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Mutagenicity and antimutagenic effect of soymilk fermented with lactic acid bacteria and bifidobacteria

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Abstract

In this study, soymilk was first fermented with lactic acid bacteria (*Streptococcus thermophilus*, *Lactobacillus acidophilus*) and bifidobacteria (*Bifidobacterium infantis*, *Bifidobacterium longum*) both individually and simultaneously. Mutagenicity and the suppression of fermented soymilk against the mutagenesis induced by 4-nitroquinoline-*N*-oxide (4-NQO), a direct-acting mutagen, and 3,2′-dimethyl-4-amino-biphenyl (DMAB), an indirect-acting mutagen, on *Salmonella typhimurium* TA 100, was then investigated.

It was found that the fermented soymilk shows no mutagenic activity on *Sal. typhimurium* TA 100. Fermentation, in general, significantly ($p<0.05$) enhanced the antimutagenicity of soymilk. The levels of increased antimutagenicity of fermented soymilk varied with the starter organism and the type of mutagen tested. Although unfermented soymilk exerted lower antimutagenic activity against DMAB than 4-NQO, the fermented soymilk, generally, showed a higher antimutagenic activity against DMAB than 4-NQO. Among the various fermented soymilk tested, soymilk fermented with both *Str. themophilus* and *B. infantis* simultaneously exhibited the highest antimutagenicity of 85.07% and 85.78%, respectively, against 4-NQO and DMAB Further investigation on this fermented soymilk revealed that both the antimutagenic factors formed during fermentation and the cells of the starter organisms contributed to the increased antimutagenic activity against DMAB, while the former led to the increased activity against 4-NQO.

Keywords: Antimutagenicity; Fermented soymilk; Lactic acid bacteria; Bifidobacteria

1. Introduction

Various researchers have suggested that the lower rate of prostate, breast and colon cancer recorded in Asian countries results from the daily consumption of soy-based foods containing antimutagenic factors (Setchell et al., 1984; Adlercreutz, 1990). A diet rich in antimutagens is thus believed to reduce the incidence of cancer in humans (Kim et al., 2000; Roy et al., 2002). Building on this research, food scientists and nutritionists have expended considerable effort in identifying and developing food ingredients containing antimutagenic factors that can reduce the rate of mutation and the corresponding incidence of cancer.

Several food ingredients have been identified to possess antimutagenic properties. Lactic acid bacteria and bifidobacteria, for example, are active ingredients in probiotic food such as bioyoghurt, dietary adjuncts and health-related products (Arunachalam, 1999; Gomes and Malcata, 1999). Lactic acid bacteria and bifidobacteria have been shown to exhibit antimutagenic activities against heterocylic-amines, *N*-nitroso compounds, benzo[*a*]pyrene and aflatoxin B (Lankaputhra and Shah, 1998; Sreekumar and Hosono, 1998a; Lo et al., 2002). In addition, milk fermented with lactic acid bacteria has also been reported to exert antimutagenic or anticarcinogenic activity (Bodana and Rao, 1990; Nadathur et al., 1995; Bakalinsky et al., 1996). Similarly, components of soybean such as isoflavones, trypsin inhibitor, saponin, and phytic acid, have been found to exhibit antimutagenic and antitumorial activities (Yavelow et al., 1983; Weed et al., 1985; Jing and Waxman, 1995; Rao and Sung, 1995). Researchers have also demonstrated that fermented soybeans exhibit enhanced antimutagenic activity (Kim et al., 2000; Park et al., 2003; Lin, 2004).

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Soymilk, a traditional oriental food beverage, is a water extract of soybean that provides a rich yet inexpensive supply of protein and calories (Bressani and Elias, 1968). To develop a probiotic dietary adjunct, we have previously conducted a series of studies on the fermentation of soymilk with the probiotic culture of lactic acid bacteria and bifidobacteria (Wang et al., 2002, 2003, 2006; Chien et al., in press). Compared with unfermented soymilk, we found that fermented soymilk contained both probiotic bacteria and a reduction in its stachyose and raffinose content (Wang et al., 2003), in addition to enhanced antioxidative activity (Wang et al., 2006). Furthermore, the fermented soymilk's content of the bioactive isoflavone aglycone, was significantly higher than its unfermented counterpart (Chien et al., in press). Besides, Abd El-Gawad et al. (2004) reported that soya yoghurt containing bifidobacteria inhibited the proliferation of Ehrlich ascites tumor cells. While Ohta et al. (2000) observed that administration of *Bifidobacterium breve*-fermented soymilk inhibited the development of mammary tumors induced by 2-amino-1methyl-6-phenylimadaxo[4,5*b*] pyridine in female Sprague–Dawley rats.

In the present study, we examined the mutagenicity and the suppression effect of fermented soymilk against mutagenesis induced by 4-nitroquinoline-*N*-oxide (4-NQO) and 3, 2′-dimethyl-4-amino-biphenyl (DMAB), an experimental indirect colon carcinogen. Additionally, changes of antimutagenic activity during the fermentation process and the role of the cells of starter organism and other possible antimutagenicity factors formed due to the fermentation process were also investigated.

2. Materials and methods

2.1. Bacterial strains and chemicals

Salmonella typhimurium TA 100, *Bifidobacterium infantis* CCRC 14633, *Lactobacillus acidophilus* CCRC 14079 and *Streptococcus thermophilus* CCRC 14085 were obtained from Culture Collection and Research Center, Hsinchu, Taiwan. *Bifidobacterium longum* B6 was obtained from Professor M. Y. Lin, Dept. of Food Science, National Chung-Shing University, Taichung, Taiwan.

Tests of histidine requirement, *rfa* mutation, *uvr*B mutation and R-factor were performed to confirm the genotypes of *Sal. typhimurium* TA 100. Prior to each mutagenicity and antimutagenicity, *Sal. typhimurium* TA 100 was grown in fresh Oxoid nutrient broth No.2 (Oxoid, Basingstoke, Hampshire, England) at 37 °C overnight (Maron and Ames, 1983).

A direct-acting mutagen, 4-NQO, and an indirect-acting mutagen, DMAB, were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). An S9 microsomal fraction (S9 mix) of rat liver was obtained from ICN Pharmaceuticals, Inc. (Chillicothe, Aurora, Ohio, USA).

2.2. Preparation of fermented soymilk

Fermented soymilk was prepared with lactic acid bacteria (*L. acidophilus* CCRC 14079 or *Str. thermophilus* CCRC 14085) and bifidobacteria (*B. infantis* CCRC 14633 or *B. longum* B6) alone and simultaneously. Fermentation was conducted at 37 °C for 32 h. Detailed procedures for the preparation of fermented soymilk were described in our previous report (Wang et al., 2002).

2.3. Simulation of fermentation

This experiment was conducted to determine whether or not antimutagenicity could be obtained by simply adding lactic acid bacteria and bifidobacteria to soymilk without subsequent fermentation. To perform this experiment, *Str. thermophilus* CCRC 14085 and *B. infantis* CCRC 14633 were grown in *Lactobacilli* MRS broth (Difco Laboratories, Sparks, MD, USA) at 37 °C for 32 h. Bacterial cells were harvested and washed by centrifugation (2250 ×*g*, 10 min) and then re-suspended in phosphate buffer solution (PBS, 0.2 M, pH 7.4) and diluted with PBS to a viable population of ca. 8.5 log cfu/ml. An aliquot of 1.0 ml each of the cell suspension of *Str. thermophilus* and *B. infantis* was added to 10.0 ml soymilk to give a final population equivalent to the fermented soymilk prepared with *Str. thermophilus* and *B. infantis* simultaneously. These samples were then immediately subjected to antimutagenicity assay without further fermentation.

2.4. Assay for antimutagenic and mutagenic effect

Two mutagens, 4-NQO and DMAB which requires liver microsomal activation were used in the present study. Both mutagens were dissolved in dimethylsulfoxide (DMSO, Wako Pure Chemical Industries, Ltd., Osaka, Japan) at a concentration of 1.0 and 80 μg/ml, for 4-NQO and DMAB, respectively.

The pre-incubation method of Maron and Ames (1983) with minor modification was employed to study the antimutagenic effect of fermented soymilk. In brief, a 10–11 h culture of *Sal. typhimurium* TA 100 (0.1 ml) in a tube was added with either fermented or unfermented soymilk (0.1 ml), PBS (0.7 ml) and mutagen dissolved in DMSO (0.1 ml). For DMAB, 0.2 ml S9 mix and 0.5 ml PBS instead of 0.7 ml PBS were added to the culture. The final concentration of mutagen was 0.1 μg/ml for 4-NQO and 8.0 μg/ml for DMAB. Following 20 min of incubation at 37 °C in a rotary shaker, 2.0 ml top agar was mixed with the tube content. The tubes were vortexed and poured onto minimal glucose agar plates and colonies were counted after 48 h of incubation at 37 °C.

Mutagenesis of soymilk and fermented soymilk was examined under the condition described for antimutagenicity testing except without the addition of mutagen.

The dosage of soymilk and fermented soymilk (0.1 ml) tested was shown to exert neither toxic effect on nor support the development of histidine-required *Sal. typhimurium* TA 100 colonies on minimal glucose agar plate in the preliminary study. Each assay was performed in triplicate, and antimutagenic activity was expressed as a percentage of mutagenic inhibition:

$$\text{Inhibition } (\%) = 1-[(A-E)/(B-D)] \times 100$$

Where A and B are numbers of mutagen-induced revertants in the presence and absence of sample, respectively. D and E are numbers of spontaneous revertants observed in the sample and DMSO control, respectively.