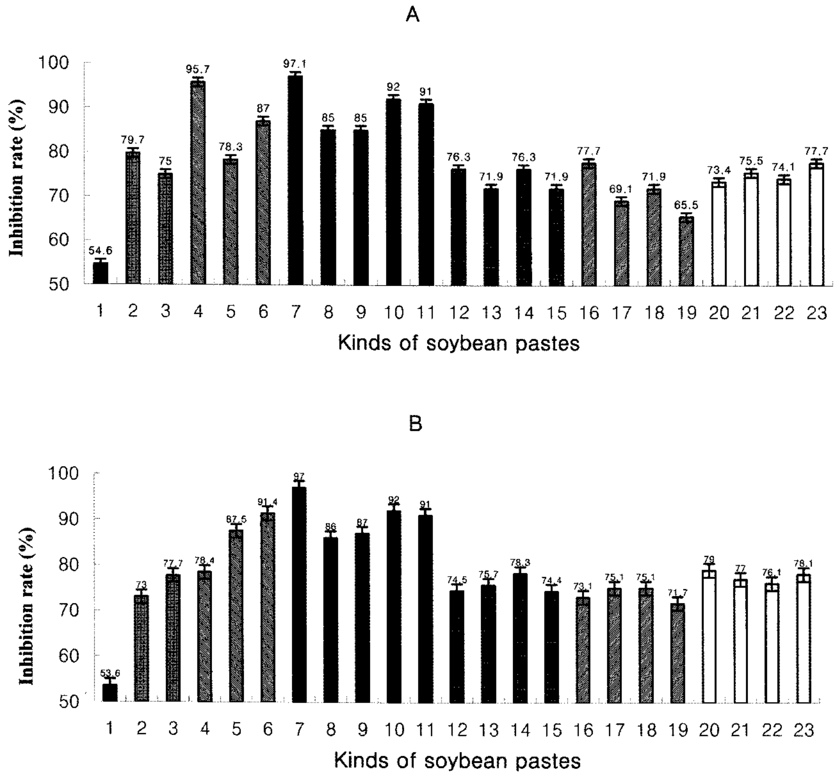

Fig. 2. Antimutagenic effects of various soybean pastes on the mutagenesis induced by aflatoxin B_1. Antimutagenic activity is expressed as inhibition ratio(%) of His^+ reversion of *Salmonella typhimurium* TA98 (A) and *Salmonella typhimurium* TA100 (B). The Values are mean±SD (standard deviation) of three experiments. The mean is significantly different from the control ($p<0.05$) using the students t-test with n=3.

No.	1	2, 3	4~6	7~11	12~15	16~19	20~23
soybean pastes	cooked soybeans	Japanese Miso	homemade soybean pastes	soybean pastes fermented by wild type *Bacillus* species	soybean pastes fermented by Mutants	soybean pastes fermented by transformants	soybean pastes fermented by cell fusants.
strains				7: *Bacillus* sp. SSA3 8: *Bacillus* sp. SS9 9: *Bacillus* sp. PM3 10:*Bacillus* sp. TKSP21 11:*Bacillus* sp. TKSP24	12: SSA3-2M1 13: PM3-M2 14: TKSP21-M8 15: TKSP24-M4	16: SSA3-NPTF1 17: SS9-NPTF1 18: PM3-NPTF2 19: SSA3-2M1-NPTF1	20: PTK324-F6 21: PTK324-F12 22: PTK324-F13 24: PTK324-F15

Table 1. Amount of methanol extracts of various soybean pastes

Sample No.	Kinds of Soybean pastes	Weight of the freezing dried extracts (g)*
1	Cooked soybean	13.3
2	Japanese Gujumac Miso	16.0
3	Japanese Suntae Miso	16.9
4	Traditional Korean soybean paste I	14.1
5	Traditional Korean soybean paste II	14.7
6	Traditional Korean soybean paste III	12.0
7	*Bacillus* sp. SSA3	10.4
8	*Bacillus* sp. SS9	11.3
9	*Bacillus* sp. PM3	13.6
10	*Bacillus* sp. TKSP21	12.4
11	*Bacillus* sp. TKSP24	13.1
12	*Bacillus* sp. SSA3-2M1	11.2
13	*Bacillus* sp. PM3-M2	13.5
14	*Bacillus* sp. TKSP21-M8	12.9
15	*Bacillus* sp. TKSP24-M4	9.5
16	*Bacillus* sp. SSA3-NPTF1	10.5
17	*Bacillus* sp. SS9-NPTF1	10.3
18	*Bacillus* sp. PM3-NPTF2	11.5
19	*Bacillus* sp. SSA3-2M1-NPTF1	14.8
20	*Bacillus* sp. PTK324-F6	13.7
21	*Bacillus* sp. PTK324-F13	13.8
22	*Bacillus* sp. TK2124-F12	11.0
23	*Bacillus* sp. TK2124-F15	14.7

*gram per 100 g of soybean paste

bacteria such as *Bacillus* species was higher than that of Japanese Miso fermented by only mold. In addition, the antimutagenic activity of soybean paste fermented with wild type strain of *Bacillus* species was similar to or higher than that of traditional Korean soybean paste. In particular, soybean pastes fermented with strains bred from wild type strains of *Bacillus* sp. showed 18 to 22% lower activities than those of soybean pastes made by wild type strains. This is the study to report that the antimutagenic activities of soybean pastes fermented by wild type strains were higher than those of the soybean pastes fermented by mutant, transformant, and cell fusant strains. Further study will be performed on the causative agents of the antimutagenic activity, and the effects of breeding strains on the activity.

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