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Antimutagenic Effect of Polysaccharides Extracted from Soybeans Fermented with Basidiomycetes on 2-Amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx)

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Abstract The antimutagenic activity of polysaccharides extracted from soybeans fermented with *Agrocybe cylindracea* (AC) or *Phellinus igniarius* (PI) against 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx) was examined using a *Salmonella*/Ames test and host-mediated assay in mice. The polysaccharides from the soybeans fermented with *A. cylindracea* and *P. igniarius* inhibited the mutagenic activity of the cooked food mutagen, MeIQx, by 31.2% and 35.3%, respectively. The polysaccharides also inhibited MeIQx genotoxicity in a dose-dependent manner in mice. These results suggest that the polysaccharides from soybeans fermented with *A. cylindracea* or *P. igniarius* exhibit antimutagenic properties against MeIQx *in vitro* and *in vivo*.

Key words: Antimutagenicity, *Salmonella*/Ames test, host-mediated assay, 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx)

It has been suggested that the use of antimutagens and anticarcinogens in daily life is the most effective way to prevent genetic diseases and human cancer [13]. The majority of antimutagenic agents are natural compounds such as ascorbic acid [14], chlorophyll, tocopherol, ellagic acid, taxol [27], aburatubolactam [6], and vitamin A. A number of studies have demonstrated the antimutagenic, anticarcinogenic, or immunomodulating activities of basidiomycetes mushrooms [19, 28]. Gruter *et al.* [11, 12] identified the antimutagenic effects of the ethanol extracts of *Craterellus cornucopioides* and other mushrooms, and Osaki *et al.* [23] showed that the fruit body of *Agaricus blazei* has an antimutagenic effect in an Ames/*Salmonella* assay. Shon *et al.* [24, 25] also reported the antimutagenic activities of the cultured broth, mycelia, and fruit body of *Phellinus igniarius* and the cultured broth of *Phellinus linteus*.

The efficacy of medicinal mushrooms against cancer is already known in China, Russia, Japan, and Korea, as well as in the United States and Canada. Some species of edible higher basidiomycetes show a marked ability to inhibit the growth of different kinds of tumors [17]. However, some medicinal mushrooms can not be cultivated artificially, and the cost of the isolation and purification of useful materials in a liquid mass culture is expensive. Accordingly, it is necessary to develop a method of fermentation using solid materials (e.g. soybean or various cereals) for culturing the mycelia of medicinal mushrooms.

The current authors previously established a method for the solid-state fermentation of basidiomycetes and demonstrated the antimutagenic activities of polysaccharides, from soybeans fermented with basidiomycetes, against both directly-acting and indirectly-acting mutagens [18] and the enhancement of phase II and antioxidant enzymes in mice [26]. In this report, the antimutagenic effects of polysaccharides extracted from soybeans fermented with basidiomycetes on MeIQx are further investigated in an *in vitro* experiment using *S. typhimurium* TA 98 and a host-mediated assay in mice.

The preparation of the inoculum, fermentation of the soybeans with basidiomycetes, and extraction of the polysaccharides from soybeans fermented with basidiomycetes were all performed as described previously [18]. The inhibitory effect of the polysaccharides from the soybeans fermented with *A. cylindracea* or *P. igniarius* on the mutagenic activity of MeIQx (5 ng per plate), using the tester strain *S. typhimurium* TA 98, was examined in a

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Table 1. Inhibitory effect of polysaccharides extracted from soybeans fermented with *A. cylindracea* or *P. igniarius* on MeIQx-induced mutagenesis in *S. typhimurium* TA 98.

Samples	Dosage per plate	Revertants per plate	Inhibition of mutagenic activity (%)
MeIQx	5 ng	266±27.1	
Polysaccharides from soybeans fermented with *A. cylindracea*	0.1 mg	230±22.7*	13.5
	1 mg	205±14.2*	22.9
	2 mg	183±19.2*	31.2
Polysaccharides from soybeans fermented with *P. igniarius*	0.1 mg	226±22.7	15.0
	1 mg	197±17.0*	25.9
	2 mg	172±7.6**	35.3

Values are mean±SD (standard deviation) of three experiments. The mean is significantly different from the control (* $p<0.05$, ** $p<0.01$) using the student's t-test with n=3.

preincubation assay, as outlined by Maron and Ames [20]. The number of histidine revertants induced by MeIQx tested without any extract (control) was given as 100%. The percentage of revertants remaining in the samples was then calculated accordingly. Based on the results in Table 1, at a concentration of 2 mg, the polysaccharides from the soybeans fermented with *A. cylindracea* and *P. igniarius* were inhibited by 31.2% and 35.3%, respectively. These results show that the polysaccharides inhibited the mutagenic activity of the cooked food mutagen, MeIQx, in a concentration-dependent manner. This is in accordance with certain previous studies, where solvent extracts from fruit and vegetable residues [9] and dichlorostearic acid [30] inhibited MeIQx-induced mutagenicity using *S. typhimurium* TA 98. Accordingly, the present study suggests that polysaccharides extracted from soybeans fermented with *A. cylindracea* or *P. igniarius* may possess antimutagenic components which can reduce the mutagenic activity induced by MeIQx.

The exact mechanisms by which the polysaccharides from soybeans fermented with basidiomycetes exert their antimutagenic effects are unknown, with respect to heterocyclic aromatic amines from cooked food. However, the polysaccharides would appear to inhibit MeIQx conversion to a bacterial mutagen in a complex manner. The type of inhibition would also seem to depend on the inhibitor concentration, being competitive at a low or mixed concentration and noncompetitive at an elevated concentration. This indicates interactions with certain components of the microsomal enzyme system, especially in relation to the activation of MeIQx. Sousa *et al.* [29] found a concentration-dependent inhibition mechanism of mixed function oxidase using quercetin, an inhibitor of ethoxyresorufin metabolism. However, with aflatoxin B_1, a competitive concentration-independent inhibition mechanism was reported for fisetin, kaempferol, and rutin and a noncompetitive one for morin [10]. From these results, it can be concluded that multifactorial inhibition takes place with competition between the polysaccharides and the substrate as one mechanism. The inhibition of NADPH-cytochrome oxidase may also be involved.

A host-mediated assay was performed, essentially using a modification of the intrasanguinous host-mediated assay of Arni *et al.* [5]. The genotoxicity of the mutagen in the livers of mice was measured by detecting the revertants of *S. typhimurium* that had previously been administered by a tail vein injection. In a preliminary study, Benzo[a]pyrene at doses of 50–200 mg/kg body weight was not found to be mutagenic in a host-mediated assay, presumably because of high levels of detoxifying enzymes, such as epoxide hydratase, in the liver (results not shown). Treatment with MeIQx (4.5 mg/kg body wt) led to a genotoxic response in the livers of the control mice. The animals that received the polysaccharides of soybeans fermented with *A. cylindracea* (intragastric application at doses of 0.1, 0.5, or 1.0 g/kg body wt for 14 consecutive days) exhibited a significantly ($p<0.05$ or $p<0.001$) lower response when challenged with MeIQx than the control mice (Fig. 1). Furthermore, those

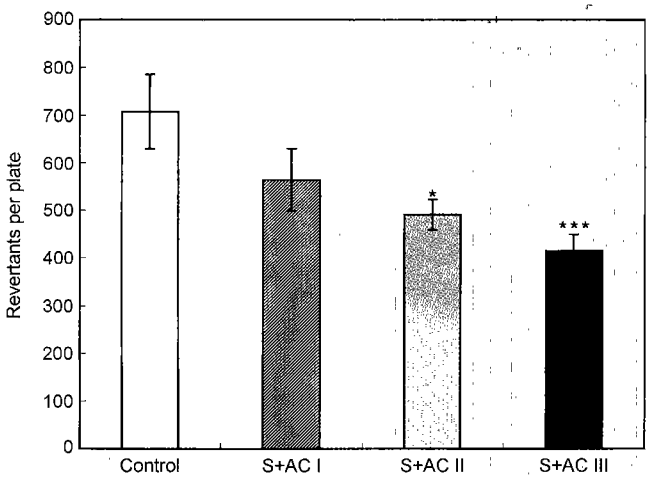

Fig. 1. Antimutagenic activity of polysaccharides extracted from soybeans fermented with *A. cylindracea* (S+AC) in a host-mediated assay.

The values are mean±SD (standard deviation). S+AC I, II, and III represent 100, 500, and 1,000 mg/kg body weight of extract from soybeans fermented with *A. cylindracea*, respectively. The mean is significantly different from the control (* $p<0.05$, *** $p<0.001$) using the student's t-test with n=3.

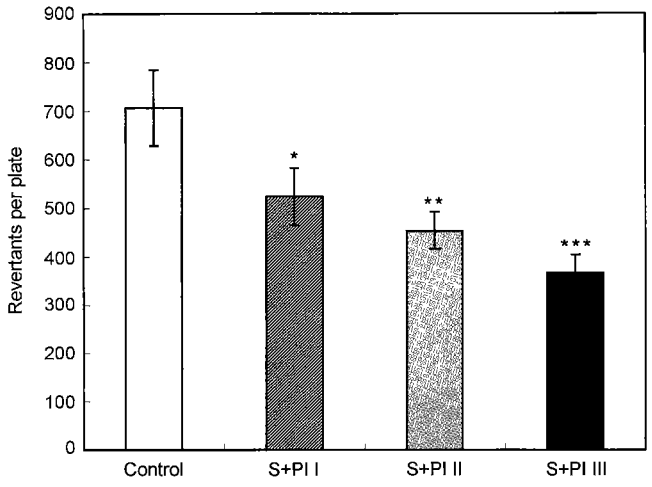

Fig. 2. Antimutagenic activity of polysaccharides extracted from soybeans fermented with *P. igniarius* (S+PI) in a host-mediated assay.

The values are mean±SD (standard deviation). S+PI I, II, and III represent 100, 500, and 1,000 mg/kg body weight of extract from soybeans fermented with *P. igniarius*, respectively. The mean is significantly different from the control (* $p<0.05$, ** $p<0.01$, *** $p<0.001$) using the student's t-test with n=3.

mice that received the polysaccharides of soybeans fermented with *P. igniarius* at doses of 0.1, 0.5, or 1.0 g/kg body wt for 14 consecutive days exhibited a significant reduction in the *in vivo* genotoxicity of MeIQx (Fig. 2). These results are consistant with those of Aldrick *et al.* [4] who reported that the consumption of caffeine led to a 47% reduction in the number of mutants induced by MeIQx in a host-mediated assay.

When proteinaceous foods such as meat and fish are cooked, a family of heterocyclic aromatic amines is produced [8]. These amines are highly mutagenic in a *Salmonella/* reversion assay. They also induce DNA damage in mammalian cells [1] and are potent multi-organ carcinogens in rodents [22]. One of the most commonly occurring heterocyclic amines is 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx), which was originally found in fried beef. MeIQx is mutagenic in both bacterial and mammalian cell mutation assays and carcinogenic in rodents [15]. Long-term feeding studies using mice and rats resulted in a high incidence of lung and liver tumors [16, 21]. The genotoxicity and carcinogenicity of MeIQx is believed to result from the metabolic conversion of MeIQx to electrophilic intermediates that can bind to DNA. The major activation step is N-hydroxylation catalyzed by cytochrome p4501A2 followed by the conjugation of the N-hydroxy moiety to any one of several leading groups, such as acetate or sulfate.

Dietary components such as fat, fiber, and plant flavonoids have been found to modify either the genotoxicity of heterocyclic amines by altering their uptake from the gut lumen, or their metabolism [2, 7]. The oral administration of MeIQx leads to its binding to the DNA of major organs, with binding to liver DNA being of a similar order to that of 2-acetylaminofluorene [3]. The biochemical basis of these toxicological effects lies in the metabolic conversion of MeIQx to electrophilic intermediates that can bind to DNA. Thus, it is possible that the polysaccharides from soybeans fermented with *A. cylindracea* or *P. igniarius* can modify MeIQx mutagenicity by altering the enzymes and cofactors in the liver responsible for its activation. Overall, the current results suggest that the polysaccharides extracted from soybeans fermented with *A. cylindracea* or *P. igniarius* may have an inhibitory effect on the activation of MeIQx to genotoxic metabolites. Additional studies are required to determine the effects of polysaccharides from soybeans fermented with basidiomycetes on various components of the microsomal enzyme system in order to elucidate the inhibition mechanisms more precisely.

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