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Production of Beauvericin, Moniliformin, Fusaproliferin, and Fumonisins B_1, B_2, and B_3 by Fifteen Ex-Type Strains of *Fusarium* Species†

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Fifteen *Fusarium* species were analyzed by high-performance liquid chromatography for the production of six mycotoxins in corn grits cultures. Production of mycotoxins ranged from 66 to 2,500 μg/kg for fumonisin B_1, 0.6 to 1,500 μg/g for moniliformin, 2.2 to 720 μg/g for beauvericin, and 12 to 130 μg/g for fusaproliferin. Fumonisin B_2 (360 μg/kg) was produced by two species, fumonisin B_3 was not detected in any of the 15 species examined, and *Fusarium bulbicola* produced none of the six mycotoxins that we analyzed.

Fifteen *Fusarium* species have been described recently by Nirenberg and O'Donnell (21), Nirenberg et al. (22), and Nirenberg and Aoki (20). All of these species are associated with the *Gibberella fujikuroi* complex, also known as *Fusarium* section *Liseola*, within which several important secondary metabolites, such as beauvericin (14, 19), fusaproliferin (16, 19), fusarins (33), and gibberellic acid (5, 23), and mycotoxins, such as fumonisins (6), moniliformin (18), and fusaric acid (3), are produced.

Fumonisins B_1, B_2, and B_3 are a group of nongenotoxic carcinogens. The consumption of fumonisin-contaminated grain has been correlated with esophageal cancer in humans (25). These mycotoxins can also cause leukoencephalomalacia in horses (11, 17, 27), pulmonary edema in swine (9, 10), and liver cancer in rats (7). Beauvericin is toxic to brine shrimp (*Artemia salina*) (8); to human hematopoietic, epithelial, and fibroblastoid cells (15); and to IARC/LCL 171 human B lymphocytes (16). Fusaproliferin can induce teratogenic effects, e.g., cephalic dichotomy, macrocephaly, and limb asymmetry, in chicken embryos (26). Moniliformin is a sodium or potassium salt of 1-hydroxycyclobut-1-ene-3,4-dione (4, 24), which has been shown to be extremely toxic to animals such as ducklings, rats, mice, chickens, and swine (1, 2, 13).

Like the other *Fusarium* species, these 15 are probably ubiquitous and recoverable from food and from feed commodities even under ideal conditions. With the establishment of new species within *Fusarium* section *Liseola*, the ability of strains representative of these new species to produce the mycotoxins produced by other members of this group needs to be determined. Our objective in this study was to determine the ability of the former type strains of these 15 recently described *Fusarium* species to produce fumonisins B_1, B_2, and B_3 and moniliformin, beauvericin, and fusaproliferin.

This experiment was conducted with three independent replicates from the same batch of grits, which then received the same treatments. Ex-type *Fusarium* cultures of each species used for these studies (strain numbers in parentheses indicate strains from Kansas State University [Manhattan, Kans.], the Medical Research Council [Tygerberg, South Africa], and the Biologische Bundesanstalt fur Land- und Forstwirtschaft [Berlin, Germany], respectively) were as follows: *F. acutatum* Nirenberg & O'Donnell (strains 10769, 7544, and 69580), *F. begoniae* Nirenberg & O'Donnell (10767, 7542, and 67781), *F. brevicatenulatum* Nirenberg, O'Donnell, Kroschel & Andrianaivo (10756, 7531, and 69197), *F. bulbicola* Nirenberg & O'Donnell (10759, 7534, and 63628), *F. circinatum* Nirenberg & O'Donnell (teleomorph *Gibberella circinata* Nirenberg & O'Donnell) (10766, 7541, and 69720), *F. concentricum* Nirenberg & O'Donnell (10765, 7540, and 64354), *F. denticulatum* Nirenberg & O'Donnell (10763, 7538, and 67772), *F. guttiforme* Nirenberg & O'Donnell (10764, 7539, and 69661), *F. lactis* (Pirotta & Riboni) Nirenberg & O'Donnell (10757, 7532, and 68590), *F. nisikadoi* Nirenberg & Aoki (10758, 7533, and 69015), *F. phyllophilum* Nirenberg & O'Donnell (10768, 7543, and 63625), *F. pseudoanthophilum* Nirenberg, O'Donnell & Mubatanhema (10755, 7530, and 69002), *F. pseudocircinatum* Nirenberg & O'Donnell (10761 7536, and 69636), *F. pseudonygamai* Nirenberg & O'Donnell (10762, 7537, and 69552), and *F. ramigenum* O'Donnell & Nirenberg (10760, 7535, and 68592).

We extracted beauvericin using a modification of the method of Thakur and Smith (31). Instead of extracting with a blender, we added 25 ml of extraction solvent (acetonitrile-H_2O, 90:10 [vol/vol]) to 250-ml boiling flasks with stoppers, and the flasks were then shaken with a wrist-action shaker (Burrel Co., Pittsburgh, Pa.) at medium speed for 30 min. We used the method of Thakur and Smith (30) to analyze for fumonisins B_1, B_2, and B_3. The method described by Kostecki et al. (12) was used for the extraction and analysis of fusaproliferin and moniliformin.

Chromatographic analyses of the extracts were made with a Hewlett-Packard (Palo Alto, Calif.) series II, model 1090A high-performance liquid chromatograph fitted with a Rheo-

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TABLE 1. Production of the mycotoxins beauvericin, moniliformin, and fusaproliferin and of fumonisins B_1 and B_2 by the ex-type strains of 15 *Fusarium* species

Fusarium species	KSU[a] strain no.	Amt of mycotoxin or fumonisin produced[b]				
		BEA (μg/g)	MON (μg/g)	FP (μg/g)	FB_1 (μg/kg)	FB_2 (μg/kg)
F. acutatum	10769	6 ± 1	ND	ND	147 ± 10	360 ± 23
F. begoniae	10767	ND	1,000 ± 64	ND	66 ± 3	ND
F. brevicatenulatum	10756	ND	ND	ND	150 ± 7	ND
F. bulbicola	10759	ND	ND	ND	ND	ND
F. circinatum	10766	57 ± 2	ND	ND	ND	ND
F. concentricum	10765	720 ± 48	ND	ND	ND	ND
F. denticulatum	10763	ND	180 ± 7	ND	ND	ND
F. guttiforme	10764	72 ± 6	ND	85 ± 5	ND	ND
F. lactis	10757	ND	51 ± 3	ND	ND	ND
F. nisikadoi	10758	ND	0.6 ± 0.1	ND	ND	ND
F. phyllophilum	10768	ND	1500 ± 73	ND	2,500 ± 100	ND
F. pseudoanthophilum	10755	2.2 ± 0.2	ND	ND	ND	ND
F. pseudocircinatum	10761	ND	100 ± 16	12 ± 0.3	280 ± 3	360 ± 30
F. pseudonygamai	10762	ND	53 ± 2	130 ± 2	ND	ND
F. ramigenum	10760	ND	46 ± 9	ND	ND	ND

[a] KSU, Department of Plant Pathology, Kansas State University, Manhattan.
[b] ND, not detected. Detection limits were as follows; for beauvericin (BEA), 5 ng; for fusaproliferin (FP), 5 ng; for moniliformin (MON), 2 ng; and for fumonisins B_1 and B_2 (FB_1 and FB_2, respectively), 0.5 ng.

dyne Inc. (Cotati, Calif.) model 7125 injector and a 50-μl loop. Chromatographic separations were made with an Alltima reversed-phase C_{18} column (250 by 4.6 mm, 5-μm particle size; Alltech Associates, Deerfield, Ill.) equilibrated at 40°C. The correlation coefficients (*r*) ranged from 0.9952 to 0.9998 (standard concentration ranges, 0.5, 1, 5, 10, 25, 50, and 100 μg/ml for beauvericin, fusaproliferin, and moniliformin and 0.3, 0.5, 1, 3, 5, and 25 μg/ml for fumonisins), and the percentages of recovery ranged from 73 to 81%. The mean response variable (mycotoxin produced) and the standard deviation were found by using the analysis of variance procedure of the SAS System, release 6.12, for personal computers (SAS Institute, Cary, N.C.). Results are presented as means ± standard deviations.

No detectable levels of any of the mycotoxins analyzed were found in the noninoculated corn grits media. Also, of the 15 *Fusarium* species we examined (Table 1), *F. bulbicola* produced none of the six mycotoxins and no species produced more than four, with most producing only one or two of these mycotoxins. Fumonisin B_1 was produced at levels of 66 to 2,500 μg/kg by representatives of five species, two of which also produced fumonisin B_2 at levels of 360 μg/kg. None of the 15 strains examined produced detectable levels of fumonisin B_3. Fusaproliferin was produced by representatives of three species (12 to 130 μg/g), beauvericin was produced by representatives of five species (2.2 to 720 μg/g), and moniliformin was produced by representatives of eight species (0.6 to 1,500 μg/g). The levels of beauvericin that we found were considerably below the highest reported levels, 3,200 μg/g (14), but are within the range of toxin production previously reported by others (14, 29). The *F. concentricum* strain in this study is a relatively high producer of beauvericin (720 μg/g).

Moniliformin production has been shown to vary widely even within a *Fusarium* species (28, 13). Therefore, the range in moniliformin production that we observed in our 15-species sample was not unexpected. Both *F. begoniae* and *F. phyllophilum* produced relatively high levels of moniliformin (1,000 and 1,500 μg/g, respectively). Moniliformin production by 12 of these 15 species was reported by Schutt et al. (28). In addition to the nonproducing species reported by Schutt et al. (28), we found that *F. acutatum*, *F. bulbicola*, *F. concentricum*, and *F. pseudoanthophillum* produced no detectable levels of moniliformin, which is understandable because not all strains of the same species are capable of producing the same metabolites.

The fusaproliferin levels that we detected (12 to 130 μg/g) are within the range previously reported by Shephard et al. (29), from a trace to 2,600 μg/g, or by Logrieco et al. (16), from 1,100 to 1,300 μg/g. By these standards, the strains that we examined are, at best, relatively poor producers of this toxin.

The levels of fumonisins that we detected were all either low or very low (66 to 2,500 μg/kg) relative to those reported for other species (6, 13, 32). The coproduction of two, three, or even four mycotoxins by 6 out of the 15 species that we examined is consistent with previous reports (29) of the production of multiple toxins by other species in *Fusarium* section *Liseola*.

In conclusion, the ability of the ex-type strains from 15 recently described *Fusarium* species to produce beauvericin, fumonisins, fusaproliferin, and moniliformin varied widely. Only one strain did not produce a detectable level of at least one of these toxins. Most of these species produced one or two of these toxins, with moniliformin being the most commonly produced (8 out of 15 species) and fusaproliferin being the least commonly produced (3 out of 15 species). These data suggest that these fungal species do not pose a uniform risk to human and animal health and that determining the substrates most commonly colonized by these species will be essential in understanding the risk that they may pose to the health of humans and domesticated animals.

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