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Incidence of *Fusarium* and Other Molds in Korean Field Crops

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Abstract

The incidence of total molds, *Fusarium* species, and the estrogenic mycotoxin, zearalenone, in Korean grain samples were investigated. The majority of molds infecting grain were identified as belonging to the genus *Alternaria*, with an average infection rate of kernels of 43% and 32% in rice and barley, respectively. *Fusarium* species were less common, with average infection rates of 13% and 19% in rice and barley, respectively. A number of field fungi including *Curvularia* and *Dactylaria* were also observed. Among the *Fusarium* species, 71 of 94 *Fusarium* isolates were identified as *F. semitectum*. A few *F. moniliforme* and *F. equiseti* were observed from both rice, barley, and beans. Zearalenone was not detected in any of the 29 samples either by enzyme-linked immunosorbent assay(ELISA) or high-performance liquid chromatography(HPLC). In addition, deoxynivalenol was not detected by ELISA. However, the presence of molds, including *Fusarium* species, may pose possible health hazards to persons consuming those grains.

Key words: incidence, *Fusarium*, molds, grain

INTRODUCTION

There are four genera of molds that are considered most important in cereal grains and foods, namely *Aspergillus, Penicillium, Fusarium,* and *Alternaria,* mainly because of their ability to produce mycotoxins(1). The ubiquitous nature of molds contributes to their worldwide occurrence and contamination of raw and processed foods with the molds from the environment. Among the four important genera of food-borne fungi, species of *Fusarium* have stimulated much interest due to their potential to produce diverse groups of mycotoxins, and the potential effects on human and animal health. *Fusarium* molds and their toxins occur commonly as contaminants of numerous agricultural products worldwide(2-4).

Zearalenone(ZEN) is a unique mycotoxin with estrogenic activity, which is produced mainly by some strains of *Fusarium graminearum*, but also by *Fusarium culmorum* (5). Though much less common, production of this mycotoxin by other *Fusarium* species including *F. acuminatum, F. equiseti, F. oxysporum, F. sambucinum, F. semitectum* and *F. solani*, has also been documented(6-8). Co-contamination of grains by other *Fusarium* toxins, especially deoxynivalenol(DON) and nivalenol(NIV) with ZEN has been reported(9).

The natural occurrence of ZEN has been reported in many countries. According to Tanaka et al.(10), Dutch cereals harvested in 1984/85 were contaminated with ZEN at an average of 61ng/g in all positive samples. The highest level of natural contamination with ZEN reported was 3,100ng/g in a corn cob mix from 1988/89 in the Netherlands(11). In Korea, 25 out of 32 samples of unpolished barley from the 1983 crop(12), and 34 out of 36 samples from the 1984 crop(13), were positive for ZEN. Average levels of ZEN contamination were 110ng/g(1,600ng/g maximum) in 1983. Park et al.(14) detected ZEN at levels of 183~1416ng/g and 40~1081ng/g for husked and polished barley, respectively, from the 1990 crop.

The objectives of this study were to assess the mycological quality of grain samples collected in Korea, determine the incidence of *Fusarium* species in the grain, and determine the levels of ZEN in grain samples by both high performance liquid chromatography(HPLC) and enzyme linked immunosorbant assay(ELISA).

MATERIALS AND METHODS

Internal infection rates and identification of molds

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Effect of Allopurinol on the Ethanol-induced Oxidative Stress : Mechanism of Allopurinol Action

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Abstract

An acute ethanol load(50mmol/kg, i.p.) resulted in an increase in lipid peroxidation and a decrease in the levels of α-tocopherol and ascorbate in rat cerebellum. Pretreatment with allopurinol(146μmol/kg, i.p.) prevented the ethanol-induced increment in lipid peroxidation and decrease in α-tocopherol content. However, the decrease of ascorbate was of greater magnitude when allopurinol was associated with ethanol. These results suggested that allopurinol, besides its action as a radical scavenger and xanthine oxidase inhibitor, might favor the regeneration of α-tocopherol by ascorbate. Therefore, the influence of allopurinol on the monoelectronic exchanges involved in α-tocopherol antioxidant activity was studied using γ-radiolysis in aerated ethanolic solutions. Even though allopurinol did not react by itself with α-hydroxyethyl-peroxyl radicals [H_3C-CH(OH)OO$^{\cdot}$], it enhanced the α-hydroxyethyl-peroxyl radical scavenging properties of α-tocopherol. The regeneration of α-tocopherol from the α-tocopherol radical by ascorbate remained as efficient in the presence of allopurinol as in its absence. The effects of allopurinol on the vitamin E oxidation-reduction mechanisms could be involved in the beneficial effect of allopurinol on the biological cellular damages linked to free radical reactions.

Key words: ethanol, allopurinol, α-hydroxyethyl-peroxyl radical, α-tocopherol

INTRODUCTION

It is generally assumed that an oxidative stress resulting from generation of free radicals in an amount exceeding the capacity of the cellular defense systems plays an important role in the pathogenesis of ethanol-induced liver injury(1). Recent studies have reported that ethanol administration to rats can also cause free radical-mediated oxidative cellular damage in extrahepatic tissues, such as the brain(2-4). Chronic ethanol treatment induced oxidative DNA damage in the hipocampus and cerebellum of rats(2). Dietary administration of ethanol to rats for 2 weeks depressed levels of glutathion and Cu/Zn superoxide dismutase in several brain regions(3). Our previous studies have also shown that an acute ethanol load to rats increased lipid peroxidation in the cerebellum and decreased the concentrations of vitamin E, the major antioxidant against peroxidative degradation of membrane lipid, and vitamin C which regenerates vitamin E from oxidation product(4).

Allopurinol(4-hydroxypyrazol(3,4)-pyrimidine), an inhibitor of xanthine oxidase(XO), has been extensively used for the treatment of hyperuricemia, both of gout and secondary to hematological disorders or antineoplastic therapy(5). Additionally, it has been suggested that the inhibition of XO by allopurinol can reduce the formation of free radicals, and thereby may prevent or ameliorate cellular injuries(6-8). However, some reports have suggested that the beneficial effects of allopurinol during ischemia/reperfusion(9) or whole body γ-irradiation(10) are due to the direct free radical scavenging properties of the drug rather than to its ability to inhibit XO. As a matter of fact allopurinol and its metabolite, oxypurinol, are powerful scavengers of hydroxyl radical($^{\cdot}$OH)(11) and free radicals generated by activated leucocytes (9,12). It has also been demonstrated that allopurinol can facilitate electron transport occurring during the oxidation -reduction mechanisms of cytochrome c(13).

These reports suggest that beneficial effects of allopurinol may be not related only to XO inhibition. It appears that allopurinol can also provide protection against oxidative cellular damage by scavenging free radicals or by other mechanisms.

Therefore, the present study was undertaken first to assess the possible protective effect of allopurinol on the ethanol-induced alterations in lipid peroxidation and contents of vitamin E and vitamin C in cerebellum, a brain region known to be particularly vulnerable to

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MATERIALS AND METHODS

Internal infection rates and identification of molds

Twenty nine grain samples from several parts of

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Korea were collected in April, 1995. These samples were shipped to the U.S. by air and kept frozen until analyzed. Internal kernel infection rates were determined for all grain samples by plating 100 single surface sanitized kernels of each sample on agar plates, allowing the molds to grow out of the kernel, and determining how many of the 100 kernels were infected. The kernels were first surface disinfected by soaking for 1 min in 5% sodium hypochlorite (full strength household bleach) and rinsed 3 times with sterile water and dried on sterile paper towels. The kernels were then placed on the surfaces of agar media and incubated at room temperature in the dark until mold growth appeared. Two media, Czapek iprodione agar (CZID; 15) and dichloran rose bengal chloramphenicol agar(DRBC; 16), were used. The CZID is more specific for *Fusarium* molds because it contains iprodione to inhibit other molds, while DRBC is used more for general or total mold counts. Both media contain antibiotics to prevent growth of bacteria.

The *Fusarium* species found in samples were isolated by a single spore technique as described previously(17). Single spore growth was transferred from water agar(WA, 1.8% agar) plates to carnation leaf agar(CLA) plates then incubated under natural lighting until sporulation. The *Fusarium* molds were identified to the species level using the key of Nelson et al.(17).

Determination of ZEN by HPLC and ELISA

For the HPLC analysis, ZEN was extracted from the grain samples by a method previously described by Thomas et al.(18) with some modification. Twenty grams of each sample was finely ground and extracted twice with 100ml of methanol-water(6 : 4) using a blender for 2 minutes at high speed. Sample extract was filtered through Whatman #4 filter paper and 100ml of filtrate was transferred to a 250ml separatory funnel. Then, 25ml of saturated NaCl solution and 40ml of hexane were added. After shaking 1 min, the lower aqueous methanol phase was transferred to a second separatory funnel and partitioned with 50ml of chloroform. The lower chloroform phase was drained into a flask containing 5g cupric carbonate and agitated. After the cupric carbonate settled, the chloroform was filtered through Whatman #2 filter paper containing 5g Na_2SO_4. The remaining cupric carbonate was washed with a 20ml portion of chloroform which was also decanted through the Na_2SO_4 and combined with the preceding 50ml. This final extract was evaporated to dryness and the residue was redissolved in 10ml chloroform.

Sample clean-up using Sep-Pak® silica cartridges (Waters Associates, Milford, MA) and HPLC detection was performed as reported by Merino et al.(19). In brief, a Sep-Pak® silica cartridge was attached to a 10ml glass syringe and conditioned with 10ml of hexane by gently pressing the plunger. Five milliliter of the final chloroform extract was transferred to the syringe, then passed through the silica cartridge. All the eluted volume was removed by passing air through the cartridge. Zearalenone was recovered with two 5ml portions of hexane/dry ethyl ether (1 : 1). Then, 5ml aliquots were transferred into 2 dram vials and evaporated to dryness under a gentle stream of nitrogen. Finally, 1ml of HPLC grade methanol was added to the vials and 10μl of the resulting solution injected into the HPLC.

The HPLC instrumentation was consisted of a 510 HPLC pump, a U6K injector, and a 474 scanning fluorescence detector(Waters Associates, Milford, MA). The wavelength of the detector was set at 274nm excitation and at 440nm emission. A reverse-phase Nova-Pak® C18 column(3.9×150mm, 4μm particle size, 60Å pore dia; Waters, Milford, MA) was employed for chromatographic separations. The mobile phase was a volumetric mixture of methanol-acetonitrile-water(1.0 : 1.6 : 2.0) at a flow rate of 1ml/min(20). The chart speed of the integrator(Hewlett Packard, model 3395) was set to 1cm/min.

In addition, ELISA based test kits(Neogen Corp., Lansing, MI) were used to determine ZEN and deoxynivalenol. Samples for ELISA were prepared by extracting 5g of finely ground sample with 25ml of 70% methanol using a wrist action shaker for 1 hr. The extract was filtered through a Whatman #1 filter paper. These tests were done in duplicate with two replications.

RESULTS AND DISCUSSION

The majority of the molds found infecting the grain were identified as members of the genus *Alternaria*, with an average kernel infection rate for Alternaria of 43% and 32% in rice and barley samples, respectively(Fig. 1, 2). *Fusarium* species were also common with average kernel infection rates of 13% and 19% in rice and barley samples, respectively. A number of field fungi such as *Curvularia* and *Dactylaria* were also observed on the

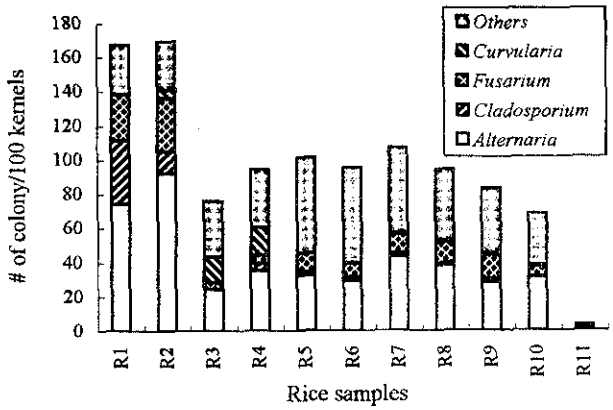

Fig. 1. Mold infection rates of rice samples as determined on DRBC.

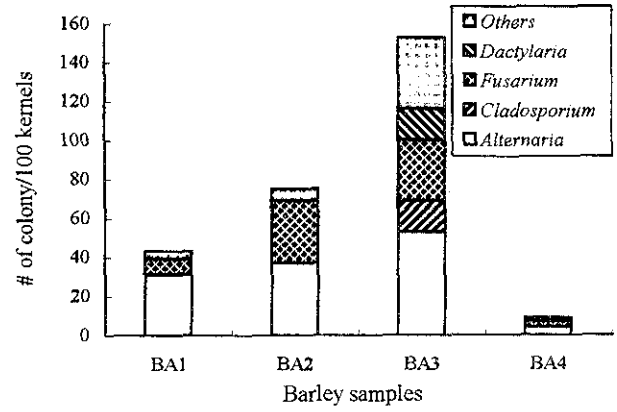

Fig. 2. Mold infection rates of barley samples as determined on DRBC.

DRBC plates. In bean samples, average kernel infection rates for *Alternaria* were only 4%(Fig. 3). Infection rates for other molds were also much lower compared to that of rice and barley. A polished rice sample(R11) had very low infection rates as shown in Fig. 1. In addition, two polished barley samples and one each of beans and wheat samples did not show any internal infection on the DRBC plates. In two millet samples, 5 isolates of *Aspergillus* spp. and one isolate each of *Fusarium* and *Cladosporium* spp. were found.

The incidence of field fungi including *Alternaria, Cladosporium,* and *Curvularia,* has been well documented (21-23). According to their reports, these field fungi are common in agricultural commodities such as rice, barley, beans, and sorghum. However, it also should be noted that grains were internally infected with all of the above fungi and hence the possibilities of subsequent growth of any toxigenic mold during storage cannot be eliminated.

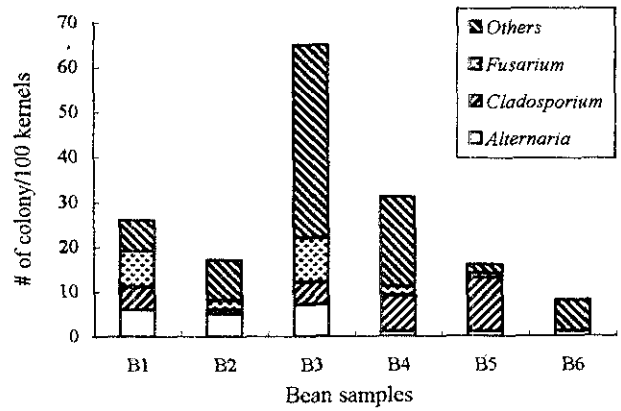

Fig. 3. Mold infection rates of bean samples as determined on DRBC.

It is particularly true since some strains of *Alternaria* and *Cladosporium* are known to be toxigenic(24,25).

Among the *Fusarium* species, 71 isolates were identified as *F. semitectum* from a total of 94 *Fusarium* isolates(Table 1). A few *F. moniliforme* and *F. equiseti* were observed from rice, barley, and beans. Lee et al.(4) also found similar populations of *Fusarium* species on corn ears and soils in Korea. Significant differences in total infection rates and populations of fungi among the

Table 1. Incidence of *Fusarium* spp. in rice and barley samples from Korea

	F. semitectum	*F. moniliforme*	*F. equiseti*	Others	Total
Rice					
R1	5	1		3 unknown	9
R2	9	1			10
R3	2				2
R4	2		1		3
R5	3	2			5
R6	2		1		3
R7	8		1		9
R8	3				3
R9	14		2	1 unknown	17
R10	13			1 *F. scirpi*	14
R11					None
Subtotal	61	4	5	5	75
Barley					
BA1		1			1
BA2					None
BA3	6		1	1 unknown	8
BA4					None
BA5					None
BA6					None
Subtotal	6	1	1	1	9
Total	67	5	6	6	84

Unit: Number of colony per 100 kernels

samples were observed which were probably due to weather differences in the growing regions. Barley is generally cultivated on the same land in between rice growing seasons which are late fall and early spring. Therefore, the microflora found in rice and barley would not differ significantly from each other. However, wheat, millet and corn, which are cultivated in more of the temperate region, seem to show different populations of molds than those of rice and barley.

The level of ZEN in all grain samples was in the non detectable range which was less than 250ng/g by the ELISA method. These results were confirmed by HPLC which has a lower detection limit of 50ng/g. Deoxynivalenol, as determined by ELISA which has a detection limit of 300ng/g, was not found in any samples, as well. This was understandable as there were no major producers of ZEN and DON, such as *F. graminearum*, found. However, the grains contained other possible toxigenic species of *Fusarium* that may be able to produce mycotoxins other than ZEN and DON. Therefore, the presence of molds including *Fusarium* species may pose potential health hazards to persons consuming those grains.

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