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Mycobiota and natural occurrence of aflatoxin, deoxynivalenol, nivalenol and zearalenone in rice freshly harvested in South Korea

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ABSTRACT

The natural fungal mycobiota and contamination by aflatoxins (AFs), deoxynivalenol (DON), nivalenol (NIV), and zearalenone (ZEN) in rice from six locations (80 farms) in South Korea were surveyed during the harvest year 2010. Mycological analysis indicated that *Fusarium* was the predominant fungal genera, followed by *Penicillium*, *Phoma*, *Myrothecium*, and *Cladosporium* spp. Within the genus *Fusarium*, *Fusarium graminearum* was found most frequently. Polymerase chain reaction analysis targeting the AFs (*aflR*, *omtB*, *ver-1* and *omtA*), DON/NIV (*tri4*, *tri5*, *tri5–tri6*, *tri7* and *tri13*) and ZEN (*pks4*) biosynthetic genes showed that potential ZEN and NIV producers were the most prevalent. A similar trend was observed for the natural occurrence of all four mycotoxins. In both white and brown rice, ZEN was the main mycotoxin contaminant (range 0.4–95.4 μg/kg), followed by NIV; AFs and DON were not prevalent. The present study provides a detailed description of the fungi and natural occurrence of these four mycotoxins in rice at harvest, which may help in understanding the population dynamics and in developing effective control measures.

1. Introduction

Rice (*Oryza sativa* L.) is an important food crop worldwide. Rice is the dominant grain for half of the world's population and provides 27% of the world's dietary energy supply and 20% of dietary protein intake in the developing world (FAO, 2004). In Korea, rice remains a staple food despite a decrease in its consumption in recent years. Currently, the average daily intake of milled rice (white rice) in Korea is 180.75 g per person, and the average daily intake of brown rice, whose consumption is increasing because of its "health food" status, is 2.9 g per person (KMHW, 2009).

During cultivation and subsequent handling of rice, kernels can be contaminated by fungi and mycotoxins if conditions are favorable. Several studies have shown that the fungi found in rice are of the genera *Fusarium*, *Alternaria*, *Penicillium*, *Rhizopus*, and *Aspergillus* (Aydin, Aksu, & Gunsen, 2011; Karunakara, Rati, & Manonmani, 2009; Lee et al., 2011; Park, Choi, Hwang, & Kim, 2005; Reddy, Reddy, & Muralidharan, 2009; Sales & Yoshizawa, 2005; Tonon, Marucci, Jerke, & Garcia, 1997). Mycotoxins that can contaminate rice grains include aflatoxins (AFs), fumonisins, deoxynivalenol (DON), nivalenol (NIV), zearalenone (ZEN), and ochratoxin (Aydin et al., 2011; Food Standards Agency, 2002; Lee et al., 2011; Makun, Dutton, Njobeh, Mwanza, & Kabiru, 2011; Park et al., 2005; Tanaka, Sago, Zheng, Nakagawa, & Kushiro, 2007; Toteja et al., 2006). Fungal and mycotoxin contamination of rice grains depends largely on the field and storage conditions. Preventing such contamination requires both careful control of storage conditions and the assurance that the rice grains are not contaminated at harvest.

The presence of mycotoxins in rice is of great concern in many countries, including Korea. AFs (B_1, B_2, G_1, and G_2) are produced mainly by the fungus *Aspergillus flavus*, both before and after harvest, in a number of plants and stored products, including rice. B_1 and B_2 are the most prevalent AFs that can contaminate agricultural products. AFs possess high acute and chronic toxicity because of their capacity to bind nucleic acids and cellular nucleoproteins, causing deleterious effects on protein synthesis and cellular integrity. They are considered potent hepatotoxins with high genotoxic activity and can cause hepatocarcinoma (IARC, 1993). DON, also known as vomitoxin, is a type B trichothecene, an epoxy-sesquiterpenoid, produced by *Fusarium graminearum* and

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Fusarium culmorum. DON is considered a potent inhibitor of protein synthesis and causes depression of the immune response, nausea, and feed refusal (Peraica, Radic, Lucic, & Pavlovic, 1999). NIV is another type B trichothecene that differs from DON only in the presence of an oxygen moiety at C-4. NIV was implicated as a chemical warfare agent with T-2 toxin in Southeast Asia. It is a strong hemorrhagic agent and has been shown to inhibit protein synthesis in rabbit reticulocytes in vitro (Ryu et al., 1987) and synthesis of nucleic acids in vitro (Thuvander, Wikman, & Gadhasson, 1999). ZEN is produced mainly by *F. graminearum* and causes estrogenic effects in various animal species, especially in swine, leading to sexual disorders, infertility, and abortion (IARC, 1993).

Identifying the contaminating mycoflora of rice at harvest is a prerequisite for establishing mycotoxin control programs. Several studies in India, Japan, Korea, Thailand, and Taiwan (Jayaraman & Kalyanasundaram, 1990; Pitt et al., 1994; Wu & Dow, 1993) have shown that paddy rice has a considerable level of fungal contamination, which is acquired primarily before and during harvesting and during storage. Which fungal genera are predominant in rice depends mainly on the soil type and climatic conditions. Despite the importance of rice as a staple food and the reported occurrence of mycotoxins, there is little information on the incidence of fungal flora and mycotoxins in rice freshly harvested in Korea.

The objectives of this study were (1) to investigate the incidence of fungal mycoflora in paddy rice harvested from six locations in Korea, (2) to survey the incidence of mycotoxigenic fungi in the paddy rice samples using polymerase chain reaction (PCR) analysis, and (3) to determine the natural occurrence of AFs, DON, NIV, and ZEN in white and brown rice after milling.

2. Materials and methods

2.1. Collection of rice samples and milling

Eighty paddy rice samples harvested in 2010 were collected from 80 farms in six different geographic locations in Korea (Table 1). All freshly harvested paddy rice samples were taken from the grain-collecting yard just before they were processed through the rice-processing complex, an integrated, computerized system of paddy postharvesting technology. The six sites were Gangneung (location A, $n = 20$), Anseong (location B, $n = 20$), Hwaseong (location C, $n = 10$), Jeongeup (location D, $n = 10$), Haenam (location E, $n = 10$), and Ulsan (location F, $n = 10$). These sites have different geographic coordinates (latitude 34.5–37.7°N, longitude 126.6–129.3°E) and climatic conditions (temperature 24.8–26.0 °C when measured over a period of 6–8 months and 17.8–19.7 °C over 9–10 months; relative humidity 72.3–81.3% over 6–8 months and 71.5–77.5% over 9–10 months; and rainfall 99.5–361.4 mm over 6–8 months and 75.4–203.0 mm over 9–10 months). Paddy rice (about 10 kg for each sample) was milled by Hansung Industrial Co., Ltd. (HSMC-4, Yesan, Korea) and was fractioned into 24% hulls and 76% brown rice per paddy rice weight. The brown rice was milled in a commercial abrasive mill (model VT-21T, Yamamoto Co. Ltd., Yamagata, Japan). The white rice yield was 76% of the brown rice weight. The milling fractions were stored at −18 °C until use. All samples were finely ground using a blender until the sample could pass through a No. 20 sieve and were kept at −18 °C in aluminum zipper bags to be subsampled before the analysis.

2.2. Materials and chemicals

Pure crystalline AFs B_1, B_2, G_1, and G_2, NIV, DON, and ZEN were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Single standards were prepared in acetonitrile, and composite working standard solutions were prepared by mixing aliquots of stock solution in acetonitrile. High-performance liquid chromatography (HPLC)-grade acetonitrile, methanol, and water were obtained from Burdick & Jackson (Muskegon, MI, USA). Immunoaffinity columns (IACs; Afla Test WB for AFs, DON Test WB for DON and NIV, and Zearala Test WB for ZEN) were purchased from Vicam Corp. (Watertown, MA, USA), and glass microfiber filters (GF/B, 110 mm internal diameter) were from Whatman (Maidstone, UK). All other inorganic chemicals and organic solvents were of reagent grade or higher.

2.3. Mycological analysis

2.3.1. Fungal isolation and culture

AF-producing strains (*A. flavus* KCCM60330 and *Aspergillus parasiticus* KCCM35078) and non-AF-producing strains (*A. flavus* KCCM60130 and *A. parasiticus* KCCM34792) were obtained from the collection of the Korea Culture Center of Microorganisms (KCCM, Seoul, Korea). DON-producing (*F. graminearum* KACC41407) and NIV-producing (*F. culmorum* KACC42099) strains were from the collection of the Korean Agricultural Culture Collection (KACC, Suwon, Korea) and were used as the reference strains for the PCR assay in this study. The fungi were isolated from rice samples according to the method of Magnoli, Hallak, Chiacchiera, and Dalcero (2006) with minor modifications. Briefly, each rice sample was surface-disinfected with a 1% sodium hypochlorite solution for 2 min, and followed by rinsed in sterile distilled water three times. One hundred rice grains were plated onto dichloran-rose bengal-chloramphenicol agar plates in duplicate. All plates were incubated at 25 °C in the dark for 7 days. The fungi showing different morphological characteristics (Nelson, Toussoun, & Marasas, 1983; Pitt & Hocking, 2009) were transferred to potato dextrose agar plates (PDA) for further identification.

2.3.2. Fungal identification

The fungal isolates were identified through their molecular characteristics for more accurate classification. The internal

Table 1
Sampling sites where the rice was harvested in Korea in 2010.

Area (administrative district)	Geographical coordinates		Climate condition (average of 2010 year)						Number of samples
			Temperature (°C)		Relative humidity (%)		Rainfall (mm)		
	Latitude (°N)	Longitude (°E)	6–8 Month	9–10 Month	6–8 Month	9–10 Month	6–8 Month	9–10 Month	
A (Gangneung)	37.7	128.8	24.8	17.8 (13.0)[a]	73.0	72.5 (62)[a]	99.5	162.3 (1102.8)[b]	20
B (Anseong)	37.0	127.2	25.2	18.3 (12.2)	80.3	77.5 (73)	231.9	203.0 (1470.6)	20
C (Hwaseong)	37.1	126.8	25.2	18.3 (12.2)	80.3	77.5 (73)	231.9	203.0 (1470.6)	10
D (Jeongeup)	35.5	126.8	26.0	19.3 (13.7)	72.3	71.5 (68)	361.4	75.4 (1748.3)	10
E (Haenam)	34.5	126.6	25.2	19.1 (13.7)	81.3	75.5 (74)	213.2	87.1 (1495.5)	10
F (Ulsan)	35.5	129.3	25.1	19.7 (14.0)	77.0	73.5 (66)	140.5	110.3 (1161.6)	10

[a] Annual mean value.
[b] Annual total value.