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Fumonisin Occurrence in Corn from High- and Low-Risk Areas for Human Esophageal Cancer in China

T. YOSHIZAWA,* A. YAMASHITA, AND Y. LUO

Department of Bioresource Science, Faculty of Agriculture, Kagawa University, Miki, Kagawa 761-07, Japan

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Forty-seven corn samples were collected in 1989 from Linxian and Shangqiu Counties in Henan Province, the high- and low-risk areas, respectively, for human esophageal cancer in the People's Republic of China. The samples were analyzed for fumonisin (fumonisin B_1 [FB_1] and FB_2) contamination. Of the fumonisin-positive samples, the mean levels in Linxian corn were found to be 872 ng/g for FB_1 and 448 ng/g for FB_2, while the Shangqiu corns had 890 ng of FB_1 and 330 ng of FB_2 per g. The incidence of fumonisin contamination of Linxian corn (48%) was about two times higher than that of Shangqiu corn (25%), and the former corn samples were frequently cocontaminated with trichothecenes. *Fusarium* species isolated from corn from Linxian County produced FB_1 at levels ranging from 1,280 to 11,300 μg/g.

Different *Fusarium* species occur worldwide on a variety of plant hosts and cause a number of human and animal diseases after consumption of fungus-damaged plant products. Trichothecenes (TRIC) and zearalenone, produced principally by *Fusarium graminearum* and *F. culmorum*, are two of the well-known causative agents of several mycotoxicoses (24, 25).

F. moniliforme Sheldon also has been suspected of involvement in chronic toxicoses of animals, e.g., equine leukoencephalomalacia and porcine pulmonary edema syndrome (7, 11). Recently, a new mycotoxin, fumonisin B_1 (FB_1), was isolated from culture materials of *F. moniliforme* and was demonstrated to be an etiologic agent associated with equine leukoencephalomalacia and porcine pulmonary edema (4, 10). FB_1 was shown to be both hepatotoxic and a promoter of carcinogenicity in rats (2). In addition, structurally related derivatives of FB_1, namely, FB_2 and FB_3 as minor toxins of the fungus, were also demonstrated to possess toxic properties similar to those of FB_1 (3). Moreover, FB_1 and FB_2 were detected in commercially available corn products intended for human consumption (13, 18, 20); hence, they are regarded as potential risks to human health.

On the other hand, the occurrence of *F. moniliforme* in corn appears to be correlated with the high rate of human esophageal cancer (HEC) in Transkei, South Africa, and in China (9, 26). FB_1 and FB_2 have been found in home-grown corn samples consumed by inhabitants of the rural areas in Transkei (22). Furthermore, a statistically significant correlation was demonstrated between the high incidence of HEC in this area and the fumonisin concentration in the staple (14, 21).

The major high-risk areas for HEC and esophagitis in the People's Republic of China are located in Henan, Hebei, and Shanxi Provinces, with Linxian County in Henan Province being the highest-risk area (5, 6). However, only limited surveys concerning the natural occurrence of mycotoxins in corn samples from HEC-prevalent areas have been made (8), and no study on the incidence of HEC in relation to levels of fumonisin has been reported. In this paper, we describe the natural occurrence of the *Fusarium* mycotoxins, including fumonisin, in corn samples from Linxian County and compare them with that in samples from Shangqiu County, an area at low risk for HEC. In addition, the ability of *Fusarium* isolates from Linxian corn to produce fumonisin is described.

MATERIALS AND METHODS

Corn samples. Corn samples analyzed in this study were those used in a previous study (8). A total of 47 corn kernel samples (at least 250 g each) were collected from Linxian and Shangqiu Counties, Henan Province, in 1989. Among these, 27 samples were obtained from 27 different families of HEC-affected patients (randomly selected) in Linxian. The remaining samples came from 20 different peasant families with no cases of esophageal cancer chosen at random in Shangqiu County. Originally, these samples were from corn stored on the cob both inside and outside. The corn was shelled, stored for a short time, and cooked without any selection for moldy ears or kernels. Individual corn samples were placed in zip-lock plastic bags and stored for approximately 18 months at −20°C prior to analysis.

Extraction and analysis. After 50-g corn samples were ground to a fine meal in a laboratory ultracentrifugal mill (Trio Blender 848-36MW; Trio Science Co. Ltd., Tokyo, Japan), a subsample (10 g) was extracted with acetonitrile-water (1:1; 50 ml) and filtered. The filtrate was partitioned with *n*-hexane, and the aqueous layer was evaporated to dryness. The residue was dissolved in acetonitrile-water (1:1; 5 ml) and applied to a preconditioned strong anion-exchange cartridge (Sep-Pak Accell Plus QMA; Millipore Corp., Milford, Mass.). The cartridge was eluted successively with methanol-water (3:1; 10 ml), methanol (5 ml), and 0.5% acetic acid in methanol (10 ml). The last eluate was evaporated to dryness and redissolved in acetonitrile-water (1:1; 2 ml) to provide a sample solution. This solution was analyzed for FB_1 and FB_2 according to the method of Shephard et al. (17), with a slight modification. Briefly, an aliquot of the sample solution was derivatized with *o*-phthaldialdehyde and analyzed on a Shimadzu LC-6A high-performance liquid chromatograph (HPLC) equipped with a spectrofluorometer under the following conditions: column, Shim-Pack CLC-ODS, 250 by 4.6 mm (inside diameter) (Shimadzu Scientific Inc., Kyoto, Japan); emission wavelength, 440 nm; excitation wavelength, 335 nm. The mobile phase was methanol-sodium dihydrogen phosphate buffer (pH 3.3; 76:24) at a flow rate of 0.5 ml/min. Quantification of fumonisin was

* Corresponding author. Mailing address: Department of Bioresource Science, Faculty of Agriculture, Kagawa University, Miki, Kagawa 761-07, Japan. Fax: 0878-98-7295.

TABLE 1. Natural occurrence of fumonisin and TRIC in corn samples from Linxian and Shangqiu Counties

Location[a]	No. of samples	No. (%) positive			Mean level (range) of positive samples (ng/g)		
		FB_1	FB_2	FB + TRIC[b]	FB_1	FB_2	TRIC[c]
Linxian	27	13 (48)	3 (11)	13 (48)	872 (186–2,964)[d]	448 (298–550)[d]	781 (17–4,213)
Shangqiu	20	5 (25)	2 (10)	1 (5)	890 (197–1,732)	330 (213–447)	102 (14–612)

[a] Linxian samples were randomly collected from the families of patients with esophageal cancer; Shangqiu samples were randomly collected from peasant families with no esophageal cancer.
[b] Corn samples cocontaminated with fumonisin (FB_1 and FB_2) and TRIC at levels above 100 ng/g.
[c] The major TRIC in corn samples were deoxynivalenol and its 15-acetate. Data are total TRIC concentrations determined from a previous study (8). Mean TRIC levels in Linxian corn samples were significantly ($P < 0.01$) higher than those in Shangqiu corn samples.
[d] No significant difference was found between mean concentrations of fumonisins in Linxian and Shangqiu County samples ($P > 0.05$).

based on externally similarly derivatized authentic standards. Standard FB_1 and FB_2 (98% purity) were purchased from the Division of Food Science and Technology, Council for Scientific and Industrial Research, Pretoria, South Africa. The detection limits were approximately 100 ng/g each for FB_1 and FB_2. Mean recoveries determined from triplicate analyses of corn samples spiked with 2 and 0.5 μg of standard per g were 98% ± 6% and 91% ± 6%, respectively, for FB_1 and FB_2.

Fungal isolation and toxin production. Seventeen strains of *Fusarium* species used in this study were isolated from Linxian corns, and single-spore isolates were prepared from each strain. The isolates were grown on potato dextrose agar and carnation leaf agar and were identified as described by Booth (1) and Nelson et al. (12). Sixty milliliters of water was added to yellow corn grits (100 g), with no detectable FB_1 and FB_2, in a 1-liter Erlenmeyer flask, and the flask was autoclaved on two consecutive days for 25 min at 121°C. The individual isolates were inoculated on these corn media, and cultures were incubated in the dark at 25°C for 3 weeks. An aliquot (10 g) of the cultured corn was extracted with acetonitrile-water (1:1; 50 ml), using a laboratory homogenizer (Physcotron NS-50; NITI-ON Co. Ltd., Nara, Japan). After filtration, the filtrate was partitioned with *n*-hexane and the aqueous layer was evaporated to dryness. The residue was dissolved in acetonitrile-water (1:1; 5 ml) and applied to a Sep-Pak C_{18} cartridge (Millipore). The cartridge was washed successively with water (2 ml) and acetonitrile-water (1:4; 2 ml), and then the fumonisins were eluted with acetonitrile-water (1:1; 5 ml). An aliquot of this eluate was reacted with *o*-phthaldialdehyde and analyzed by HPLC under the conditions described above.

RESULTS AND DISCUSSION

The range and mean levels of FB_1 and FB_2 concentrations together with the number of samples positive for each fumonisin are given in Table 1.

FB_1 was found in concentrations ranging from 186 to 2,964 ng/g (mean of positive samples, 872 ng/g) in 48.1% (13 of 27) of corn samples from Linxian, among which three samples were coincidentally contaminated with FB_2 (298, 495, and 550 ng/g). On the other hand, 5 of the 20 Shangqiu corn samples (25%) contained FB_1 in concentrations ranging from 197 to 1,732 ng/g (mean of positive corn samples, 890 ng/g). FB_2 was also detected together with FB_1 in two of the samples (213 and 447 ng/g). Thus, the incidence of fumonisin in corn samples from Linxian, the high-risk area, was about two times higher than in samples from Shangqiu, the low-risk area, although no significant ($P > 0.05$) difference in FB_1 and FB_2 levels between the two areas was found.

The incidence and mean levels of fumonisin in Linxian corns were close to those reported in corn-based human food products in the United States (18, 20) and Switzerland (13). In the United States, 94% of corn meal for human consumption analyzed in 1991 was contaminated, with mean levels of 1,048 and 298 ng/g for FB_1 and FB_2, respectively. However, fumonisin levels obtained from Linxian corns were apparently much lower than those found in corn samples from Transkei, the high-risk area for HEC in South Africa (21), where fumonisin levels were comparable to those determined in feed samples associated with field outbreaks of equine leukoencephalomalacia in several countries (16, 19, 23). Despite these facts, appreciably high levels of fumonisin were found in two corn samples (7.4%; 3,395 and 3,459 ng/g) from the high-risk area in China (Linxian), and similar levels of fumonisin have not been detected in commercially available corn-based human food products.

More remarkable was the co-occurrence of fumonisin and TRIC in the Chinese corn samples. Previously, we reported that the major TRIC found in these corn samples were deoxynivalenol and its 15-acetate and that the incidence and levels of these mycotoxins in Linxian corn samples were significantly ($P < 0.01$) higher than those in Shangqiu corns (8). Figure 1 illustrates the distribution of Linxian and Shangqiu corn samples (28 samples) contaminated with fumonisin (FB_1 plus FB_2; 18 samples) and/or TRIC (deoxynivalenol plus its 15-acetate; 24 samples) at concentrations of >100 ng/g; this level was the detection limit of the analytical method used. Among the 22 corn samples from Linxian County, only TRIC

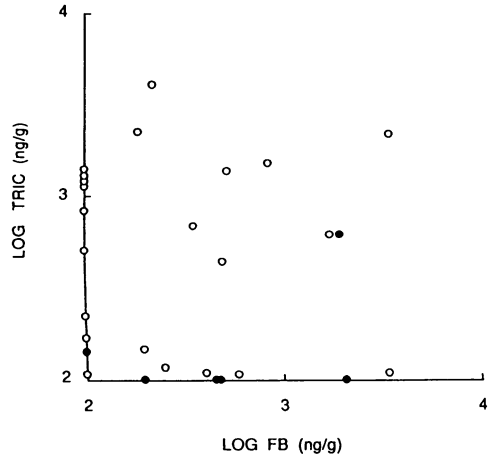

FIG. 1. Distribution of Linxian and Shangqiu corn samples contaminated with FB and/or TRIC at concentrations of >100 ng/g. FB, FB_1 plus FB_2; TRIC, principally deoxynivalenol and its 15-acetate. ○, Linxian corn samples ($n = 22$); ●, Shangqiu corn samples ($n = 6$).

TABLE 2. FB_1 and FB_2 concentrations in corn cultures of *F. moniliforme* and *F. proliferatum* isolates from corn as a staple food of families of patients with HEC in Linxian County

Corn sample code	Concn (ng/g) in corn		Species and isolate[a]	Concn (μg/g) in culture		Ratio, FB_1/FB_2
	FB_1	FB_2		FB_1	FB_2	
27H	2,964	495	*F. moniliforme* KU2324	8,800	510	17.4
			F. moniliforme KU2326	6,980	620	11.2
			F. proliferatum KU2325	5,510	790	7.0
10H	2,845	550	*F. moniliforme* KU2320	5,710	910	6.3
			F. proliferatum KU2317	11,300	1,950	5.8
			F. proliferatum KU2318	7,300	1,590	4.6
			F. proliferatum KU2319	7,090	1,140	6.2
13H	1,446	298	*F. proliferatum* KU2321	7,100	1,070	6.6
			F. proliferatum KU2322	6,170	910	6.8
			F. proliferatum KU2323	8,180	1,440	5.7
14H	855	ND[b]	*F. moniliforme* KU1560	1,280	210	6.2
			F. proliferatum KU1558	4,250	420	10.0
			F. proliferatum KU1559	3,920	830	4.7
			F. proliferatum KU1561	1,560	140	11.1
			F. proliferatum KU1564	4,980	970	5.2
22H	ND	ND	*F. moniliforme* KU1548	10,200	1,130	9.0
			F. proliferatum KU1547	1,960	420	4.7

[a] Identification number of authors' laboratory.
[b] ND, not determined.

was detected in 9 samples, while both fumonisin and TRIC were present in the remaining 13 samples. Only one of six corn samples from Shangqiu was contaminated with both toxins. Thus, the incidence (48%) of Linxian corn samples cocontaminated with both fumonisin and TRIC was approximately 10 times higher than that of similarly contaminated Shangqiu corn samples (Table 1).

The relative incidence of *Fusarium* species, including fumonisin-producing fungi, was not determined because of limited amounts of corn samples. From five corn samples from Linxian County, however, *F. proliferatum* and *F. moniliforme* were isolated and examined for fumonisin production. As shown in Table 2, all of the 12 isolates of *F. proliferatum* showed high levels of production of FB_1 ranging from 1,500 to 11,300 μg/g and produced FB_2 at levels ranging from 140 to 1,950 μg/g, while *F. moniliforme* (5 isolates) produced FB_1 at levels ranging from 1,280 to 10,200 μg/g and FB_2 at levels ranging from 210 to 1,130 μg/g. Although the toxin levels shown in Table 2 were determined on the basis of the wet weights of corn cultures, the isolates from Linxian corn samples had considerably higher toxin production levels than reported in previous papers (15). The FB_1/FB_2 ratios, varying from 4.6 to 17.4, for all isolates were similar to those reported previously (15, 23). The results suggest that corn samples from the high-risk area infected with both *F. moniliforme* and *F. proliferatum* contained high levels of fumonisin, which may be largely due to the latter organism.

This is the first report of a comparative study of the natural occurrence of fumonisin in staple foods in relation to the incidence of HEC in the People's Republic of China. There are several differences in mean levels and incidence of this mycotoxin contamination when South Africa and China are compared. The levels of FB_1 and FB_2 in corn samples were significantly correlated with the incidence of HEC in South Africa (14, 21, 22), but the correlation was insignificant in China, as shown in this paper. On the other hand, the incidence and levels of TRIC in corn samples were clearly correlated with the incidence of HEC in China (8) but not in South Africa (21). Hence, continuous studies are needed to clarify the occurrence and level of *Fusarium* toxins in corn intended for human consumption in both areas.

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