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Production of Fumonisins by *Fusarium moniliforme* Strains from Various Substrates and Geographic Areas†

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Strains of *Fusarium moniliforme* from different geographic areas and from corn and other substrates were tested for the ability to produce fumonisins in culture. The test results indicate that the potential exists for production of fumonisins by such strains in agricultural commodities and other substrates in widespread geographic areas.

The fungus *Fusarium moniliforme* Sheldon (13) occurs worldwide on a variety of plant hosts and is one of the most prevalent fungi associated with dietary staples such as corn. Since its original description in 1904 (7), this fungus has been suspected of being involved in human and animal diseases and has been shown to be toxic to a variety of experimental animals (4, 5). In 1988, Bezuidenhout et al. (1) characterized the structures of fumonisins, a new group of mycotoxins that had been purified from cultures of *F. moniliforme* (3). Fumonisin B_1 (Fig. 1) is the major fumonisin present both in cultures and in naturally contaminated samples (10, 12, 15). It is the only fumonisin for which toxicity data have been reported. Fumonisin B_1 has been shown to have cancer-promoting activity in rats (3), to cause equine leukoencephalomalacia (6), and to be associated with porcine pulmonary edema (11). Much of the work reported has been on fumonisins from *F. moniliforme* MRC 826 isolated from moldy home-grown corn in an area of southern Africa with a high rate of human esophageal cancer (5). The purpose of this study was to examine strains of *F. moniliforme* other than MRC 826 from different geographic areas and from corn, other cereals, and miscellaneous substrates for the ability to produce fumonisins in culture.

The cultures of *F. moniliforme* used in this study were obtained from the culture collection of the Fusarium Research Center, The Pennsylvania State University. All cultures were isolated originally on a modified pentachloronitrobenzene medium (8) selective for *Fusarium* species, mass transferred to potato dextrose agar and carnation leaf agar, and identified (2, 8). Selected cultures were initiated from single conidia on carnation leaf agar, lyophilized, and stored at −40°C (8). The accession number and source of each culture are given in Table 1.

Slants containing 8 ml of V-8 juice agar medium (14) were inoculated with lyophilized cultures. The slants were grown for 1 to 2 weeks on an alternating 12-h, 25°C light-20°C dark schedule and then washed with sterile distilled water to produce conidial suspensions. Erlenmeyer flasks (300 ml) with Morton closures were filled with 50 g of coarsely cracked corn and 11 ml of distilled water and autoclaved for 30 min. After autoclaving, an additional 11 ml of sterile distilled water was added aseptically to each flask. Each flask was inoculated with 10^7 conidia and shaken once or twice daily for 3 days to aid in even distribution of the inoculum. The flask cultures were incubated in the dark at 25 ± 2°C for 28 days.

Each corn culture was assayed for fumonisins by soaking 5 g of culture material in 100 ml of distilled water for 2 to 3 h, with mixing every half hour. This suspension was filtered through Whatman no. 1 paper in a Buchner funnel, and the filtrate was poured through a Millipore SC filter with an AP25 prefilter (Millipore Corp., Bedford, Mass.), collected, and evaporated to dryness at 65°C on a rotary evaporator.

The residue was suspended in 10 ml of water, and a 2-ml aliquot (1 g equivalent) was evaporated to dryness at 70°C under nitrogen and hydrolyzed in 2 N aqueous KOH, and the hydrolysis product was derivatized with trifluoroacetic anhydride and analyzed by gas chromatography-mass spectroscopy (GC-MS) as previously reported (10). The detection limit for this GC-MS method for fumonisin B_1 was about 1 ppm in corn cultures. Results obtained by thin-layer chromatography for cultures that produced more than 300 to 400 ppm of fumonisin B_1 agreed well with the GC-MS results. One microliter of the suspended residues was spotted on a silica thin-layer chromatography plate along with standards and developed in acetonitrile-water (85/15). Fumonisin B_1 was visualized by spraying the plate with *p*-anisal-

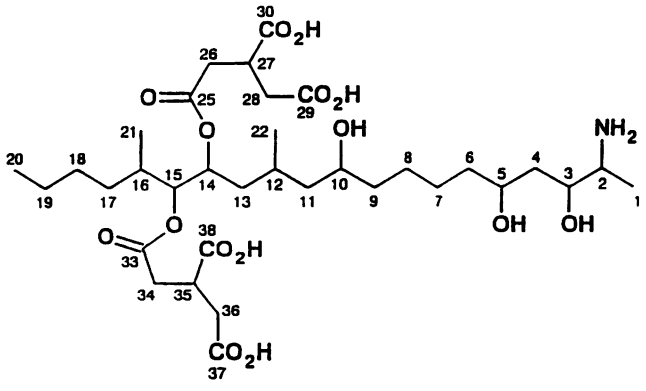

FIG. 1. Structure of fumonisin B_1.

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TABLE 1. The production of fumonisin B_1 by cultures of *F. moniliforme* isolated from various substrates and geographic areas

FRC[a] accession no. (fumonisin B_1 concn [ppm])							
Corn-based feed samples associated with ELEM[b] outbreaks in Ga., Ind., Miss., N.C., and Pa.	Millet and sorghum grain from Nigeria and Zimbabwe	Corn-based laboratory rat diet samples from the United States	Corn kernels from Nepal	Sorghum stalks and grain, corn, sugarcane, and soil from Queensland and New South Wales, Australia	Mycotic keratitis, and various types of cancer in humans from Canada and the United States	Good quality corn for use in poultry feed from Md., Va., and Pa.	Corn silks from Iowa
M-2546 (2,589)	M-5042 (2,448)	M-3089 (825)	M-5496 (ND)[d]	M-1775 (trace)	M-772 (1,914)	M-2111 (1,927)	M-5630 (1,526)
M-2552 (6,421)	M-5067 (733)	M-3090 (300)	M-5500 (trace)	M-1776 (trace)	M-773 (2,500)	M-2232 (3,091)	M-5633 (1,973)
M-2647 (1,047)	M-5068 (539)	M-3092 (10)	M-5507 (trace)	M-1792 (trace)	M-775 (1,300)	M-2258 (1,891)	M-5648 (1,000)
M-2650 (144)	M-5076 (1,782)	M-3093 (780)	M-5515 (trace)	M-1794 (trace)	M-1006 (1,150)	M-2270 (459)	M-5664 (1,094)
M-2669 (1,391)	M-5081 (trace)	M-3094 (10)	M-5519 (60)	M-1795 (trace)	M-1086 (300)	M-2285 (2,716)	M-5672 (1,529)
M-2672 (2,912)	M-5193 (trace)		M-5525 (6,397)	M-1796 (trace)	M-1102 (ND)	M-2310 (1,402)	M-5679 (310)
M-2677 (1,335)	M-5199 (trace)		M-5534 (trace)	M-1797 (trace)	M-1810 (130)	M-2325 (1,919)	M-5693 (3,253)
M-2958 (195)	M-5207 (trace)		M-5538 (trace)	M-3673 (trace)	M-2507 (2,150)	M-2326 (1,406)	M-5697 (3,482)
M-2962 (917)	M-5210 (trace)		M-5542 (48)	M-3674 (trace)	M-2768 (ND)		M-5705 (2,183)
M-2999 (826)	M-5223 (trace)		M-5550 (trace)	M-3675 (trace)	M-2978 (1,700)		
M-3030 (629)	M-5234 (95)				M-3632 (1,400)		
M-3031 (trace)[c]	M-5243 (104)				M-5114 (2,900)		
M-3034 (6,090)	M-5252 (trace)				M-5187 (800)		
M-3035 (739)	M-5254 (trace)						
M-3036 (1,973)	M-5257 (trace)						
M-3037 (1,422)							
M-3038 (2,234)							
M-3039 (2,078)							
M-3040 (2,000)							
M-3041 (323)							

[a] FRC, Fusarium Research Center.
[b] ELEM, equine leukoencephalomalacia.
[c] Culture produced less than 10 ppm.
[d] ND, none detected.

dehyde reagent and charring it (3). The detection limit of this confirmatory procedure was 300 to 400 ppm.

Ninety strains of *F. moniliforme* were grown on cracked corn, and the cultures were analyzed for fumonisins by hydrolysis–GC-MS and confirmed by thin-layer chromatography. The fumonisin B_1 levels produced are reported in Table 1. These strains were selected for analysis specifically to represent a wide range of substrates and geographic areas. In most cultures, three fumonisin homologs were detected by the GC-MS procedure. The predominant fumonisin homolog present was B_1, which typically accounted for 70 to 95% of the total fumonisins measured. The other two fumonisins detected were B_2, which lacks the hydroxyl group at C-10 of the backbone (3, 4), and a newly characterized isomer of B_2, the structure of which will be reported separately (9). None of the A series fumonisins were detected in any cultures. The corn used as the substrate for this study had a low but detectable level of naturally occurring fumonisin B_1 (1 to 2 ppm).

The fumonisin B_1 level of replicate cultures grown at the same time was reproduced within ±20 to 30%, which was approximately the standard deviation of the GC-MS analysis procedure. In addition, replicate cultures were grown at different times and tested for production of fumonisin B_1. Some cultures were initiated from V-8 slants grown for 1 to 2 weeks and stored at 4°C for 0 to 9 weeks. Other cultures tested were initiated from fresh V-8 slants subcultured from V-8 slants that were stored at 4°C for 1 year. Cultures initiated from either source had only slightly greater variability in fumonisin B_1 production than did replicate cultures grown at the same time.

There was considerable variation in the amounts of fumonisins produced by strains from different sources but, in general, less variation among strains from the same source. For example, fumonisin B_1 was produced by most (95%) of the strains from corn-based feed that had been associated with equine leukoencephalomalacia, all of the strains from good quality corn for use in poultry feed, and all of the strains from corn silks in Iowa at either the intermediate (50 to 500 ppm) or high (>500 ppm) level. In contrast, of the 10 strains from Nepal, all but 1 were low-level producers (trace to 49 ppm). Strains from millet and sorghum grain from Africa, from a corn-based laboratory rat diet, and from sorghum from Australia were low- and intermediate-level producers primarily, although a few strains in some groups were high-level producers. It is of particular interest that most (85%) of the strains from mycotic keratitis, ulcers, and various types of cancer in humans were intermediate- or high-level producers. Although there is some variation in the amounts of fumonisins produced, the potential for production in natural substrates and agricultural commodities certainly exists in strains from a variety of substrates and geographic areas.

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