Production of Fumonisin B and C Analogues by Several *Fusarium* Species

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Six strains of *Fusarium verticillioides*, two of *F. oxysporum*, one strain of *F. proliferatum*, and a strain of an unidentified species were cultured on maize patties and rice and evaluated for their ability to simultaneously produce fumonisin B (FB) and C (FC) series analogues. Fumonisins were quantified by LC-MS-MS using positive ion electrospray ionization. FC_1 provided characteristic fragment ions at *m/z* 690, 672, 654, 532, 514, and 338 corresponding to sequential loss of H_2O and tricarboxylic acid moieties from the alkyl backbone, while FC_3 and FC_4 provided equivalent product ions 16 and 32 amu lower than the corresponding FC_1 fragments, respectively. All isolates cultured on maize produced FC_4. All isolates except for that of *F. proliferatum* also produced FC_1, and three of the six strains of *F. verticillioides* produced FC_3. All isolates except those of *F. oxysporum* produced detectable amounts of FB_1, FB_2, and FB_3. Isolates that produced fumonisin B analogues produced at least 10 fold more of the B series analogues than they did of the C series analogues. The results confirm that at least some strains of *F. oxysporum* produce FC, but not FB, fumonisin analogues and also suggest that the genetics and physiological regulation of fumonisin production may be more complicated than previously envisaged since some strains of *F. verticillioides* and *F. proliferatum* as well as the strain of the unidentified species can simultaneously produce both FB and FC analogues.

KEYWORDS: *Fusarium*; fumonisins; tandem mass spectrometry; DNA; amplified fragment length polymorphisms

INTRODUCTION

Fusarium species are important fungal contaminants in agricultural crops that produce a variety of secondary metabolites, including the fumonisins, that are potential risks to human and animal health. Fumonisins (*1*) are a group of structurally related mycotoxins produced by *Fusarium verticillioides* (Sacc.) Nirenberg, formerly known as *F. moniliforme* Sheldon (*2*), and related species. Twenty-eight structural fumonisin analogues are known (*3*), but most research has focused on the most widespread natural forms, which are members of the B-series (FB), for example, fumonisin B_1 (FB_1), fumonisin B_2 (FB_2), and fumonisin B_3 (FB_3). The fumonisins have been shown to affect several target tissues in animals resulting in disease syndromes such as equine leukoencephalomalacia and porcine pulmonary edema as well as causing nephrotoxicity, hepato-

toxicity, and hepatocellular carcinoma in rats (*4*). The consumption of fumonisin-contaminated corn has been statistically associated with the high incidence of esophageal cancer in rural areas of South Africa (*5*) and China (*6*).

In addition to the B-series fumonisins (FB), related compounds belonging to the A-, P-, and C-series also have been described. The A-series fumonisins (FA) are *N*-acetyl analogues of the B-series and have little or no biological activity (*7*). The P- and C-series of fumonisins differ from the B-series in that the fumonisin P analogues have a 3-hydroxypyridinium functional group in place of the C-2 amine group (*8*) and the fumonisin C analogues lack a terminal methyl group at C-1 (**Figure 1**). Fumonisin C_1 (FC_1) was the first fumonisin C analogue to be characterized (*9*) and was isolated from a culture of "*F. moniliforme*" (FRC M-2326) that originated on maize from Maryland (Jean Juba, Fusarium Research Center, Pennsylvania State University, personal communication, October 8, 2003). Fumonisins C_3 (FC_3) and C_4 (FC_4) subsequently were detected from strains (KSU A-00816, KSU A-00817, and KSU A-00819) first identified as *F. moniliforme* from maize in South Carolina (*10, 11*) and *F. oxysporum* Schlecht. emend Snyd. &
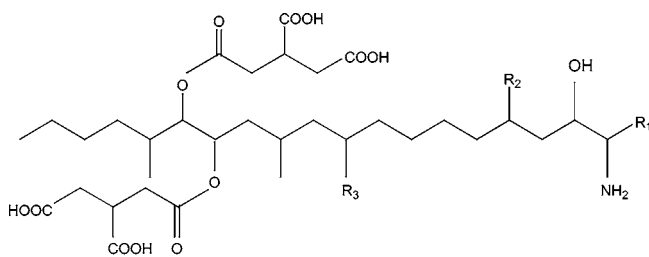
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Fumonisin Analog	R_1	R_2	R_3	Molecular Weight
FB_1	CH_3	OH	OH	721
FB_2	CH_3	OH	H	705
FB_3	CH_3	H	OH	705
FC_1	H	OH	OH	707
FC_3	H	H	OH	691
FC_4	H	H	H	675

Figure 1. Chemical structures of the fumonisin B and C analogues investigated in this study.

Hans (*12, 13*) isolated from carnation and asparagus in Korea. *N*-acetyl derivatives of fumonisin C analogues also have been reported from wheat cultures of one of the Korean strains of *F. oxysporum* (*13*). The natural occurrence of the fumonisin C analogues has not been widely tested, but Seo and Lee (*14*) reported both their natural occurrence and co-occurrence with fumonisin B analogues in samples of moldy maize from Korea.

The FC and FP fumonisins are phytotoxic to duckweed (*Lemna pausicotata* L.) and cytotoxic to mammalian cell lines H4TG and MDCK (*15*). FC_1, FC_2, and hydroxy-FC_1 had phytotoxic effects similar to those caused by FB_1 or AAL-toxin, indicating that the C-1 terminal methyl group is not required for this biological activity. FC_3 and FC_4 were less phytotoxic than FC_1 and FC_2, and FP_1 was even less phytotoxic than FC_3 or FC_4. The cytotoxicity of these analogues to cell lines paralleled their phytotoxicity toward duckweed (*15*).

Our objectives in this study were to confirm earlier reports of fumonisin C analogue production by *F. oxysporum* (*12, 13*), to describe additional strains or species producing fumonisin C analogues, and to confirm whether the FB and FC fumonisins are synthesized simultaneously by the same strain of *Fusarium verticillioides*, an observation also reported previously by Branham and Plattner (*9*).

MATERIALS AND METHODS

Reagents and Standards. Acetonitrile, HPLC grade (>99.9% purity), was purchased from Romil (Cambridge, U.K.). Formic acid (analytical grade) was obtained from Merck (Darmstadt, Germany). Water was purified in a Milli-Q system (Millipore, Bedford, MA). Fumonisin B standards were isolated in the PROMEC Unit by the method of Cawood et al. (*16*), and fumonisin C standards were prepared by Prof. Y.-W. Lee as described by Seo et al. (*13*).

Fusarium Strains. Two Korean strains of *Fusarium oxysporum*, CAR (MRC 7547 = KSU X-12910) and KCTC 16654 (MRC 8184 = KSU X-12915), have been reported to produce fumonisin C analogues (*13, 14*). MRC 7547 was isolated from carnation wilt and produces FC_1, FC_3, FC_4, and hydroxy-FC_1 in cultures grown on wheat (*12*). MRC 8184 was isolated from asparagus wilt and produces an isomer of FC_1 (iso-FC_1) and three *N*-acetyl derivatives of hydroxy-FC_1 (*13*). Two other putative *F. oxysporum* strains from Korea, *F. oxysporum* No. 4 (MRC 8171 = KSU D-12913) from sesame seeds and *F. oxysporum* No. 53 (MRC 8172 = KSU X-12914) from red beans, have not been previously described but can produce moniliformin (Y.-W. Lee, School of Agricultural Biotechnology and Center for Agricultural Biomaterials,

Seoul National University, October 11, 2003, unpublished). *F. moniliforme* 94-cob-01 was isolated from corn in Korea that contained more FC_1 than FB_1. Two single-conidial isolates, MRC 8169 (=KSU A-12911) and MRC 8170 (=KSU A-12912), were obtained from different sectors of the original culture. The South African *F. verticillioides* strains, MRC 826, 4315, 4319, and 4321, were isolated from home-grown corn in Centane, Eastern Cape, South Africa (*7*). These isolates can produce FB_2, FB_3, and high levels of FB_1 (*17*). These strains are all sexually cross-fertile with the mating type testers for *Gibberella moniliformis* (*Gibberella fujikuroi* mating population A) (*11*).

DNA Analyses and Comparisons. Strains for DNA extraction were cultured by inoculating approximately 1 mL of a spore suspension (typically 10^6-10^7 conidia) into 40 mL of liquid complete media (*18*). Isolates were grown on a rotary shaker (150 rpm) for 2 days at room temperature (23-26 °C) and were harvested by filtration through milk filters (KenAG, Ashland, OH). Mycelia were blotted dry between paper towels and the dried mycelia were stored at −20 °C until DNA extraction.

DNA was extracted with a cetyltrimethylammonium bromide (CTAB) protocol as described in Kerényi et al. (*19*). All buffers and DNA modifying enzymes were used following either the manufacturer's instructions or standard protocols (*20*). Amplified fragment length polymorphisms (AFLPs) were generated with primer pairs EcoRI+TT and MseI+AC used in the final amplifications as described by Zeller et al. (*21*). *Eco*RI primers used in the final specific PCR amplifications were labeled with $^{33}\gamma$P-ATP. The PCR machine used was a PTC-2000 Thermal Cycler (MJ Research, Inc., Watertown, Massachusetts).

The presence or absence of polymorphic AFLP bands ranging in size from 100 to 800 bp in each gel was scored manually and the data were recorded in a binary format. All polymorphic markers in this size range were scored, even those that were unique to a single individual. Bands appearing at the same mobility in different individuals were assumed to represent the same allele. Each band of differing mobility was treated as a single independent locus with two alleles (present or absent), and unresolved bands or missing data were scored as ambiguous.

We amplified and sequenced portions of the DNA sequences for β-tubulin, translation elongation factor 1α, and the ribosomal internally transcribed spacer (ITS) region. For the β-tubulin gene, we used PCR primers T1 and T2 and the PCR reaction conditions described by O'Donnell and Cigelnik (*22*), except that we increased the annealing temperature to 58 °C, to amplify a homologous region of the β-tubulin gene. For translation elongation factor 1α gene, we used primers EF1 and EF2 (*23*) and an amplification program of 60 s at 94 °C followed by 34 cycles of 58 °C for 60 s, 72 °C for 60 s, and 94 °C for 30 s and then held at 4 °C. For the ITS region, we used primers ITS4 and ITS5 (*24*) with the same amplification program as for the transcription elongation factor 1α gene except that the annealing temperature was decreased to 52 °C.

PCR products were purified and desalted with the Wizard PCR Preps DNA Purification System (Promega, Madison, WI). DNA sequences were obtained by direct sequencing of the PCR products by using the same PCR primers as used for the amplifications from the genomic DNA with an ABI 3700 automated sequencer at the Kansas State University sequencing facility. Sequences from both strands were aligned to minimize ambiguous nucleotide positions and each sequence was then aligned with ClustalW (*25*), as implemented in the program BioEdit v4.7.8 (*26*). Preliminarily aligned sequences were further aligned and edited manually with BioEdit.

Culture Conditions. Six *F. verticillioides* isolates, four from South Africa (MRC 826, 4315, 4319, and 4321) and two from Korea (MRC 8169 and MRC 8170), two confirmed Korean *F. oxysporum* strains (strain CAR = MRC 7547 and KCTC 16654 = MRC 8184), and the two putative Korean *F. oxysporum* strains (MRC 8171 and MRC 8172) were evaluated. All isolates were cultured on maize patties (300 per strain) consisting of ground whole yellow maize (30 g) mixed with 30 mL distilled water placed in 90-mm Petri dishes and autoclaved at 121 °C for 1 h on two consecutive days. Patties were inoculated with 1 mL of a spore suspension made from lyophilized cultures (1:100 distilled water dilution per 2 mL lyophilized culture vial) and were incubated in the dark at 25 °C for 21 days. The patty cultures were dried at 50

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°C for 24 h, ground to a meal, and then stored at 4 °C until analyzed for fumonisins in triplicate.

The four South African *F. verticillioides* strains also were grown on rice. Rice cultures consisted of polished rice (1 kg) soaked overnight in distilled water (245 mL), with occasional shaking. Portions of 40−50 g were placed in 250-mL Erlenmeyer flasks and were autoclaved for 20 min at 121 °C. The rice cultures were inoculated, incubated, and then processed in the same manner as were the cultures grown on maize patties.

Sexual crosses were made with standard mating type tester strains of *Gibberella moniliformis*, FGSC 7600 (*MATA-1*) and FGSC 7603 (*MATA-2*); *Gibberella fujikuroi*, FGSC 8931 (*MATC-1*) and FGSC 8932 (*MATC-2*); and *Gibberella intermedia*, FGSC 7615 (*MATD-1*) and FGSC 7614 (*MATD-2*) available from the Fungal Genetics Stock Center (University of Missouri, Kansas City). Crosses were made on carrot agar by using standard crossing procedures as described by Klittich and Leslie (*27*).

Determination of Fumonisins. Sample extracts from the cultures were prepared by the method of Sydenham et al. (*28*). In brief, culture material (5 g) from each strain in 100 mL methanol/water (3:1, v/v) was homogenized for 3 min using a Polytron homogenizer (Kinematica, Luzern, Switzerland). Extracts were centrifuged at 4 °C and 4000*g* for 10 min, filtered (Whatman No. 4), and the filtrate adjusted to pH 6.0 with 1 M NaOH. The solution (10 mL) cleanup was performed on a Bond Elut SAX solid-phase extraction cartridge (500 mg sorbent) (Varian, Harbor City, CA). The eluate from the SAX cartridge was evaporated to dryness at 60 °C under nitrogen, and the residue was redissolved in the mobile phase prior to injection.

HPLC-MS Conditions. The HPLC system consisted of a Spectra-SERIES P2000 pump and an AS 1000 autosampler (Thermo Separation Products Inc., Riviera Beach, FL) with a 20-μL injection loop. Reversed-phase HPLC was performed using a binary gradient at a flow rate of 0.7 mL/min on a Luna C_{18} column (4.6 × 150 mm, 5 μm ODS-2) (Phenomenex, Torrance, CA). The mobile phase consisted of mixtures of water/acetonitrile/formic acid in the ratio 90:10:0.1 v/v (solvent A) and 10:90:0.1 v/v (solvent B). The initial composition of 80:20 A:B was adjusted linearly over a 35-min period to 65:35 A:B and held for 1 min. The composition was adjusted back to 80:20 A:B in 2 min and retained for the last 2 min. Fumonisin analogues were detected on the basis of their retention times.

Positive ion electrospray ionization (ESI) mass spectrometry was performed with a Finnigan MAT (San Jose, CA) LCQ ion-trap mass spectrometer. The MS parameters were optimized separately by direct infusion of each fumonisin B and C standard (1 mg/mL) into the source at a flow rate of 5 μL/min. The use of appropriate segments during the chromatographic run made scanning at optimum conditions possible for each of the toxins. Capillary and source voltages were 3 V and 4.5 kV, respectively, with the heated capillary temperature maintained at 220 °C. Nitrogen was used as both sheath and auxiliary gas (80 and 20 arbitrary units, respectively). Mass spectrometry experiments were initially carried out by scanning from *m/z* 190 to 730 to detect the protonated molecular ion signals for FB_1, FB_2, FB_3, FC_1, FC_3, and FC_4 at *m/z* 722, 706, 706, 708, 692, and 676, respectively. MS-MS experiments were subsequently undertaken through collision-induced dissociation (34% collision energy) of the protonated molecular ions to yield fingerprint profiles for each of the toxins. Toxin levels were quantified (limit of detection = 2 mg/kg) by comparing the resultant peak areas from the extracts with a calibration plot of the standards.

RESULTS

Identification of Korean *Fusarium* Strains. MRC 7547 and MRC 8184 are *F. oxysporum* on the basis of morphological characters. On the basis of AFLP fingerprint patterns, the two strains are clones (identity at 72/72 AFLP bands), even though they originated from different hosts. The AFLP fingerprints are not particularly similar to those of other known strains of *F. oxysporum*, for example, KSU nos. X-11391 (40% identity, 39/97 matches) and X-11394 (44% identity, 39/89 matches). On the basis of DNA sequences of portions of the genes encoding

β-tubulin (GenBank no. AY627278), translation elongation factor 1α (GenBank no. AY627279), and the ITS region of the ribosomal DNA repeat unit data (GenBank no. AY627280), these strains also belong to *F. oxysporum*.

F. oxysporum No. 4 (MRC 8171) is not *F. oxysporum* on morphological criteria but is *F. proliferatum* (Matsushima) Nirenberg and is cross-fertile (*MAT-1*) with standard mating type testers of *G. intermedia*. The AFLP fingerprint is somewhat similar to that of one of the standard mating type strains of *F. proliferatum*, FGSC (KSU4853) (44% identity). On the basis of DNA sequences of portions of the genes encoding β-tubulin (GenBank no. AY660013), translation elongation factor 1α (GenBank no. AY660014), and the ITS region of the ribosomal DNA repeat unit data (GenBank no. AY660015), this strain also is *F. proliferatum*.

F. oxysporum No. 53 (MRC 8172) is not *F. oxysporum* on morphological criteria but is *Fusarium nygamai*. The standard mating type testers of *G. nygamai* were not cross-fertile with one another, so this strain was not tested with them for cross-fertility. MRC 8172 was not cross-fertile with the standard testers of either *G. fujikuroi* or *G. intermedia*. The AFLP fingerprints differ significantly from those of mating type standards of *F. nygamai* (G5111, 19% similarity) and *F. proliferatum* (D4853, 31% similarity) and most closely resemble those of *F. fujikuroi* (C1993, 55% similarity). The identity of MRC 8172 as *F. fujikuroi* is supported by its ITS (Genbank no. AY662329) and β-tubulin (Genbank no. AY662327) sequences. The translocation elongation factor 1α (Genbank no. AY662328) sequence is considerably more distant from other available sequences, for example, Genbank no. AF160279, from which it differs at least 13/641 nucleotides sequenced. This strain could represent a previously undescribed species.

F. verticillioides MRC 8169 and MRC 8170 were identified as *F. verticillioides* on the basis of morphological characters. On the basis of AFLP fingerprint patterns, the two strains are clones (identity at 48/48 AFLP bands), which was not unexpected as these strains are duplicate subcultures of a single wild collection. The strains are cross-fertile (*MAT-1*) with standard tester strains of *G. moniliformis*. The AFLP fingerprints resemble those of one of the mating type tester strains of *F. verticillioides*, KSU A-00149 (67% identity). On the basis of DNA sequences of portions of the genes encoding translation elongation factor 1α (GenBank no. AY662325), the ITS region of the ribosomal DNA repeat unit data (GenBank no. AY662326), and the gene encoding β-tubulin (GenBank no. AY665679), this strain also is *F. verticillioides*.

Production of Fumonisin B and Fumonisin C Analogues by Strains of *F. verticillioides*. Tandem MS experiments resulted in successive losses of water and tricarboxyllic acid (TCA) groups from the alkyl backbone of each fumonisin analogue hence producing product ion mass spectra characteristic of the structural moieties present in the compounds (**Table 2**). The use of appropriate segments during the chromatographic run made scanning at optimum conditions possible for each of the analogues. FB_1 and FC_1, and FB_3 and FC_3, have similar retention times. The MS-MS experiment was thus undertaken so as to selectively excite the respective ions under a single segment, which provided for the robust detection and quantification of these closely eluting analytes.

When cultured on maize, the fumonisin B analogues constituted 56−84% of the total fumonisins produced (**Table 3**). All of the *F. verticillioides* strains (**Table 3**) produced FC_1 (11−410 mg/kg) and FC_4 (8−570 mg/kg). Three strains, MRC 8169, MRC 8170, and MRC 4319 (**Table 3**), also produced FC_3 (4−

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Table 1. *Fusarium* Strains Used for Fumonisin B and C Series Production

MRC[a]	*Fusarium* strain number Korean	KSU[b]	received as	current identification	strain source
7547	CAR	X-12910	F. oxysporum	F. oxysporum	Korean, carnation wilt
8184	KCTC 16654	X-12915	F. oxysporum	F. oxysporum	Korea, asparagus wilt
8171	no. 4	D-12913	F. oxysporum	F. proliferatum	Korea, sesame seed
8172	no. 53	X-12914	F. oxysporum	*Fusarium* sp. (undescribed)	Korea, red bean
8169	94-cob-01[c]	A-12911	F. moniliforme	F. verticillioides	Korea, corn
8170	94-cob-01[c]	A-12912	F. moniliforme	F. verticillioides	Korea, corn
826				F. verticillioides	Centane, South Africa, home-grown corn
4315				F. verticillioides	Centane, South Africa, home-grown corn
4319				F. verticillioides	Centane, South Africa, home-grown corn
4321				F. verticillioides	Centane, South Africa, home-grown corn

[a] Culture collection of the PROMEC Unit, Medical Research Council, Tygerberg, South Africa. [b] Culture collection of the Department of Plant Pathology, Kansas State University, Manhattan, Kansas. [c] Two single-spored cultures made from original Korean culture because of slight culture differences (sectors).

Table 2. Product Ions (*m/z*) of the Fumonisin B and C Series

product ion	FB_1	FB_2	FB_3	FC_1	FC_3	FC_4
$[M + H-H_2O]^+$	704	688	688	690	674	658
$[M + H-2H_2O]^+$	686	670	670	672	656	640
$[M + H-TCA]^+$	546	530	530	532	516	500
$[M + H-H_2O-TCA]^+$	528	512	512	514	498	482
$[M + H-2TCA]^+$	370	354	354	356	340	324
$[M + H-2TCA-H_2O]^+$	352	336	336	338	322	306

**Table 3.** Production of Fumonisin Analogues by Korean and South African *Fusarium* Strains

Fusarium strains	MRC number	Fumonisin analogues (mg/kg)[a] FC_1	FC_3	FC_4	FB_1	FB_2	FB_3
		Korean Strains					
F. verticillioides	8169[b]	15	4	8	220	25	30
	8170[b]	170	48	300	3800	630	530
F. proliferatum	8171[b]	n.d.[c]	n.d.	5	130	14	6
Fusarium sp.	8172[b]	9	n.d.	71	2100	1400	110
F. oxysporum	7547[b]	150	n.d.	440	n.d.	n.d.	n.d.
	8184[b]	130	n.d.	330	n.d.	n.d.	n.d.
South African Strains							
F. verticillioides	826 (batch 1)[b]	92	n.d.	230	2100	720	170
	826 (batch 2)[b]	35	n.d.	120	1400	600	86
	4315[b]	11	n.d.	63	260	87	12
	4319 (batch 1)[b]	410	33	570	5900	1800	650
	4319 (batch 2)[b]	120	10	150	2700	740	190
	4321[b]	17	n.d.	69	360	77	49
	826[d]	12	n.d.	n.d.	190	59	19
	4315[d]	13	n.d.	n.d.	220	64	22
	4319[d]	73	7	67	980	340	71
	4321[d]	6	n.d.	n.d.	94	17	14

[a] Each sample was analyzed in triplicate. [b] Cultured on corn patties. [c] n.d.: not detected (<2 mg/kg). [d] Cultured on rice.

48 mg/kg). MRC 4319 was the highest producer of fumonisin C series fumonisins. The fumonisin C analogues were present at 5−20% of the levels of the fumonisin B series analogues.

All of the South African strains of *F. verticillioides* also were cultured on rice, which generally supports the production of smaller amounts of fumonisins. Again, FB_1 was the major toxin, accounting for 64−72% of the total fumonisins produced. Of the FC fumonisins, FC_1 was produced by all of the strains on rice, but only MRC 4319 produced either FC_3 or FC_4 on this substrate.

In addition to FC_1, FC_3, and FC_4, an unknown HPLC peak, at approximately 17.5-min retention time, was observed to elute after FB_3 from the extracts of all of the strains except MRC 4321 (**Figure 2**). On the basis of its fragment ion profile, this analogue may be FC_2 or an isomer of FC_3 (iso-FC_3), both of
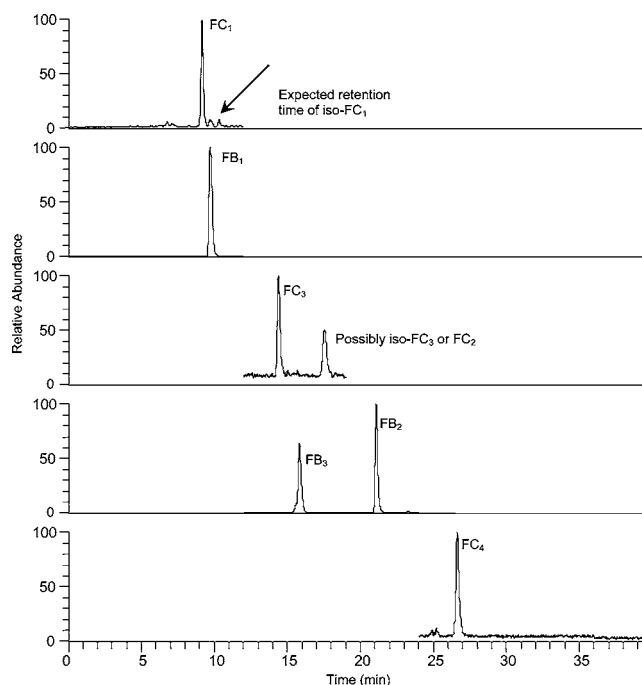
Figure 2. Product ion mass chromatogram of *F. verticillioides* strain MRC 8170 showing the chromatographic profile in each MS segment.

which would be isobaric with FC_3. Techniques other than those used here, for example, NMR spectroscopy, are required to fully characterize this compound and to confirm its structure.

Production of Fumonisin B and Fumonisin C Analogues by Other Korean Strains of *Fusarium*. The *F. proliferatum* strain, MRC 8171, produced FC_4, but no other fumonisin C analogues, and moderate levels of FB_1, FB_2, and FB_3 (**Table 3**). Strain MRC 8172, which was not assigned to a *Fusarium* species, produced small amounts of FC_1 and FC_4 but much larger amounts of FB_1, FB_2, and FB_3 (**Table 3**).

The two *F. oxysporum* strains, MRC 7547 and MRC 8184, produced only fumonisin C analogues, that is, FC_1 and FC_4, with the levels of FC_4 almost 3 times higher than those of FC_1 (**Table 3**). These strains did not produce any of the fumonisin B analogues but did produce two unknown compounds that eluted after FB_1 and FC_3, respectively. The first unknown peak had a fragmentation profile similar to that of FC_1, suggesting that the unidentified compound could be an isomer of FC_1. In this study, only the *F. oxysporum* strains produced this compound. The second unknown peak is similar to the unknown peak in the *F. verticillioides* strains and presumably represents either FC_2 or iso-FC_3.

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DISCUSSION

The present study confirms the identity of two unusual strains of *F. oxysporum* and their ability to produce fumonisin C analogues. We also found that two other putative strains of *F. oxysporum* were not identified correctly, as one of these strains is *F. proliferatum* and the other may belong to an as yet undescribed *Fusarium* species. The two *F. oxysporum* strains were reportedly isolated from different host plants with wilt disease, that is, carnation (*12*) and asparagus (*13*). However, the AFLP and sequence data indicate that these two strains are clones and their fumonisin profiles are similar enough for the differences to be due to experimental error.

The ability of strains of *F. oxysporum* to produce fumonisins has not been widely studied. Isolates of *F. oxysporum* and *F. oxysporum* var. *redolens* from root lesions of Eastern white pine have been reported to produce fumonisin B analogues in some cases (*29*) but not in others (*30*). At present, these two Korean isolates are the only *F. oxysporum* strains that are known to produce fumonisin C analogues. Clearly, additional isolates of *F. oxysporum* from Korea and elsewhere need to be analyzed for fumonisin production.

The present study provides LC-MS confirmation for the simultaneous production of the B and C series fumonisin analogues by several strains of *F. verticillioides*. The B and C fumonisin series differ in whether there is a terminal methyl group at the C-1 position (**Figure 1**). This portion of the molecule results from the condensation of an amino acid to the terminal end of a polyketide carbon chain, thus ending the chain elongation process and completing the synthesis of the fumonisin carbon backbone. Isotope feeding studies have shown that the fumonisin B series is generated by the addition of alanine to the polyketide (*31*). Hence, it may be assumed that the C series is formed by the utilization of glycine for this reaction. Seo et al. (*32*) have characterized a *F. verticillioides* gene, *FUM8*, whose predicted translation product is similar to class II α-aminotransferases, enzymes that catalyze the condensation of amino acids and acyl-CoAs. For a strain to simultaneously produce both the B and C series of fumonisin analogues, this aminotransferase either must be able to use both amino acids or there must be two forms of the enzyme in the cell, one for each of the two different substrates. If there are two enzymes, then there must be either two sequences coding for this gene or there must be a splicing of the single genomic sequence in such a manner that two similar, but functionally different, proteins result. If there is only a single enzyme, then factors determining conditions under which the substrates can be used need to be determined. This enzyme and the physiology of fumonisin biosynthesis in *F. verticillioides* also must differ from that found in *F. oxysporum* since *F. oxysporum* produces only fumonisin C, and not fumonisin B, analogues.

The fragment ions of FC$_1$, FC$_3$, and FC$_4$ provided unequivocal confirmation of the presence of these fumonisin analogues in the culture samples as they were identical to those of the authentic standards and were consistent with the chemical structure of the compounds. The fragmentation patterns all were consistent with the previous results reported on fast atom bombardment mass spectrometry (FAB-MS) (*12*, *13*). Although FB$_1$ is generally the most abundant of the B series, these results suggest that in the C series, FC$_4$ is produced on corn culture at similar or greater levels than the other C series analogues. In general, *F. verticillioides* strains produced lower levels of both the FB and FC fumonisins in cultures grown on rice than in cultures grown on maize.

This report is the first on the production of C series fumonisins by South African strains of *F. verticillioides*. However, within the strains investigated, the B series remained the dominant analogues produced. This report also is the first to show the presence of an unidentified fumonisin analogue in cultures of *Fusarium* species. As our knowledge of fumonisin mycotoxicology grows, so the extent to which individual *Fusarium* strains can produce a range of major and minor fumonisin secondary metabolites becomes more apparent.

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