The Journal of Microbiology, September 1999, p. 136-140 Vol. 37, No. 3


# Antimutagenicity of *Phellinus linteus* in *Salmonella typhimurium*

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(Received July 31, 1999 / Accepted August 27, 1999)

**The mutagenicities and antimutagenicities of butanol (PL I) and water (PL II) extracts from the filtrate of the cultured broth of *Phellinus linteus* were examined using the Ames/*Salmonella* test. No mutagenic activity of PL I and PL II was found in *Salmonella typhimurium* strains TA98 and TA100, either with or without S9 activation. In contrast, PL I and PL II showed inhibitory effects on the mutagenic activities induced by the directly-acting mutagens, 4-nitro-*o*-phenylenediamine (NPD) using the tester strain TA 98 and sodium azide ($NaN_3$) using the tester strain TA 100 in the absence of S9 mix. PL I and PL II also showed inhibitory effects on the mutagenicities of the indirectly-acting mutagens, 2-aminofluorene (2-AF) using the tester strain TA 98 and benzo[a]pyrene (B[a]P) using the tester strain TA 100 in the presence of S9. These results suggest that *P. linteus* has an antimutagenic activity and may play a role in the prevention of cancer.**

**Key words: Mutagenicity, antimutagenicity, *Phellinus linteus*, *Salmonella typhimurium***

The short-term tests including the Ames test, which are being used to evaluate the genotoxic potential of chemicals, have been helpful in recognizing the carcinogenic potential of several environmental chemicals (1). Since it is difficult to remove a particular chemical from atmosphere, much attention has recently been devoted to antimutagenic factors that could act to decrease the mutagen rate either by inactivating the mutagens or interfering in the process of mutagenesis. Antimutagenic substances can prevent cancer because they are able to either destroy mutagens in or out of body cells or block mutagens which damage DNA and cause mutations in cells (6). The search for compounds possessing antimutagenic properties is warranted not only in view of eventual practical applications, but also because of the possibilities in unraveling some mechanisms of the mutagenesis.

There are many studies associated with the antimutagenic or anticarcinogenic activity of basidiomycetes mushrooms (2, 7, 16, 17). It is known that water-soluble extracts of some basidiomycetes (7), polysaccharides from *Lentinus edodes* (2), and polysaccharopeptide isolated from *Coriolus versicolor* (9) exhibit antitumor activies in allogenic and syngeneic tumor systems. Gruter *et al.* (4) also reported on the antimutagenic effects of ethanol extracts of *Craterellus cornucopioides* and other mushrooms.

The present report describes the mutagenic activity of *P. linteus* and its antimutagenic property with directly-acting mutagens [4-nitro-*o*-phenylenediamine (NPD), sodium azide ($NaN_3$)] and indirectly-acting mutagens {2-aminofluorene (2-AF) and benzo[a]pyrene (B[a]P)} using the *S. typhimurium* tester strains TA 98 and TA 100.

## Materials and Methods

### Materials

Rats were supplied by the Daehan Laboratory Animal Research Center Co. (Eumsung, Korea). *S. typhimurium* strains TA98 and TA100 were obtained from Korean Collection for Type Cultures (Taejeon, Korea). Bactor agar, malt extract, and yeast extract were purchased from Difco Laboratories (Detroit, USA). All other reagents were purchased from Sigma Chemical

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Co. (St. Louis, USA).

### Preparation of S9 mixture

Rats (male Sprague-Dawley) received a combined intraperitoneal injection of phenobarbitad and β-naphthoflavone (12). The treated rats were starved 12 hr before decapitation, then their livers were aseptically removed, minced, and homogenized in three volumes of 0.15 M KCl. The liver homogenate was centrifuged at 9000 × g for 20 min, and the supernatant (S9 fraction) was stored as aliquots at −80°C. The S9 mixture was then prepared according to the method of Maron and Ames (11). Briefly, 0.1 M NADP, 1 M glucose-6-phosphate, $MgCl_2$-KCl salts, and 0.2 M of sodium phosphate buffer (pH 7.4) were mixed with the S9 fraction.

### Bacterial tester strains

*S. typhimurium* strains TA98 and TA100 were tested for presence of the ampicillin resistance factor (R-factor) and cultured for 13~14 hr before use. The *Salmonella* strain TA 98 can detect mutagens that cause a frameshift in a G-C base pair region. The strain TA 100 can detect mutagens that cause a base-pair substitution in a G-C pair.

### Preparation of samples

The *P. linteus* strain was grown for 13 days at 28°C with MYG (malt extract: yeast extract: glucose=1:0.4: 0.4) media in a shaking incubator (Hanback Scientific Co., HB201S, Korea). The cultured broth was fractionated into filtrate and mycelia by filtration. The filtrate of the cultured broth was sequentially extracted with hexane, chloroform, butanol, ethylacetate and water, and each extract was lyophilized. The lyophilizates of butanol (PL I) and water (PL II) extracts were dissolved in DMSO and then filtered through a 0.22 μm sterile membrane filter disc before being subjected to mutagenicity and antimutagenicity testings.

### Mutagenicity assay

The *Salmonella* mutagenicity tests were performed essentially as described by Maron and Ames (11). PL I and PL II at concentrations of 0.02, 0.1, 0.5, and 1 mg in 100 μl of DMSO were studied with *S. typhimurium* tester strain TA98 or TA100 with or without the addition of 0.5 ml of the S9 mixture. The positive control plates for TA98 contained NPD and 2-AF. The positive control plates for TA100 contained $NaN_3$ and B[a]P. The plates for the negative control also contained 100 μl of DMSO both with and without the S9 mixture. The colonies were counted manually using a colony counter (Suntex Korea Company, Model 560). The presence of background lawn on all plates was confirmed. All samples were tested on duplicate plates, and the experiments were repeated three times. A sample was considered mutagenic when the observed number of colonies was at least 2-fold over the spontaneous level.

### Antimutagenicity assay

The plate incorporation assay as outlined by Maron and Ames (11) was used. A brief outline of the protocol is: to 2 ml of top agar containing 0.5 mM histidine/biotin, 0.1 ml of fresh overnight grown *Salmonella* culture of tester strains (TA98 or TA100), 0.1 ml of NPD (10) or $NaN_3$ (1), and 0.1 ml of samples were added and mixed thoroughly. After pouring on minimal glucose agar plates, the plates were tilted and rotated immediately to ensure uniform spreading of top agar. In order to unravel the impact of the samples on S-9 dependent mutagens (2-AF or B[a]P), the antimutagenicity tests consisted of combining 0.1 ml of a fresh overnight culture of the tester strain, 0.1 ml of the test samples, 0.1 ml of mutagens (5 μg of 2-AF or B[a]P), and 0.5 ml of S9 mix in soft agar, which was poured onto a minimal agar plate. The plates were placed in an incubator at 37°C for 48 hr. Revertant colonies were counted to determine the inhibitory effects, expressed as an inhibition rate. The number of histidine revertants induced by NPD, $NaN_3$, 2-AF, and B[a]P tested without any extract was given as a 100%. The percentage of revertants remaining in the samples was then calculated accordingly.

## Results and Discussion

### Mutagenic activity of *P. linteus*

No mutagenic activity of PL I and PL II was

**Table 1.** Mutagenicity testings of the butanol (PL I) and water (PL II) extracts from the filtrate of the cultured broth of *P. linteus*

| Samples | Concentration (μg) | Histidine revertants per plate | | | |
|---|---|---|---|---|---|
| | | −S9 mix | | +S9 mix | |
| | | TA98 | TA100 | TA98 | TA100 |
| DMSO | 0.1 ml | 19±0.9 | 91±0.7 | 20±1.0 | 86±5.9 |
| NPD | 10 | 363±23.6 | – | – | – |
| $NaN_3$ | 1 | – | 548±36 3 | – | – |
| 2-AF | 5 | – | – | 311±29 5 | – |
| B[a]P | 5 | – | – | – | 167±13.1 |
| PL I | 1000 | 20±1.1 | 82±6.9 | 18±1.2 | 88±4.2 |
| | 500 | 17±1.0 | 84±7.0 | 22±2.1 | 89±5.9 |
| | 100 | 12±0 8 | 60±5.4 | 16±1.8 | 85±4.9 |
| | 20 | 14±1.1 | 73±6.1 | 20±2.2 | 79±6.7 |
| PL II | 1000 | 22±1.8 | 100±7.8 | 14±1.9 | 89±6.9 |
| | 500 | 23±2.0 | 60±5.9 | 15±1.3 | 79±5.5 |
| | 100 | 15±1.1 | 79±6.1 | 7±0.4 | 85±6.7 |
| | 20 | 13±1.4 | 82±9.1 | 16±1.7 | 88±8.9 |

Values are mean ± standard deviation (SD) of three experiments.

detected using *S. typhimurium* tester strains TA98 and TA100 either with or without S9 activation (Table 1). The number of spontaneous revertants was normal, and the background growth was typical in all experiments.

**Antimutagenic activity of *P. linteus***

The effects of PL I and PL II were checked against NPD and $NaN_3$ induced histidine revertants in *S. typhimurium*. PL I and PL II possessed antimutagenic potentials against NPD (Fig. 1A). With strain TA100, PL I and PL II did inhibit the mutagenicity of $NaN_3$ (Fig. 1B). The addition of PL I caused about 46% inhibition in mutagenic activity of $NaN_3$. At 0.1 and 1 mg doses of PL II, the inhibitory effects varied from 33.5% to 48.3%. The results of the present study demonstrate that *P. linteus* contains antimutagens capable of inhibiting the mutagenicity of directly-acting mutagens such as NPD and $NaN_3$. These results are consistent with those of Gruter *et al.* (4) who identified the antimutagenic activity of ethanol extract of *C. cornucopioides* toward directly-acting mutagens, 2-nitrofluorene (2-NF) and acridine half mustard ICR-191, on *S. typhimurium* TM677. After addition of the ethanol extract of *C. cornucopioides*, the mutagenicities induced by 2-NF and ICR-191 were inhibited by about 97% and 98%, respectively. Antimutagenic properties of ethanol extracts from other edible fungi (*Agaricus abruptibulbus, Agaricus bisporus, Antharellus cibarius, Lactarius lilacinus, Lyophyllum connatum*, and *Xerocomus chrysenteron*) against 2-NF were also found (4).

The inhibitory effects of PL I and PL II on the mutagenic activities of the indirectly-acting mutagens, 2-AF (5 μg per plate) using tester strain TA 98 and B[a]P (5 μg per plate) using tester strain TA 100 along

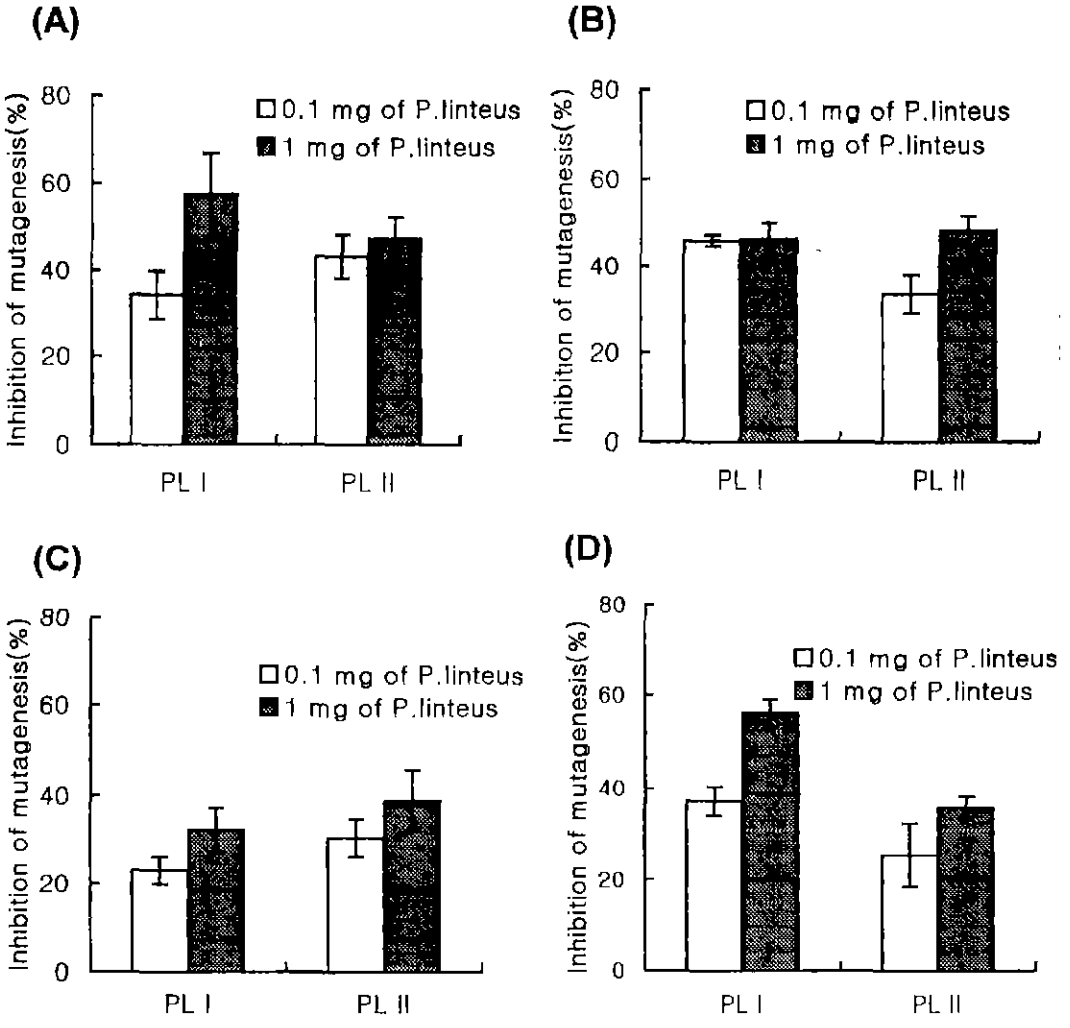


**Fig. 1.** Inhibition of the mutagenicity induced by (A) NPD in *S. typhimurium* 98, (B) $NaN_3$ in *S. typhimurium* 100, (C) 2-AF in *S. typhimurium* 98, and (D) B[a]P in *S. typhimurium* 100 by the butanol (PL I) and water (PL II) extracts from the filtrate of the cultured broth of *P. linteus*. Data are mean values (n=3), and bars indicate the standard deviations.

with the S9 mixture, were examined in plate incorporation assay. The addition of 0.1 mg of *P. linteus* inhibited the mutagenicity of 2-AF by 23.0% to 30.4%. At the concentration of 1 mg, PL I and PL II inhibited the mutagenicity of 2-AF by 32.1% and 38.8%, respectively (Fig. 1C). Gichner *et al.*, (3) found that aminobenzoic acid isomer repressed the mutagenicity of 2-AF in *S. typhimurium* strain YG1024. Approximately 70% repression of 2-AF-induced mutagenesis was observed with the addition of 20 mM aminobenzoic acid. The antimutagenic effect of aminobenzoic acid on 2-AF was probably due to the inhibition of plant cell peroxidases and bacterial acetyltransferases that are required for the activaton of 2-AF to mutagenic product(s) (3).

The effects of PL I and PL II on the B[a]P-induced mutation frequency are also presented in Fig. 1D. The addition of 0.1 mg of PL I and PL II inhibited the mutagenicity of B[a]P by 37.2% and 25.4%, respectively, and the addition of 1 mg of PL I and PL II inhibited by 56.2% and 35.7%, respectively. PL I was more effective than PL II (Fig. 1D). This is in accordance with some previous studies, where basidiomycetes mushrooms inhibited mutagenicities induced by indirectly-acting mutagens. With the addition of a heat-resistant factor in ethanol extract of *C. cornucopioides* at a concentration of 2.5 mg, the mutagenicities induced by aflatoxin B1 and B[a]P were inhibited 97% and 98%, respectively (4). B[a]P induced mutagenicity was also inhibited by ethanol extract of mushroom *Tirmania pinoyi* in a dose-dependent manner (5). Kusamran *et al.*, (8) also found that some Thai vegetables contain chemical compounds capable of inhibiting the mutagenicity of B[a]P.

The inhibiting mechanism of *P. linteus* on the mutagenicity of B[a]P is unknown. However, some natural compounds have been reported to inhibit the mutagenicities of B[a]P and smoke condensate, and the mechanism has been suggested as an interaction between antimutagen and the enzymes in the liver homogenate (8, 17). The mutagenicity of S9-activated B[a]P is due to the production of primary and secondary metabolites which are themselves directlyacting mutagens. The major primary mutagenic metabolites of B[a]P are a 4,5-oxide and a 6-phenol, with the 4,5-oxide being about 4 times more mutagenic than the 6-phenol in TA 100 (18). The primary metabolite B[a]P-7.8-oxide is further oxidized to a 7,8-diol-9,10-epoxide, which is the major secondary mutagenic metabolite of B[a]P. The diol-epoxide is at least 5 times as mutagenic as B[a]P-4,5-oxide [10, 19, 20]. Osborne and Crosby (13) also suggested that B[a]P can form a number of reactive intermediates with the activating S9 system.

It was suggested that most of the agents antimutagenic to B[a]P can be classified as blocking agents since they inhibit the conversion to ultimate carcinogens, increase the activity of detoxifying enzymes, or react directly with electrophiles (14). Many natural compounds could induce the activity of detoxifying enzymes (6). *P. linteus* may interfere with the production of primary and secondary metabolites either by blocking an enzyme system necessary for their production or favoring synthesis of metabolites that are not carcinogens.

The evidence that *P. linteus* is antimutagenic against different mutagens is significant because the ingestion of natural anticarcinogens is recognized to play a role in counteracting the unavoidable intake of carcinogenic substances present in food (15). One explanation for the activities of *P. linteus* against mutagens (NPD, $NaN_3$, 2-AF, and B[a]P) that do not necessarily share a common mode of action or activation pathway is that more than one antimutagenic factor may be present. Diverse antimutagenic factors-one acting against directly-acting mutagens and others against S9-dependent mutagens may be there. However, further studies are required to determine such factors.

## Acknowledgment

This study was supported by a Highly Advanced National (HAN) project grant from the Korean Ministry of Science and Technology.

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