J. Microbiol. Biotechnol. (1999), **9**(4), 525–528

JOURNAL OF
MICROBIOLOGY
AND
BIOTECHNOLOGY

Antimutagenic Potential of *Phellinus igniarius*

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Received: May 19, 1999

Abstract Mutagenic activities of extracts from the filtrate of the cultured broth (PI-I), mycelia (PI-II), and the fruiting bodies (PI-III) of *Phellinus igniarius* were examined by Ames/*Salmonella* tests. No mutagenic activity was found in *Salmonella typhimurium* strains TA98 and TA100, either with or without S9 activation. In contrast, PI-I, PI-II, and PI-III showed inhibitory effects on the mutagenic activities by the directly-acting mutagens, 4-nitro-*o*-phenylenediamine(NPD) and sodium azide (NaN_3), and also by the indirectly-acting mutagens, 2-aminofluorene (2-AF) and benzo[*a*]pyrene (B[*a*]P). These results suggest that *P. igniarius* possesses some antimutagenic activity and may contain some chemopreventive agents.

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

There are many antimutagenic factors that could reduce the frequency or rate of spontaneous or induced mutation by either inactivating mutagens or interfering in the process of mutagenesis. Antimutagenic substances may prevent cancer because they can either destroy mutagens in or out of body cells or block mutagens which damage DNA and cause mutations in cells. The majority of antimutagenic or anticarcinogenic agents are natural compounds such as ascorbic acid [7], tocopherol [2], taxol [13], vitamin A [3], and aburatubolactam C [1]. There are many studies associated with the antimutagenic, anticarcinogenic, or immunomodulating activities of basidiomycetes mushrooms [8, 14, 17]. TML-1 and TML-2 isolated from the mycelium of *Tricholoma mongoolicum* caused inhibition of tumor cell proliferations and possessed antitumor activities in, mice [16]. Liu *et al.* [9] reported that a polysaccharopeptide from *Coriolus versicolor* was an effective growth inhibitor of sarcoma 180 *in vivo*. Gruter *et al.* [5] also reported antimutagenic effects of ethanol extracts of *Craterellus cornucopioides* and other mushrooms. The aim of the present study was to examine the mutagenic activity of *P. igniarius* and to explore its possible 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.

The *P. igniarius* 26005 strain was obtained from the National Agricultural Science and Technology Institute. This strain was then grown for 7–10 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 fresh fruiting bodies were extracted with boiling water for 3 h at 100°C. After centrifugation, the supernatant was collected and lyophilized. The procedure used for the extraction from the mycelia and the filtrate of the cultured broth of *P. igniarius* is shown in Fig. 1.

The *Salmonella* mutagenicity tests were performed essentially as described by Maron and Ames [11]. PI-I, PI-II, and PI-III at concentrations of 0.02, 0.1, 0.5, and 1 mg in 100 μl of DMSO were tested with *S. typhimurium* tester strain TA 98 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. A sample was considered mutagenic when the observed number of colonies was at least 2-fold over the spontaneous level. After using a plate incorporation assay, no mutagenic activity of PI-I, PI-II, and PI-III was detected when investigated on *S. typhimurium* tester strains TA98 and TA100 with and without S9 activation (Table 1). These results indicate that PI-I, PI-II, and PI-III are not mutagenic in the Ames test.

The inhibitory effects of PI-I, PI-II, and PI-III on the mutagenic activities of these directly-acting mutagens, NPD (10 μg per plate) using tester strain TA 98 and NaN_3 (1 μg per plate) using tester strain TA100, were examined in a plate incorporation assay. The number of histidine

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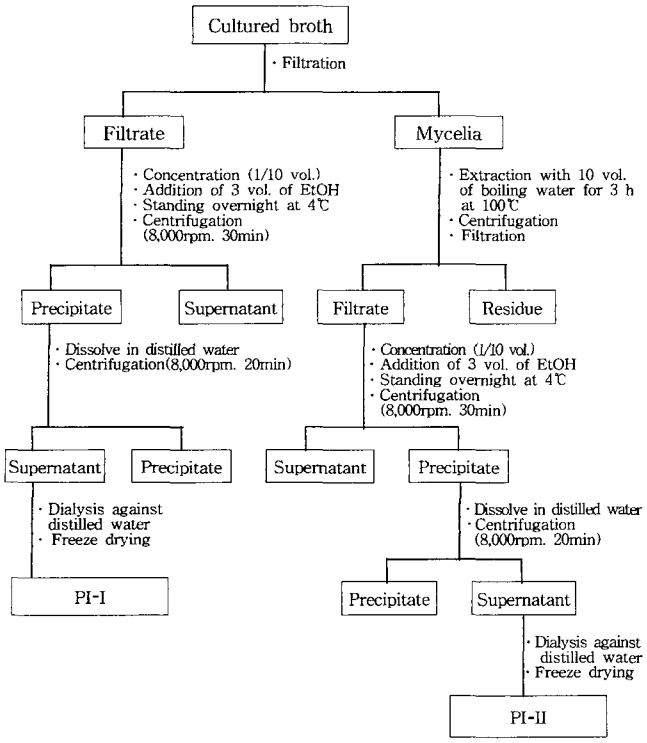

Fig. 1. Procedure for the extraction of the filtrate of the cultured broth (PI-I) and mycelia (PI-II) of *P. igniarius*; EtOH, ethanol.

Table 1. Mutagenicity testings of the extracts from the filtrate of the cultured broth (PI-I), mycelia (PI-II), and the fruiting bodies (PI-III) of *P. igniarius*.

Samples	Concentration (μg)	Histidine revertants per plate			
		-S9 mix		+S9 mix	
		TA98	TA100	TA98	TA100
DMSO	0.1	19±0.9	91±0.7	20±1.0	86±5.9
NPD	10	363±23.6	N.D.	N.D.	N.D.
NaN_3	1	N.D.	548±36.3	N.D.	N.D.
2-AF	5	N.D.	N.D.	311±29.5	N.D.
B[*a*]P	5	N.D.	N.D.	N.D.	167±13.1
PI-I	1000	22±1.9	67±4.5	27±2.1	61±5.2
	500	10±0.7	81±6.2	33±2.5	55±4.3
	100	9±0.5	38±2.6	17±1.9	86±4.3
	20	10±0.4	61±5.3	16±1.0	52±3.0
PI-II	1000	20±1.0	64±5.8	22±2.1	78±4.2
	500	19±1.1	77±6.9	28±1.6	54±3.2
	100	22±2.0	71±5.6	17±0.9	63±4.2
	20	11±0.8	60±4.2	19±1.1	60±4.7
PI-III	1000	21±1.2	67±5.6	19±2.0	62±3.6
	500	12±0.9	34±2.9	22±1.3	55±4.3
	100	18±1.4	55±5.8	25±1.9	64±3.9
	20	23±0.9	78±4.3	30±1.5	58±2.9

N.D., Not determined.
Values are mean standard deviation (SD) of three experiments.

Table 2. Antimutagenic activities of the extracts from the filtrate of the cultured broth (PI-I), mycelia (PI-II), and the fruiting bodies (PI-III) of *P. igniarius* against direct-acting and indirect-acting mutagens.

Samples	Concentration (mg)	Inhibition of induced mutagenesis (%)			
		Direct-acting mutagens		Indirect-acting mutagens	
		NPD	NaN_3	2-AF	B[*a*]P
PI-I	0.1	35.5±5.4	35.6±7.2	32.3±3.7	33.4±4.2
	1	47.2±4.3	58.0±5.4	43.8±5.3	32.7±2.6
PI-II	0.1	37.0±5.0	40.1±6.4	34.3±6.5	22.3±2.2
	1	53.3±1.4	58.8±4.7	49.1±4.6	46.8±6.2
PI-III	0.1	38.9±6.0	31.3±6.1	26.3±4.0	31.0±6.0
	1	51.5±7.1	48.1±3.1	43.9±7.7	43.0±7.1

Values are means±standard deviations (SD) of three experiments.

revertants induced by mutagens without any extract was given as 100%. The percentage of revertants remaining in the samples was then calculated accordingly. PI-I, PI-II, and PI-III possessed strong antimutagenic potential against NPD (Table 2). With strain TA100, PI-I, PI-II, and PI-III did inhibit the mutagenicity of NaN_3 (Table 2). At 0.1 and 1 mg doses, the inhibitory effects varied from 31.3% to 58.8%, and PI-II possessed the strongest antimutagenicity. The present results demonstrate that *P. igniarius* contains antimutagens capable of inhibiting the mutagenicity of direct-acting mutagens such as NPD and NaN_3. These results are consistent with those of Gruter *et al.* [5] who identified the antimutagenic activity of an ethanol extract of *C. cornucopioides* toward directly-acting mutagens, 2-nitrofluorene (2-NF) and acridine half mustard ICR-191 on *S. typhimurium* TM677. After the addition of the ethanol extract of *C. cornucopioides*, the mutagenicities induced by 2-NF and ICR-191 were inhibited 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 [5].

The inhibitory effects of PI-I, PI-II, and PI-III 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 with the S9 mixture were examined in a plate incorporation assay. The addition of 0.1 mg of *P. igniarius* caused about a 30% inhibition of the mutagenic activity of 2-AF. A 44% to 49% inhibition of mutagenic activity of 2-AF was observed when 1 mg each of PI-I, PI-II, and PI-III was added (Table 2). The effects of PI-I, PI-II, and PI-III on the B[*a*]P-induced mutation frequency are also presented in Table 2. The addition of 0.1 mg of *P. igniarius* caused 22.3% to 33.4% inhibition of the mutagenic activity of

B[*a*]P. A 32.7% to 46.8% reduction of the number of revertants was observed at a dose of 1 mg each of PI-I, PI-II, and PI-III (Table 2). This is in good agreement with previous studies, where basidiomycetes mushrooms inhibited mutagenicities induced by indirectly-acting mutagens. With the addition of a heat-resistant factor in an ethanol extract of *C. cornucopioides* at a concentration of 2.5 mg, the mutagenicities induced by aflatoxin B1 and B[*a*]P were inhibited by 97% and 98%, respectively [5]. B[*a*]P-induced mutagenicity was also inhibited by an ethanol extract of mushroom *Tirmania pinoyi* in a dose-dependent manner [6].

The mechanism by which the antimutagens in *P. igniarius* inhibit the mutagenicity of either 2-AF or B[*a*]P is not known. However, some natural compounds have been reported to inhibit the mutagenicities of B[*a*]P and smoke condensate, and a mechanism has been suggested as an interaction between antimutagen and the enzymes in the liver homogenate. The metabolism of B[*a*]P with a cytochrome P-450-dependent microsomal mixed-function oxidase results in the formation of reactive epoxides at various positions in the hydrocarbon skeleton [4]. The epoxides either isomerize spontaneously to phenols or are hydrated via epoxide hydrase to dihydrodiols. The mutagenicity of S9-activated B[*a*]P is due to the production of primary and secondary metabolites which are themselves direct-acting 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]. *P. igniarius* may interfere with the production of primary and secondary metabolites by either blocking an enzyme system necessary for their production or favoring the synthesis of metabolites that are not carcinogens. It is also possible that *P. igniarius* may affect an enzyme activity such as aryl hydrocarbon hydroxylase in the S9 mixture. Ramel *et al.* [12] 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 produce a direct reaction with electrophiles.

The evidence that *P. igniarius* is antimutagenic against different mutagens is significant, because 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. igniarius* 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 multiple antimutagenic compounds may be present in the complex of the extracts of *P. igniarius*. Further studies on the purification and identification of the active constituents of *P. igniarius* will help efforts to determine whether they are antimutagenic/anticarcinogenic in appropriate animal models, and ultimately to apply to anticarcinogenesis in humans.

Acknowledgments

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

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