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Antioxidative and Antimutagenic Activities of 70% Ethanolic Extracts from Four Fungal Mycelia-Fermented Specialty Rices

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Summary **The health-promoting potential of 70% ethanolic extracts of 4 rice varieties fermented with *Monascus ruber*, *Phellinus linteus*, *Cordyceps sinensis* and *Agaricus blazei* was evaluated mainly focusing on their antioxidative and antimutagenic capacities based on the following parameters: phenolic compound and phytic acid content; inhibitory activity on lipid peroxidation; scavenging activity on DPPH radical; suppressing ability on mitomycin C-induced mutagenesis in *E. coli* cells; and protective effect on 4-nitroquinoline oxide-triggered DNA lesion in V79 hamster cells. The fermented rice extracts were superior in overall health-promoting parameters compared to the source material. The higher antimutagenic activity of the fermented rice extracts might be in part caused by a larger amount of antioxidant constituents such as phenolic compounds or phytic acid. Of the fungal species, *Monascus ruber* was found to impart a marked increase in both the antioxidative and antimutagenic abilities to the source material. The current study suggests a possibility that such fermented rice may contribute to the prevention of lifestyle-related diseases such as cancer through a daily intake of rice-based diets.**

Key Words**: antioxidation, antimutagenesis, anticarcinogenesis, fermented rice, fungal mycelia**

Introduction

The spontaneous generation of reactive oxygen species (ROS) has widely been accepted as an inevitable phenomenon that occurs in the process of the aerobic cell respiration. Despite its advantage in the efficient free energy transition from fuel molecules to ATP, concomitant ROS generation much greater than a physiologically relevant level has been reported as a harmful event causing the oxidative destruction of constituents in the aerobic cells [1]. Emerging evidence indicates that such a cellular dysfunction caused by ROS might play a pivotal role in the pathogenesis of chronic lifestyle-related diseases including atherosclerosis and cancer as well as aging [2–4].

For protecting against oxidative damages, aerobic cells are equipped with a variety of antioxidants with different functions and antioxidative enzymes, by which cellular redox-status homeostasis is ensured through the scavenging of various preformed ROSs [5–7]. Therefore, much attention has been paid to the prevention or amelioration of oxidative stress-related chronic diseases through daily intake of antioxidant-rich functional foods.

Recently, the medicinal use of rice has been regarded as one of the cost-effective ways to ensure quality of life in the aging society through the prevention of lifestyle-related diseases without creating any immense economic burden. A variety of health-promoting specialty rice is now commercially available, the majority of which is polished rice coated with well-known functional materials such as antioxidant

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vitamins or herbal extracts which have already demonstrated their pharmaceutical potency in folk medicine. As well, germinated rice or fermented rice made by cultivating fungal mycelia on the rice grain is commercially available specialty rice [8]. The fungal mycelia growth on rice is implicated as a valuable process to enhance health-promoting ability of rice grain; however, critical evaluation for its medicinal potency has to be done with a range of tests for elucidation of its validity as a marketable healthy food.

In this context, antioxidant and antimutagenic activities, regarded as the primary health-promoting parameters in medicinal potencies, were examined with the specialty rice fermented with each of 4 mycelia of *Agaricus blazei*, *Phellinus linteus*, *Cordyceps sinensis* and *Monascus ruber*, especially focusing on the phenolic compound and phytic acid contents, inhibitory activity on lipid autoxidation and scavenging ability on free radical, antimutagenic activities in both bacterial cell system and cultured mammalian cell system, respectively. The current study showed that the fermented rice varieties tested had both improved antioxidative and antimutagenic activities, and that *Monascus rubber* was the most effective in imparting these health-promoting functionalities to the source material.

Materials and Methods

Materials

1,1-Diphenyl-2-picrylhydrazyl (DPPH), ferric chloride, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide, 2-nitrophenyl-β-D-galactopyranoside (ONPG), 4-nitrophenyl phosphate (PNPP), mitomycin C, 4-nitroquinoline-1-oxide (4-NQO), and other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO). All reagents were of analytical grade and were used without further purification. *E. coli* PQ37 strain was kindly provided by Dr. I.M. Chang, Natural Products Research Institute, Seoul National University (Seoul, Korea). V79 strain of Chinese hamster lung cells was purchased from Health Science Research Resources Bank (Osaka, Japan). Eagle's minimum essential media, Hanks' balanced salt solution (HBSS), fetal bovine serum (FBS) and other reagents for mammalian cell culture were the products of Life Technologies (Grand Island, NY).

Rice extracts

Four commercially available fermented rice varieties, made by cultivating the mycelia of *Agaricus blazei*, *Phellinus linteus*, *Cordyceps sinensis* and *Monascus ruber* on unpolished rice, together with the source material, were provided by Shinzi Co. (Suwon, Korea). The rice was ground into powder with a blender (Food processor, J World Tech Co., Korea), and passed through a 100-mesh sieve to obtain fine powders. The active compounds in the rice were extracted by shaking overnight at an ambient temperature

with 5-times the sample weight of 70%–30% ethanol-water [9]. The filtrate was passed through Whatman No. 1 filter paper (Whatman International Ltd., Maidstone, UK). The solvent was then removed from the extract by rotary evaporation (EYELA, Tokyo, Japan) at room temperature. The extracts were dissolved in dimethyl sulfoxid (DMSO) and stored at −20°C until analyzed. For convenience, the extracts from the rice varieties fermented with *Agaricus blazei*, *Phellinus linteus*, *Cordyceps sinensis* and *Monascus ruber* and the unpolished rice used as source material, are hereafter termed as ABRE, PLRE, CSRE, MRRE and UBRE, respectively.

Analysis of general components

Moisture, protein, lipid and other macrocomponents were measured following the procedure previously reported [10]. Rice was burned at 550°C electric furnace (HMF-3M, Dae Duck Hi-Tech Co., Seoul, Korea) to produce ash for subsequent quantification. Dietary fiber was quantified according to the Prosky-AOAC method based on the enzymatic gravimetric procedure [11]. The crude protein content was measured by the Kjeldahl method using an auto analyzer (1035, Kjeltec Co., Tecator, Sweden). Moisture content was measured using the Infrared moisture determination balance (FD-240, Kett Electronic Lab., Tokyo, Japan).

Quantification of phenolic compound and phytic acid

The phenolic compound content was determined according to the method of Singleton and Rossi [12] with slight modifications. Briefly, deionized water was added to a rice extract (0.5 ml) to a volume of 7 ml, followed by addition of 50% Folin-ciocalteu's phenol (0.5 ml). After standing for 3 min at room temperature, Na_2CO_3 solution (1 ml) was added to the mixture, and the reaction continued for another 1 h at room temperature with intermittent mixing. The phenolic compound in the mixture was measured spectrophotometrically at 725 nm using a UV/Vis spectrophotometer (V-550, JASCO International Co., Tokyo, Japan) to express its quantity as gallic acid equivalents (mg GAE/g sample) with reference to the standard curve using gallic acid as a standard compound.

To measure phytic acid, the method of Fruhbeck and others [13] was applied with a slight modification. Briefly, the rice powder was extracted with 50 ml of 1.2% HCl containing 10% Na_2SO_4 by shaking at room temperature. After recovery of the filtrated solution, aliquot (10 ml) was mixed with 12 ml ferric chloride ($FeCl_3$), followed by heating in boiling water for 75 min and subsequent cooling for 1 h. The mixture was then centrifuged at 3,500 rpm for 15 min to precipitate the resultant ferric phytate. After dissolving the precipitants in 50 ml of distilled water, 4 ml of aliquot from the ferric phytate solution were mixed with 1 ml of Wade reagent. The phytic acid in the solution was

measured spectrophotometrically at 500 nm, and its quantity was expressed with reference to the standard curve using a sodium phytate reagent.

Inhibition of linoleic acid peroxidation

The *in vitro* antioxidant activity of the rice extracts was measured following the thiocyanate method [14]. Briefly, rice extracts (200 µg) were mixed with five volumes of 0.02 mM linoleic acid in ethanolic emulsion and 4 volumes of 0.2 M phosphate buffer (pH 7.0) in a test tube. The mixture was then placed in the dark for 8 days at 40°C to accelerate lipid oxidation. At appropriate intervals, the reaction mixture was withdrawn, and mixed with the ferric chloride and thiocyanate solution. The resultant chromophore production was measured at 500 nm using a UV/Vis spectrophotometer, and the absorbance value was used to calculate the magnitude of lipid peroxidation level.

Free radical-scavenging activity on DPPH

The electron-donating ability of the rice extracts toward the DPPH radical was examined according to the method described by Yen and Chen [15] as follows. The assay mixture consisting of the rice extract and DPPH (final concentration of 100 µg/ml and 0.2 mM, respectively) was shaken vigorously for 5 min and placed for 30 min at room temperature in the dark, and then the absorbance of the mixture was recorded at 517 nm. Special care was taken to minimize the loss of free radical activity of the DPPH stock solution as recommended in the original article, *i.e.*, preliminarily, the integrity of the DPPH solution is tested using 100 mM sodium acetate buffer (pH 5.5) as the replacement for the DPPH solution. The radical scavenging activity of the rice extracts was expressed as the percent inhibition relative to the control.

SOS chromotest

A SOS chromotest was carried out according to the procedure described by Quillardet and Hofnung [16] in a modified version recommended by Mersch-Sundermann and others [17]. An overnight culture of *E. coli* PQ37 was 10-fold diluted with LB medium, and cultured for 2 h at 37°C. Then, rice extracts (final concentration 100 µg/ml) and/or mitomycin C (final concentration 6 ng/ml) were added to the aliquots, previously 4-fold prediluted with the flesh medium. After incubation for 2 h at 37°C, the bacterial cells were used for the following assays: To measure the β-galactosidase activity, culture aliquots (0.2 ml) were 10-fold diluted with B buffer (120 mM Na_2HPO_4, 40 mM NaH_2PO_4, 10 mM KCl, 1 mM $MgSO_4$, 50 mM β-mercaptoethanol, 1% SDS, pH 7.0). The enzyme assay was started by adding 1.6 mg of ONPG, followed by incubation for 20 min at 37°C with continual shaking. Then, Na_2CO_3 was added to terminate the reaction, and the enzyme activity was measured spectro-

photometrically at 420 nm. For the alkaline phosphatase assay, culture aliquots were 10-fold diluted with P buffer (1 M Tris, 0.1% SDS), and then incubated for 30 min at 37°C in the presence of 1.6 mg PNPP. After terminating the reaction with HCl, the enzyme activity was also measured spectrophotometrically at 420 nm. The enzyme activities were calculated according to the following formula:

Enzyme units
= absorbance at 420 nm × 1000/t [t = substrate conversion time (min)].

The induction factor (R) was expressed as a ratio of β-galactosidase unit to alkaline phosphatase unit. Anti-mutagenicity was defined as follows:

Antimutagenicity (%)
= $[1 - (R_x - R_0) / (R_c - R_0)] \times 100$ (R_x, R_c, and R_0; Induction factor of experimental group, only mitomycin C-treated positive control and blank, respectively)

Cytotoxicity assay in Chinese hamster V79 cells

The V79 strain of Chinese hamster lung cells was maintained in Eagle's minimum essential medium (MEM) supplemented with 10% heat-inactivated fetal bovine serum (FBS) with 100 units/ml penicillin and 100 µg/ml streptomycin under 5% CO_2-contained humidified air. The cytotoxicity on the V79 cells was determined by measuring the colony-forming activity (CFA) of cells pretreated with the rice extracts. Briefly, triplicate inocula of 5×10^2 cells were incubated in 100-mm cell dishes in the medium for 24 h, followed by treatments with various doses of rice extracts for 3 h. After rinsing with HBSS, the media were added, and incubation continued for 7 days. The cell colonies formed on the culture dishes were fixed with methanol and stained with Giemsa solution (Sigma Chemical Co.). Colonies containing more than 50 cells were scored under a microscope, and the CFA was calculated from the average number of colonies as a percentage of the number of cells initially inoculated. The effect of the rice extracts on cell survival is expressed as the surviving fractions of the CFA with reference to that of the untreated control cultures.

The antimutagenic ability of the rice extracts was determined following the re-plating method previously reported [2]. The inocula of 2×10^5 cells in 100-mm dishes were incubated for 24 h and then treated with the mutagen, 4-nitroquinoline oxide (4-NQO), at a concentration of 1.5×10^{-7} M for 3 h. After rinsing twice with HBSS to remove the mutagen completely, the cells were incubated for 7 days in the medium to allow for maximal expression of mutagenicity. Then, the cells were dissociated by treatment with 0.25% trypsin containing 0.02% EDTA, followed by replating 2×10^5 cells in five 100-mm dishes in the medium containing 5 µg/ml of 6-thiaguanine (6TG) for 8 days. The resultant 6TG-resistant mutant colonies were fixed, stained, and scored for the number of colony-forming units. In

parallel experiments, triplicate inocular of 5×10^2 cells, each in 100-mm dishes, were incubated in the medium without 6TG for 7 days. The CFA of the replated cells was determined as described above. The number of induced mutants was calculated by subtracting the number of colonies in the untreated control cultures from those in the treated cultures. The induced mutation frequency (IMF) was expressed as the number of induced mutants per 10^5 colony-forming cells.

Statistical analysis

All statistical analyses were conducted using the statistical package for social science software program (SPSS ver. 11, SPSS Inc., Chicago, IL). Data were presented as the mean ± SD. Significant differences among the groups were determined by a one-way analysis of variance (ANOVA) with a *post hoc* Duncan's multiple-range test. Probability values<0.05 were considered to be statistically significant.

Results

General chemical composition

The protein, lipid, ash, dietary fiber and moisture contents of 4 fermented rice extracts were significantly altered compared to those of the source material (Table 1); cultivation of the fungal mycelia increased the crude protein and dietary fiber contents, while *vice versa* for the ash and moisture contents. The fermented rice extracts, except for MRRE, tended to show a lower lipid content. MRRE exhibited the greatest increase in the protein and dietary fiber contents, and CSRE the greatest decrease in the lipid and ash contents.

Phenolic compounds and phytic acid contents

The phenolic compound and phytic acid contents were quantified as an index to evaluate the health-promoting potentials of the fermented rice varieties. The 4 fungal mycelia growths on the rice significantly gave rise to the overall increase in the phenolic compounds content compared to that of the source material. Among them, the highest increase in the phenolic compounds content (about
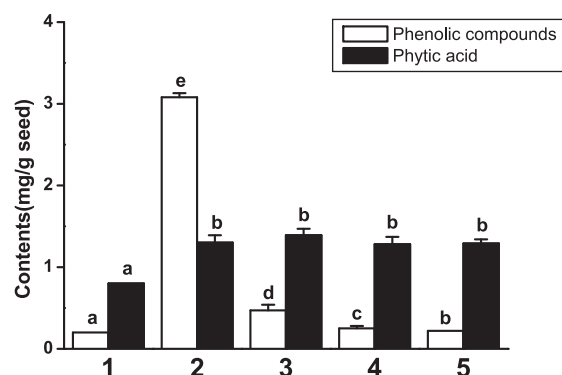

Fig. 1. Phenolic compounds and phytic acid contents in 70% ethanol extract of the following rices. Lane 1, unpolished brown rice used as the source material; lane 2, *Monascus rubber*-fermented rice; lane 3, *Phellinus linteus*-fermented rice; lane 4, *Cordyceps sinensis*-fermented rice; lane 5, *Agaricus blazeis*-fermented rice. Values are expressed as mean ± SD. The means not sharing a common letter are significantly different between groups at $p<0.05$ ($n = 3$).

16-fold) was observed with MRRE compared with the source material, and the phenolic compound content of each fermented rice extract decreased in the order of PLRE > CSRE > ABRE (Fig. 1). The fungal mycelia growth also indicated overall increase in the phytic acid content compared to the source material, however, no significant quantitative differences could be found among the 4 fermented rice extracts.

Antioxidative activity

The antioxidant activity of the fermented rice extracts was determined as a valuable parameter to simply evaluate their health-promoting capacities. Therefore, the inhibitory activity of the fermented rice extracts on lipid autoxidation was first measured in the assay system using linoleic acid as a substrate for oxidation reaction. The results show that the inhibitory activity was higher in the fermented rice extracts than the source material extract (Table 2), and that the inhibitory

Table 1. General chemical composition of the fermented rices[†]

(%)

Rice[‡]	Protein	Lipid	Ash	Dietary fiber	Moisture
UBR	5.87 ± 0.26[a]	2.0 ± 0.08[c]	0.3 ± 0.03[e]	0.78 ± 0.01[a]	12.32 ± 0.36[d]
MRR	8.29 ± 0.12[d]	3.48 ± 0.21[d]	0.16 ± 0.01[b]	4.84 ± 0.08[d]	11.3 ± 0.26[d]
PLR	7.19 ± 0.11[bc]	1.29 ± 0.03[b]	0.22 ± 0.01[c]	3.98 ± 0.88[c]	6.31 ± 0.16[cd]
CSR	6.99 ± 0.14[b]	0.55 ± 0.03[a]	0.12 ± 0.01[a]	3.25 ± 0.29[bc]	7.51 ± 0.08[bc]
ABR	7.32 ± 0.1[c]	1.5 ± 0.08[b]	0.25 ± 0.02[d]	2.87 ± 0.05[b]	6.68 ± 0.34[a]

[†]Values in each column with the same superscript are not significantly different at $p<0.05$. Values are expressed as mean ± SD ($n = 3$).
[‡]The rice fermented with the mycelia of *Monascus ruber*, *Phellinus linteus*, *Cordyceps sinensis* and *Agaricus blazei* are termed as MRR, PLR, CSR and ABR, respectively. UBR means unpolished brown rice used as the source material.

Table 2. Antioxidative activities of the fermented rice extracts[†]

Rice extract[‡]	Inhibition of linoleic acid peroxidation		Scavenging of DPPH radical	
	Absorbance at 500 nm	Inhibition (%)	Absorbance at 517 nm	Inhibition (%)
Control	1.059 ± 0.054	0	3.098 ± 0.254	0
BHT	0.097 ± 0.004^a	90.8	0.325 ± 0.027^a	89.5
UBRE	0.509 ± 0.051^e	51.9	2.447 ± 0.151^f	21
MRRE	0.163 ± 0.024^b	84.6	0.504 ± 0.034^b	84.6
PLRE	0.223 ± 0.032^c	78.9	1.115 ± 0.092^c	64
CSRE	0.431 ± 0.022^d	59.3	1.379 ± 0.102^d	45.2
ABRE	0.485 ± 0.024^e	54.2	1.902 ± 0.112^e	38.6

[†]Values in each column with the same superscript are not significantly different at $p < 0.05$. Values are expressed as mean $\pm$ SD ($n = 3$). [‡]The extracts from rice fermented with the mycelia of *Monascus ruber*, *Phellinus linteus*, *Cordyceps sinensis* and *Agaricus blazei* are termed as MRRE, PLRE, CSRE and ABRE, respectively. UBRE, used as an internal control, means the extract from unpolished brown rice, source material. See sample concentration in Materials and Methods. BHT was used at a dose of 200 µg.

Table 3. Inhibitory effects of the fermented rice extracts on mitomycin C-induced mutagenesis in *E. coli* PQ37 cells

Rice extract[†]	β-galactosidase activity (unit)	Alkaline phosphatase activity (unit)	R factor	Antimutagenicity[‡] (%)
Control (−)	6.71 ± 0.57	40.37 ± 0.97	0.17	100
Control (+)	19.19 ± 0.24	14.69 ± 1.07	1.31	0
UBRE[1)]	18.18 ± 1.43	15.77 ± 1.10	1.15	14
MRRE	16.34 ± 1.99	19.62 ± 1.27	0.83	42.1
PLRE	14.17 ± 0.39	16.88 ± 1.02	0.84	41.2
CSRE	9.55 ± 0.39	14.16 ± 1.15	0.67	56.1
ABRE	15.35 ± 0.93	15.97 ± 0.91	0.96	30.7

[†]See footnote to Table 2. [‡]Values represent percent inhibition of cells loaded simultaneously with mitomycin C and rice extracts compared to maximum values from cells stimulated with only mitomycin C treatment (positive control).

action of MRRE, PLRE, CSRE and ABRE decreased in that order. Second, the free radical scavenging ability was also measured as another parameter of the antioxidativity. The results show that the radical scavenging ability of the fermented rice extracts was higher than that of the source material, and that the activity decreased in the order of MRRE > PLRE > CSRE > ABRE.

Antimutagenic activity in SOS chromotest system

The inhibitory effect of the fermented rice extracts on chemically induced mutagenesis was assessed in the SOS chromotest using *E. coli* as an indicator cell. Data in Table 3 shows that CSRE have suppressed mitomycin C-induced mutagenesis more potently than other fermented rice extracts such as MRRE and PLRE (about 56% *vs.* 41~42% inhibitions). On the other hand, ABRE exhibited the lowest antimutagenic activity (about 30% inhibition).

Cytotoxicity of the rice extracts in mammalian cell system

To assess the antimutagenicity of the fermented rice extracts accurately, the rice extract doses without any marked cytotoxicity and mutagenicity were first measured in V79 Chinese hamster cells. The results show that the optimal dose of rice extracts varied with each of the rice extracts; at 120, 20, 160 and 80 µg/ml or a higher dose of UBRE, MRRE, PLRE and CSRE, respectively, cell viabilities were markedly diminished to a level less than 0.94 of the surviving fraction of cells (Table 4). Therefore, 80, 10, 120, 40 and 5 µg/ml of UBRE, MRRE, PLRE, CSRE and ABRE were determined to be the satisfactory doses for conducting further assessments of the antimutagenicity of the rice extracts.

Antimutagenic activity of the rice extracts in mammalian cell system

A preliminary test has demonstrated that a value of induced mutation frequency by mutagen 4-NQO reached its

Table 4. Cytotoxicity and mutagenicity test of the fermented rice extracts in V79 cells

Concentration (µg/mL)	Surviving fraction of cells[†]				
	UBRE[‡]	MRRE	PLRE	CSRE	ABRE
0	1	1	1	1	1
5	1.109	0.934	1.276	1.133	0.923
10	1.048	0.948	1.146	1.191	0.812
20	1.018	0.913	1.233	1.133	0.895
40	1	0.918	1.22	1.122	0.789
80	0.971	0.747	1.187	0.82	0.516
120	0.912	0.329	1.152	0.028	0.394
160	0.695	ND	0.896	ND	ND

[†]Induced mutation frequency per 10^5 surviving cells was 0.000. [‡]See footnote to Table 2. ND; not determined.

maximum at a dose of 1.5×10^{-7} M in V79 cells (data not shown). Therefore, under these mutagen and rice extract concentrations, the protective effect of rice extracts on 4-NQO-induced mutagenesis was measured to see if the antimutagenic potency was enhanced in the fermented rice extracts compared to the source material. Data show that MRRE, PLRE and ABRE exhibited over 5-fold greater antimutagenic potencies relative to the source material, and that the activity decreased in the order of MRRE > ABRE > PLRE > CSRE > UBRE if was taken no account of the difference in sample doses.

Discussion

A growing number of chemical carcinogens are now encountered in the environment at suspected doses to induce mutagenesis or carcinogenesis. To count the impact of cancer in an aging society, dietary control seems likely to be the most desirable preventive way which is not accompanied by any immense social economic burden on public health care. In this consequence, the current study is undertaken to examine whether 4 commercially available rice varieties made by cultivating fungal mycelia on unpolished rice grains have health-promoting potencies worthy for daily intake. Experiments were mainly conducted to evaluate the antioxidant and antimutagenic effects of fermented rice varieties. First, this study noted the alteration in both phenolic compound and phytic acid contents, well-known health-promoting phytochemicals [18, 19]. The data show a significant increase in both phenolic compound and phytic acid contents in the fermented rice varieties compared to the source material itself (Fig. 1). Especially, a remarkable increase in the phenolic compound content was observed with MRRE. These findings might be a clue that the enhanced antioxidant capacities of the 4 fermented rice extracts are associated with the increase in the phenolic compound and phytic acid contents (Table 2). This notion is supported by a number of studies on the action of phenolic

compound and phytic acid in suppressing ROS-triggered oxidative DNA damage [18, 20]. As it is considered that irreversible mutations in DNA trigger tumorigenesis through so-called 'tumor-initiation' step [21], it is crucial to examine whether the enhanced antioxidant activity observed with the fermented rice extracts is coupled with the raised antimutagenic activity in cell milieu. Data in Table 3 show that the extent of inhibitory actions of the rice extracts, except for CSRE, on mitomycin C-induced mutagenesis tends to be parallel with their antioxidant potentials in *E. coli* PQ37 cells. This might indicate a pivotal role of phenolic compound in the expression of antimutagenic potential [22]. MRRE would be the representative example, and a number of earlier studies indicate that phenolic antioxidants inhibit a substantial variety of chemical carcinogens including mitomycin C by their radical scavenging action toward highly reactive electrophiles of reactive forms of carcinogens. This again supports a possibility that the mutation-suppressing action of the rice extracts observed in the current study is closely coupled with the antioxidant potential [22–24]. However, the current study failed to find such a relationship for CSRE. Alternatively, the non-phenolic components of the fungal mycelia might be involved in the suppression of chemically induced mutagenesis in desmutagenic and/or bio-antimutagenic manners [25], and in that case, the most probable candidate would be the antioxidant polysaccharides known as antitumorigenic components in earlier studies [26–28]. Although a SOS chromotest is a convenient tool to examine the alteration in DNA, the lack of a mixed function oxidase system in bacterial cells hampers an accurate evaluation of the xenotoxicity prerequisite for cellular metabolic conversions [29, 30]. In this consequence, V79 Chinese hamster lung fibroblast cells were employed to evaluate the protective effect of the rice extracts on a direct mutagen 4-NQO-induced mutagenesis; the antimutagenic potential of the rice extracts was assessed by measuring the reduction rate of 6-thioguanine resistant cell colony formation resulting from 4-NQO-induced genetic alteration at the

Table 5.   Effects of the fermented rice extracts on 4-NQO-triggered IMF in V79 cells

Rice extract[†]	Concentration (μg/mL)	6-TG resistant Colony number	Induced Mutation Frequency (IMF)	Inhibition (%)
Control[‡]	—	13.40 ± 2.07	34.86	0
UBRE	80	14.78 ± 1.64	29.86	14.3
MRRE	10	4.22 ± 2.17	7.7	77.9
PLRE	120	6.33 ± 1.79	9.76	72
CSRE	40	9.14 ± 2.61	15.4	55.8
ABRE	5	5.63 ± 1.52	8.5	75.6

[†]See footnote to Table 2. [‡]Cells were treated only with 1.5×10^{-7} M 4-NQO.

hypoxanthine-guanine phosphoribosyltransferase gene locus [31]. The prominent antimutagenic action of MRRE could also be explained based on a much larger amount of phenolic compounds, as discussed in the results from the SOS chromotest (Table 3), whereas those of other fermented rice extracts did not agree directly with their phenolic compound contents (Table 3). Such a tendency was evident especially in the case of CSRE and ABRE. Moreover, the mutation-suppressing profile seemed inversely related in between the assay systems. In the current study, the antimutagenic effect of the rice extracts on the V79 cells was assessed under the condition that the cells were treated with the extracts during the mutation expression time after treatment with 4-NQO, not simultaneously with the extracts and the mutagen as for the SOS chromotest (see Materials and Methods). Consequently, the mutation-suppressing effects of the rice extracts on the V79 cells might be bio-antimutagenic (Table 5). On the contrary, the SOS chromotest might, in part, exhibit a desmutagenic ability of the rice extracts [32], possibly explaining the discrepancy in antimutagenicity according to the assay system employed.

In conclusion, the current study demonstrates that 4 fungal mycelia-fermented rice extracts have greater mutation-suppressing effects on both chemically induced mutagenesis in the SOS chromotest and the 6-TG resistant colony forming assay using V79 Chinese hamster cells. In addition, their antioxidative potentials might, in large part, play a role in the expression of their antimutagenic activities. Considering that accumulation of irreversible mutations is a primary cause leading to the transformation of normal cell to a cancerous cell, daily intake of the fermented rice is desirable for the prevention of cancer, a most threatening lifestyle-related chronic disease. Therefore, more intensive studies are needed to clarify the mechanism for the mutation-suppressing action of the fermented rice varieties for a wider use of fermented rice as a food with health implications.

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