Natural Occurrence of the C Series of Fumonisins in Moldy Corn

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We analyzed 44 moldy corn samples for the B and C series of fumonisins by high-performance liquid chromatography. Of the 44 samples, 32 (73%) were contaminated with both the B and C series of fumonisins and 6 were contaminated with only the B series of fumonisins. The incidence of fumonisin C1 in moldy corn was 71%; the incidence was 11% for fumonisin C3 and 43% for fumonisin C4. Their mean levels ranged from 500 to 1,900 ng/g. This is the first report on the natural occurrence of the C series of fumonisins and fumonisin B4 in moldy corn.

Fumonisins are a structurally related group of mycotoxins that are frequently found in corn and corn-based food products (12). Consumption of fumonisin-contaminated corn is the causal factor of leukoencephalomalacia in horses (11, 16) and of pulmonary edema in swine (2, 9) and is correlated with the high incidences of human esophageal cancer in South Africa and China (6, 20).

Chemically, fumonisins are characterized by a 19- or 20-carbon aminopolyhydroxyalkyl chain which is diesterified with propane-1,2,3-tricarboxylic acid. The A, B, C, and P series, as well as some partially hydrolyzed fumonisins, have been isolated from cultures of Fusarium moniliforme (= F. verticillioides) and characterized (3–5, 13, 17). Among these, the C series of fumonisins are chemically similar to the B series; the chemical structures of fumonisin C1 (FC1), fumonisin C3 (FC3), and fumonisin C4 (FC4) are identical to those of fumonisin B1 (FB1), fumonisin B3 (FB3), and fumonisin B4 (FB4), respectively, except that the C-1 terminal methyl group is missing (4, 17, 19). Hydroxylated FC1 (OH-FC1) has one more hydroxy group at the C-3 position than does FC1. Since the first report of the natural occurrence of FB1 in corn (21), contamination of corn and corn-based feed or food samples by FB1, fumonisin B2 (FB2), FB3, fumonisin A2 (FA2) and by partially hydrolyzed fumonisins has been reported by many laboratories (2, 7, 10, 15, 18, 22–24), whereas the natural occurrence of FB4, fumonisin A1 (FA1), and the C series of fumonisins has not been reported.

During the screening of fumonisins produced in cultures of Fusarium species, one F. oxysporum isolate produced the C series of fumonisins as its major toxins (19). This isolate (MRC 7547) (PROMEC, Medical Research Council, Tygerberg, South Africa) was submitted to W. F. O. Marasas for identification. He confirmed that the isolate is F. oxysporum according to the criteria of Nelson et al. (14). We obtained substantial amounts of the C series of fumonisins from wheat cultures of this isolate for chemical and toxicological studies. The objective of this study was to survey the C series of fumonisins in moldy corn samples.

Forty-four samples of visibly moldy corn cobs were collected after corn harvest in the Kangwon province of Korea during November 1997. This grain was intended for animal consumption. All samples were shelled, milled to 120–250 mesh with a laboratory mill, and stored at −20°C before analysis. The standard toxins of the B and C series of fumonisins were prepared in our laboratory from wheat cultures of F. moniliforme and F. oxysporum MRC 7547, respectively.

Fumonisins were extracted from corn samples according to

![FIG. 1. HPLC chromatogram of the extract of a moldy corn sample.](http://aem.asm.org/content/65/3/1331.f1)

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**TABLE 1. Natural occurrence of the B and C series of fumonisins in 44 moldy corn samples from Korea**

<table>
<thead>
<tr>
<th>Fumonisin</th>
<th>No. of positive samples</th>
<th>Mean level (range [ng/g]) in positive samples*</th>
</tr>
</thead>
<tbody>
<tr>
<td>FB1</td>
<td>38</td>
<td>18,500 (100–160,000)</td>
</tr>
<tr>
<td>FB2</td>
<td>34</td>
<td>5,600 (90–46,000)</td>
</tr>
<tr>
<td>FB3</td>
<td>32</td>
<td>2,500 (50–31,000)</td>
</tr>
<tr>
<td>FB4</td>
<td>23</td>
<td>1,600 (80–11,000)</td>
</tr>
<tr>
<td>FC1</td>
<td>31</td>
<td>1,900 (60–11,000)</td>
</tr>
<tr>
<td>FC3</td>
<td>5</td>
<td>1,700 (100–12,000)</td>
</tr>
<tr>
<td>FC4</td>
<td>18</td>
<td>500 (50–3,300)</td>
</tr>
</tbody>
</table>

* Fumonisins were quantified by HPLC by use of a fluorescence detector with OPA derivatization.
the procedure of Xie et al. (24). A 0.5-ml portion of the cleanup samples was reacted with 0.1 ml of ortho-phthalaldehyde (OPA) solution (40 mg of OPA per ml of methanol followed by dilution with 5 ml of disodium tetraborate buffer [pH 9.0] and with 50 μl of mercaptoethanol) at room temperature and then injected into a high-performance liquid chromatography (HPLC) column within 1 min. For HPLC analysis, the following equipment and conditions were used and maintained: tsp Spectra SYSTEM (Thermo Separation Products Inc., San Jose, Calif.); Bondclone10 C_{18} column (30 cm by 4.9 mm [inside diameter]; particle size, 10 μm; Phenomenex Co., Torrance, Calif.); mobile phase, an acetonitrile gradient containing 1% acetic acid beginning with acetonitrile-water (20:80, vol/vol) with a hold of 1 min and changing linearly to acetonitrile-water (80:20, vol/vol) at 30 min with a final hold of 10 min; flow rate, 1 ml/min; FL3000 fluorescence detector, excitation at 336 nm and emission at 440 nm. Retention times of fumonisins were 21.9 min for FC_{1}, 22.7 min for FB_{1}, 25.7 min for FC_{3}, 26.2 min for FB_{2}, 26.7 min for FB_{3}, 29.6 min for FC_{4}, and 30.5 min for FB_{4}. The calculation of values for each fumonisin was

Fig. 2. Total-ion chromatograms of a moldy corn extract (A) and electrospray mass spectra of FB_{1} (B) with [M+H]^{+} at m/z 690, FC_{3} (C) with [M+H]^{+} at m/z 708, FC_{3} (D) with [M+H]^{+} at m/z 692, and FC_{4} (E) with [M+H]^{+} at m/z 676 in the sample extract.
based on the external standards of fumonisins. When control corn was spiked with 1,000 ng of each fumonisin per g of corn sample, recoveries ranged from 85% ± 2.3% to 103% ± 1.2%.

The presence of the B and C series fumonisins detected by HPLC was confirmed by liquid chromatography-mass spectrometry (LC-MS) on a Finnigan LCQ mass spectrometer (Finnigan Analytical, San Jose, Calif.) with the standard Finnigan electrospray ion source. An HP 1050 LC pump (Hewlett-Packard Co., Palo Alto, Calif.) was used, and LC was performed on a Capcell-Pak C18 LC column (25 cm by 4.6 mm inside diameter; particle size, 5 μm; Shiseido, Tokyo, Japan). Chromatographic elution was accomplished with a gradient system beginning with acetonitrile-water (20:80, vol/vol) and 0.1% trifluoroacetate for 3 min and then with a linear gradient to acetonitrile (80:20; vol/vol) at 40 min with a final hold of 10 min. Under these conditions, the underivatized mixture of the B and C series of fumonisins was resolved at 17.3 min for FC1, 17.7 min for FB1, 19.1 min for FC2, 20.2 min for FB3, 21.6 min for FB2, 23.6 min for FC3, and 24.2 min for FB4.

It was difficult to resolve each peak of the B and C series of fumonisins when they were mixed together. For HPLC analysis, various solvent gradient programs were tested for separation of the fumonisins. An acetonitrile gradient containing 1% acetic acid beginning at an acetonitrile-water ratio of 20:80 and changing linearly to a ratio of 80:20 was selected as the best program to separate the fumonisins (Fig. 1). However, the peaks of FB2 and FB4 were not completely separated under these conditions. Of the 44 moldy corn samples, 32 were contaminated with both the B and C series of fumonisins and 6 were contaminated with only the B series of fumonisins (Table 1). The levels of the C series of fumonisins were approximately 10% of those of the B series of fumonisins. Of the 32 samples with both the B and C series of fumonisins, FB1 was the major toxin in 27 samples and FB3 and FC1 were the major toxins in 2 and 3 samples, respectively. In the 3 samples contaminated with FC1 as the major toxin, the levels of FC1 and FB1 were 200/100, 4,000/300, and 11,000/1,000 ng/g, respectively.

To verify the simultaneous presence of the B and C series of fumonisins, one corn sample extract, which was positive for the C series of fumonisins in HPLC analyses, was subjected to LC-MS. The peaks of FB3 and FC1 (Fig. 2A) were not separated because the concentration of FB3 was much higher than that of FC1, but mass spectra of both compounds were obtained. Figure 2B to E shows electrospray mass spectra of FB3, FC1, FB2, FC3, and FC4 naturally occurring in corn. The protonated molecular ions for FB1, FB2, FB3, FB4, FC1, FC2, FC3, and FC4 are m/z 722, 706, 706, 690, 708, 692, and 676, respectively. The agreement of retention times and mass spectra of these fumonisins with those of standard toxins verified the presence of the C series of fumonisins in the sample extract.

The ubiquitous nature of fumonisins in corn and the toxicity associated with fumonisin ingestion have instigated a number of surveys to determine levels of FB1 or the B series of fumonisins in corn and corn products throughout the world (2, 7, 10, 15, 18, 20–23). The occurrence of the C series of fumonisins in corn might be significant. Two of the moldy corn samples were contaminated with more than 10 μg of both FC1 and FC3 per g, and the incidence of FC1 was similar to that of FB3 in this study.

The B series of fumonisins initiated cancer in the rat liver, whereas the A series, including FA1 and FA2, and the hydrolysis products of fumonisins did not (8). Thus, the free amino group and an intact molecule are required for cancer initiation. Although the C series of fumonisins have not been tested as cancer initiators, they are potential carcinogens because the C series of fumonisins, like the B series, have a free amino group and their structures are similar to the B series of fumonisins except for the terminal methyl group. In addition, FC1 exhibited phytotoxicity and cytotoxicity at concentrations similar to those of FB1 (1). This is the first report of the natural occurrence of the C series of fumonisins and FB3 in corn.

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REFERENCES