APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Apr. 1991, p. 1089–1093
0099-2240/91/041089-05$02.00/0

Vol. 57, No. 4

Survey of Fumonisin Production by *Fusarium* Species

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Received 5 November 1990/Accepted 15 January 1991

Fumonisins B_1 (FB_1) and B_2 (FB_2), two structurally related mycotoxins with cancer-promoting activity, were recently isolated from corn cultures of *Fusarium moniliforme* MRC 826. These toxins have been reported to be produced also by isolates of *F. proliferatum*. Contamination of foods and feeds by *F. moniliforme* has been associated with human esophageal cancer risk, and FB_1 has been shown to be the causative agent of the neurotoxic disease leukoencephalomalacia in horses. Because of the toxicological importance of the fumonisins, the potential to produce FB_1 and FB_2 was determined in a study of 40 toxic *Fusarium* isolates representing 27 taxa in 9 of the 12 sections of *Fusarium*, as well as two recently described species not yet classified into sections. With the exception of one isolate of *F. nygamai*, fumonisin production was restricted to isolates of *F. moniliforme* and *F. proliferatum*, in the section Liseola. The *F. nygamai* isolate produced 605 μg of FB_1 g^{-1} and 530 μg of FB_2 g^{-1}, and the identity of the toxins was confirmed by capillary gas chromatography-mass spectrometry. This is the first report of the production of the fumonisins by *F. nygamai*.

Fusarium moniliforme contamination of feeds has been associated with several diseases in animals, including leukoencephalomalacia (LEM) in horses (12), and the incidence of *F. moniliforme* infection of home-grown corn has been shown to be correlated with the high incidence of human esophageal cancer in Transkei, southern Africa (9, 10, 16), and in China (8, 27).

Fumonisins B_1 (FB_1) and B_2 (FB_2), structurally related mycotoxins with cancer-promoting activity, were recently isolated from corn cultures of *F. moniliforme* Sheldon strain MRC 826 (3), and their structures were elucidated (1). FB_1 has since been shown to induce LEM in horses after either intravenous (11) or per os (6) administration and to induce pulmonary edema in swine after intravenous administration (5).

Both FB_1 and FB_2 have been found to occur naturally in corn and in feeds associated with field outbreaks of LEM in horses (20, 21, 23, 25, 26). Higher concentrations of both toxins have been detected in corn samples from areas with a high esophageal cancer rate in Transkei than in samples from low-rate areas (22).

At present there is no experimental proof that the fumonisins, which are rat liver cancer promoters, are causative agents of esophageal cancer in humans. However, the toxicological importance of the fumonisins emphasizes the need to establish the extent of human and animal exposure to these toxins. One approach is to screen different fungal isolates that commonly occur on foods and feeds for their potential to produce the fumonisins. A recent report (19) documented the production of FB_1 and FB_2 by cultures of both *F. moniliforme* and *F. proliferatum* (Matsushima) Nirenberg isolated from feeds associated with LEM in horses and pulmonary edema in swine in the United States.

In the present study 40 toxic *Fusarium* isolates representing 27 taxa in 9 of the 12 sections of *Fusarium* as classified by Nelson et al. (17), as well as two recently described species not yet classified into sections, were screened for their potential to produce FB_1 and FB_2.

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MATERIALS AND METHODS

***Fusarium* isolates.** All *Fusarium* isolates used in this investigation were obtained from the culture collection of the Research Institute for Nutritional Diseases, Medical Research Council, Tygerberg, South Africa. Cultures on corn of all the isolates tested were known to be highly toxic to ducklings, causing four of four deaths when fed to 1-day-old Pekin ducklings for 14 days, as previously described (15). The origin of each isolate, the substrate from which it was isolated, and the *Fusarium* taxon to which it belongs are given in Table 1. The *Fusarium* taxa are also arranged in sections as described by Nelson et al. (17), except for the two recently described species *F. nygamai* Burgess & Trimboli (2) and *F. napiforme* Marasas, Nelson & Rabie (13), which have not yet been classified into sections.

Culture techniques. Lyophilized conidia of the different *Fusarium* isolates were suspended in sterile water and used to inoculate moistened yellow corn kernels (400 g of kernels and 400 ml of water) in 2-liter glass fruit jars previously autoclaved at 121°C for 1 h on each of 2 consecutive days. Cultures were incubated in the dark at 25°C for 21 days, dried overnight at 45°C, ground to a fine meal in a laboratory mill, and stored at 0°C until analyzed.

Reference standards. FB_1 and FB_2 standards were isolated and purified from cultures of *F. moniliforme* MRC 826 as previously described (3). The identity and purity of each standard were assessed by thin-layer chromatography, high-performance liquid chromatography, and nuclear magnetic resonance spectroscopy.

Determination of fumonisins. FB_1 and FB_2 were determined by a recently developed high-performance liquid chromatography method (20). Briefly, fumonisins were extracted from a sample of the culture material with methanol-water (3:1, vol/vol). The extract was purified on a strong anion-exchange cartridge, and an aliquot was derivatized with *o*-phthaldialdehyde. The derivatized fumonisins were separated on a reversed-phase column, monitored by fluorescence detection, and quantified by comparison of peak areas with those obtained with reference standards of FB_1 and FB_2.

TABLE 1. Fumonisin production by *Fusarium* species

Section, MRC[a] no., and species	Origin	Substrate	Fumonisin concn $\mu g\ g^{-1}$	
			FB_1	FB_2
Spicarioides				
2801 *F. decemcellulare*	South Africa	Avocado	ND[b]	ND
Sporotrichiella				
43 *F. sporotrichioides*	France	Corn	ND	ND
3424 *F. poae*	South Africa	Barley	ND	ND
1577 *F. tricinctum*	United States	Ivy	ND	ND
Roseum				
3302 *F. avenaceum*	South Africa	Medicago	ND	ND
Arthrosporiella				
2905 *F. semitectum*	South Africa	Corn	ND	ND
4815 *F. camptoceras*	Costa Rica	Cocoa	ND	ND
3520 *F. camptoceras*[c]	South Africa	Soil	ND	ND
Gibbosum				
2330 *F. equiseti*	United States	Cereal	ND	ND
4463 *F. equiseti*	Transkei	Soil	ND	ND
3311 *F. acuminatum*	South Africa	Medicago	ND	ND
4028 *F. acuminatum*[d]	South Africa	Soil	ND	ND
3687 *F. scirpi*	Australia	Soil	ND	ND
3528 *F. longipes*[e]	South Africa	Soil	ND	ND
4458 *F. longipes*	New Guinea	Soil	ND	ND
Discolor				
3307 *F. sambucinum*	South Africa	Medicago	ND	ND
4666 *F. sambucinum*	South Africa	Potato	ND	ND
1115 *F. graminearum* Gr.2	Transkei	Corn	ND	ND
4917 *F. graminearum* Gr.1	South Africa	Barley	ND	ND
5052 *F. graminearum* Gr.2	South Africa	Wheat	ND	ND
3636 *F. reticulatum*	South Africa	Medicago	ND	ND
1386 *F. compactum*	South Africa	Groundnut	ND	ND
Lateritium				
1925 *F. lateritium*	Zimbabwe	Coffee	ND	ND
Liseola				
826 *F. moniliforme*	Transkei	Corn	7,100	3,000
1065 *F. moniliforme*	Transkei	Corn	85	10
4315 *F. moniliforme*	Transkei	Corn	2,645	325
4317 *F. moniliforme*	Transkei	Corn	205	40
4318 *F. moniliforme*	Transkei	Corn	105	ND
4319 *F. moniliforme*	Transkei	Corn	180	40
4321 *F. moniliforme*	Transkei	Corn	1,330	140
2059 *F. proliferatum*	South Africa	Sorghum	20	160
2301 *F. proliferatum*	United States	Corn	870	450
2302 *F. proliferatum*	United States	Corn	290	65
2383 *F. proliferatum*	Sierra Leone	Corn	660	200
1077 *F. subglutinans*	Transkei	Corn	ND	ND
3823 *F. anthophilum*	South Africa	Oats	ND	ND
Elegans				
1492 *F. oxysporum*	South Africa	Groundnut	ND	ND
Newly described species				
4003 *F. nygamai*	South Africa	Soil	605	530
4150 *F. nygamai*[f]	Namibia	Millet	ND	ND
4134 *F. napiforme*	Namibia	Sorghum	ND	ND

[a] MRC, Medical Research Council.
[b] ND, Not detected ($<1\ \mu g\ g^{-1}$).
[c] Lamprecht et al. (7).
[d] Rabie et al. (18).
[e] Van Wyk et al. (24).
[f] Marasas et al. (14).

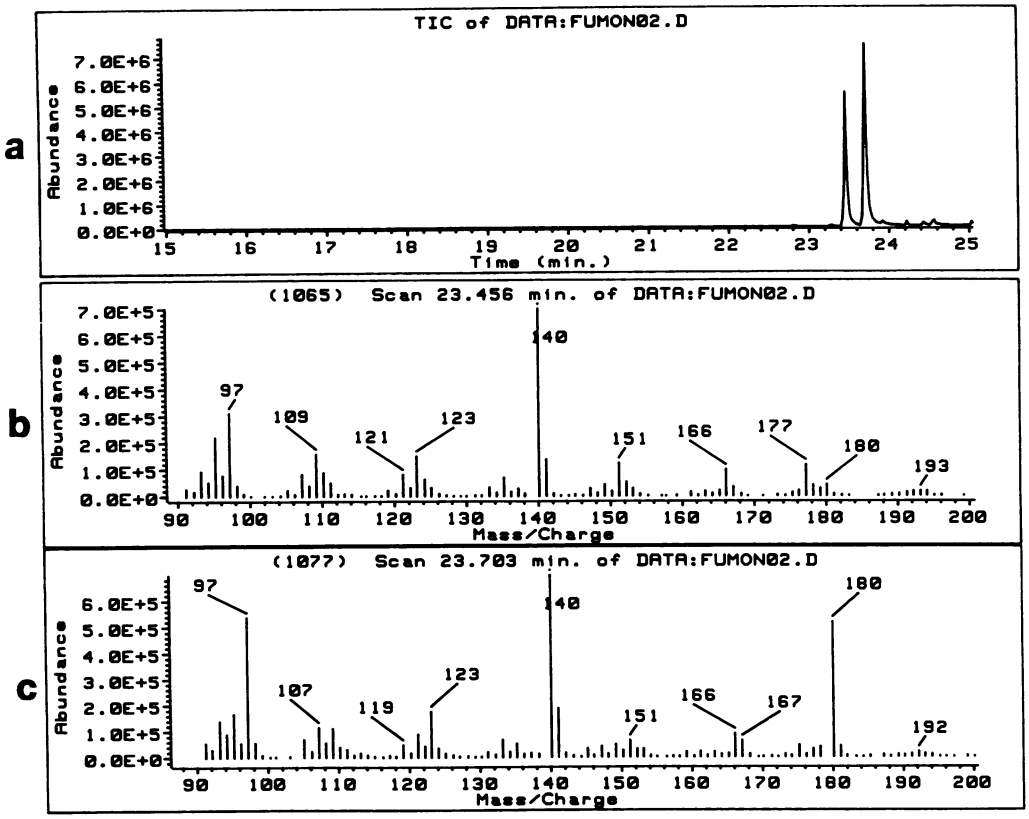

FIG. 1. (a) Total ion chromatogram (TIC) of the acylated hydrolysates of authentic fumonisin standards. (b) Partial mass spectrum of the amino-tetraol moiety of FB_2. (c) Similar mass spectrum of the amino-pentol moiety of FB_1.

Confirmation of the presence of FB_1 and FB_2. Corn culture extracts were prepared and purified on strong anion-exchange cartridges and then hydrolyzed with 2 M potassium hydroxide (25). The hydrolysates were acidified with 1 M hydrochloric acid and purified further on short Amberlite XAD-4 columns (25). Aliquots of the purified hydrolysates were either derivatized with *o*-phthaldialdehyde and analyzed by high-performance liquid chromatography or derivatized with trifluoroacetylimidazole and analyzed by capillary gas chromatography-mass spectrometry. The amino-pentol moiety of FB_1 and the amino-tetraol moiety of FB_2 (present in the hydrolysates) were determined by both chromatographic techniques. The retention times and mass spectra observed in the culture extracts were compared with those obtained for the amino-pentol and amino-tetraol present in similarly hydrolyzed fumonisin standards.

RESULTS AND DISCUSSION

Results of fumonisin analyses of corn cultures of 40 toxic *Fusarium* isolates are summarized in Table 1. With the exception of one isolate of *F. nygamai*, fumonisin production was restricted to section Liseola. All the isolates tested of two of the *Fusarium* spp. in section Liseola, *F. moniliforme* and *F. proliferatum*, produced both FB_1 and FB_2 except for *F. moniliforme* strain MRC 4318, which produced only FB_1. One isolate of each of two other species in this section, *F. subglutinans* (Wollenweber & Reinking) Nelson, Toussoun & Marasas, and *F. anthophilum* (A. Braun) Wollenweber, did not produce any chemically detectable FB_1 or FB_2.

Six of the seven *F. moniliforme* isolates from corn from a high-risk area of human esophageal cancer in the Transkei (4) produced both FB_1 and FB_2, whereas the seventh isolate (MRC 4318) produced only FB_1. The highest producer of both FB_1 (7,100 $\mu g\ g^{-1}$) and FB_2 (3,000 $\mu g\ g^{-1}$) was *F. moniliforme* MRC 826, the strain from which the fumonisins were originally isolated and characterized (1, 3). Cultures of MRC 826 also had the highest cancer promotion activity compared with those of 10 other isolates of *F. moniliforme* from Transkeian corn, in a short-term cancer initiation-promotion bioassay in rat liver (4). The two other highest producers of fumonisins (MRC 4315 and MRC 4321, Table 1) similarly exhibited high cancer promotion activity in the cancer initiation-promotion bioassay (4). Of the three lowest producers of fumonisins (MRC 4317, MRC 4318, and MRC 4319; Table 1), two (MRC 4317 and MRC 4318) exhibited no cancer promotion activity, whereas the third (MRC 4319) registered the second-highest activity (4). With the exception of *F. moniliforme* MRC 4319, the fumonisin production by all *F. moniliforme* strains correlated well with the cancer promotion activity of the cultures.

The observation that all four isolates of *F. proliferatum* tested produced both FB_1 and FB_2 (Table 1) confirms a previous report (19) on fumonisin production by two isolates of *F. proliferatum* from feeds associated with field outbreaks of equine LEM and porcine pulmonary edema, respectively. The concentrations reported by Ross et al. (19) were in excess of 1,600 $\mu g\ g^{-1}$ for FB_1 and 150 $\mu g\ g^{-1}$ for FB_2. In the present study, four *F. proliferatum* isolates, either from sorghum in South Africa or from corn in the United States

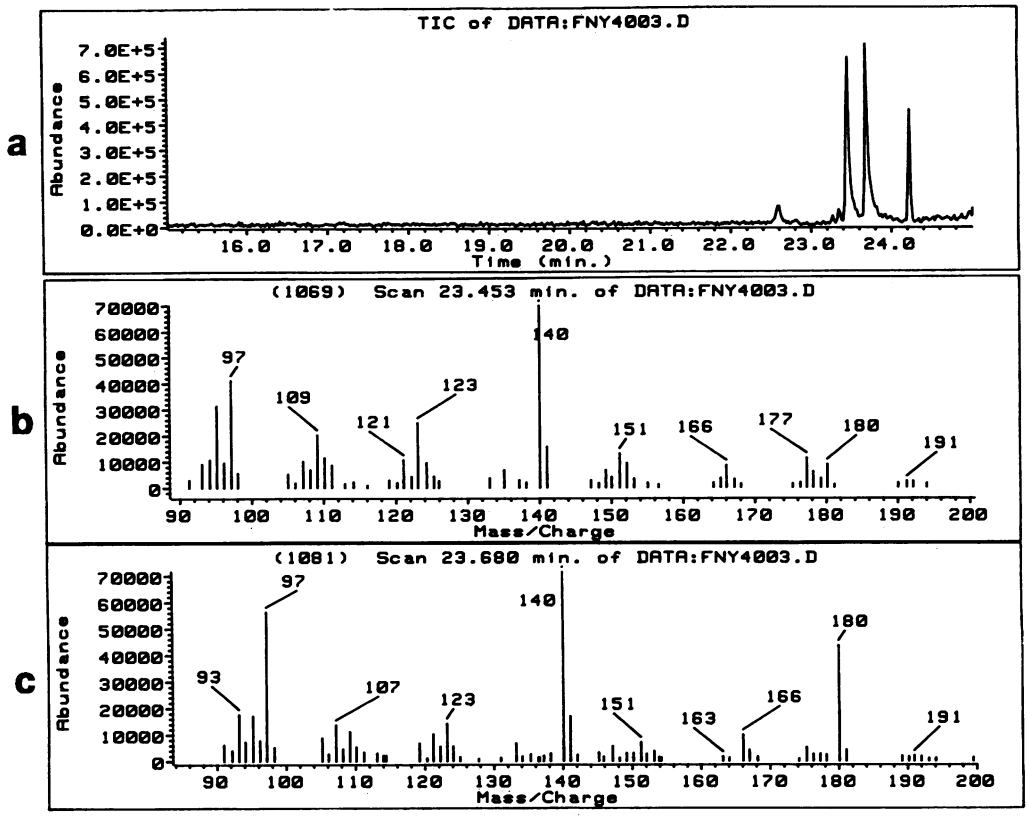

FIG. 2. (a) Total ion chromatogram (TIC) of the acylated hydrolysate of an extract of *F. nygamai* MRC 4003. (b) Partial mass spectrum of the peak eluting at 23.45 min. (c) Similar mass spectrum of the peak eluting at 23.68 min.

and Sierra Leone, produced FB_1 and FB_2 in cultures on corn, at levels ranging from 20 to 660 μg g^{-1} and 65 to 450 μg g^{-1}, respectively. One South African isolate of *F. proliferatum* (MRC 2059) from sorghum differed from all other fumonisin-producing strains thus far analyzed, in that it produced more FB_2 (160 μg g^{-1}) than FB_1 (20 μg g^{-1}).

In addition to the two fumonisin-producing *Fusarium* species in section Liseola, i.e., *F. moniliforme* and *F. proliferatum*, only one other toxic *Fusarium* isolate produced chemically detectable levels of FB_1 and FB_2; this was *F. nygamai* MRC 4003 (Table 1). The identity of the fumonisins produced by this isolate was confirmed by the hydrolysis of a sample extract followed by *o*-phthaldialdehyde derivatization and high-performance liquid chromatographic analysis as well as by acylation and analysis by capillary gas chromatography-mass spectrometry.

Figure 1a shows the total ion chromatogram of the hydrolyzed, acylated products of authentic fumonisin standards. The two chromatographic peaks eluting at 23.45 and 23.70 min correspond to the amino-tetraol and -pentol moieties of FB_2 and FB_1, respectively. Figures 1b and c show the partial mass spectra (90 to 200 *m/z*) of the peaks.

Figure 2a illustrates the total ion chromatogram of an extract of *F. nygamai* MRC 4003, and Fig. 2b and c show the mass spectra of the two major peaks observed in Fig. 2a. The excellent agreement between the two sets of retention times and mass spectra confirmed the production of both FB_1 and FB_2 by *F. nygamai* MRC 4003. The presence of the amino-pentol moiety of FB_1 and the amino-tetraol moiety of FB_2 in the hydrolyzed extracts of a culture of *F. nygamai* MRC 4003 was also confirmed by HPLC analysis of the *o*-phthaldialdehyde-derivatized hydrolysate.

The recently described species *F. nygamai* (2) is related to but excluded from the section Liseola by the production of chlamydospores but has not yet been assigned to a section. *F. nygamai* MRC 4003 was isolated from soil in the Kruger National Park, South Africa, and is one of the authentic isolates cited (as M-2368) in the original description (2). Cultures of *F. nygamai* MRC 4003 on corn produced 605 μg of FB_1 g^{-1} and 530 μg of FB_2 g^{-1} (Table 1). This is the first report of fumonisin production by *F. nygamai*.

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