Mycopathologia **113**: 11–14, 1991.
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Mycotoxin formation by different geographic isolates of *Fusarium crookwellense*

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Received: 2 November 1989; accepted in revised form 4 April 1990

Key words: Trichothecenes, mycotoxin, *Fusarium crookwellense*, zearalenone, fusarian C

Abstract

Eighteen *Fusarium crookwellense* isolates from the continents of Australia, Europe, and North America were compared for their ability to produce mycotoxins on corn at 25 °C after 2 weeks. Extracts from corn fermented with each *Fusarium* isolate were analyzed by thin-layer chromatography (TLC) and gas chromatography/mass spectroscopy (GS/MS) for mycotoxins. Toxins detected were zearalenone (13 isolates), fusarin C (11 isolates), nivalenol (4 isolates), and diacetoxyscirpenol (2 isolates). Zearalenone and fusarin C were produced by isolates from each continent, while nivalenol was detected in the *Fusarium* isolates originating from Australia and one isolate from the United States.

Introduction

Fusarium crookwellense Burgess, Nelson, and Toussoun, a member of the Section Discolor has been isolated from wheat crowns, potato tubers, corn stalks, corn stubble, and soil debris [1, 6, 7, 9]. This fungus has widespread geographic distribution and is generally more abundant in temperate zones or areas of moderate rainfall [1]. Recently, *F. crookwellense* has been isolated from pasture herbage from several areas of New Zealand [2, 4, 5]. Livestock grazing in these pastures had reproductive problems and were generally unhealthy. Zearalenone, fusarin C and several trichothecenes and related compounds have been reported as metabolites of *F. crookwellense* [3–5]. These metabolites are listed in Table 1 along with isolate source and production media.

We recently reported on the mycotoxins produced by *F. crookwellense* KF 748 isolate from potato tuber [3] and now report on the toxigenic potential of eighteen *F. crookwellense* isolates from North America, Australia, and Poland. Each isolate was grown on corn substrate, and the extracts from the fermented corn were assayed by thin-layer chromatographic methods for the presence of trichothecenes (e.g., nivalenol, fusarenone X, diacetoxyscirpenol), zearalenone,

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Table 1. Metabolites of *Fusarium crookwellense*

Metabolite	Culture source	Production substrate	Reference
Apotrichothecene, 3-hydroxy	a, b	d, e	4
Butenolide	a, b	d	4
Fusarenone X	c	f, g	3
Fusarin C	c	f, g	3
Isotrichodermin	a, b	d, e	5
7-hydroxy	a, b	d, e	4
8-hydroxy	a, b	d	4
8-hydroxy	a	e	
7,8-dihydroxy	a, b	d	4
8-keto	a, b	d	4
Isotrichodermal	a, b	d	5
7-hydroxy	a	d	5
8-hydroxy	a	d	5
Nivalenol	c	f, g	3
4,15-diacetyl	a, b	d, e	4
4,15-diacetyl-7-deoxy	a, b	d	5
Sambucinol	1, b	d	5
3-deoxy	a, b	d	5
Sambucoin	a	d	5
Zearalenone	a, b, c	c, d, f, g	3, 4, 5
Zearalenols			
α-trans, β-cis	c	f, g	3

a = DAOM [Department of Agriculture, Ottawa, Ontario, Canada] 193611; b = DAOM 193861; c = NRRL [A-28100]; d = MYRO (Liquid medium); e = GYEP (Liquid medium); f = rice; g = corn

zearalenol and fusarin C. The identity of the metabolites was confirmed by gas chromatography-mass spectroscopy (GC/MS).

Materials and methods

Origin of Fusarium crookwellense *isolates*

Eighteen isolates of *F. crookwellense* were received from the following sources: Six isolates originating in Poland were obtained from the Institute of Food Technology Culture Collection, Agricultural University of Poznan, Poland; the remaining twelve isolates (five Australian isolates, five United States isolates and two Canadian isolates) were kindly supplied by Paul E. Nelson, the Pennsylvania State University, University Park. The *F. crookwellense* isolate accession numbers to the specific Culture Collection, and the substrate source are summarized in Table 2.

Culture techniques

Inoculum was prepared by growing single-spored isolates of each culture on hay-agar for seven days. Spore suspensions (1 ml of a 5 ml spore suspension per hay-agar [broth from 50 g dry hay and 1 l H_2O steamed 30 min., pH 6.0–6.5 and 20 g agar] tube) were used to inoculate 50 g of autoclaved (0.5 h at 121 °C) whole corn (adjusted

Table 2. Mycotoxins produced in corn by isolates of *F. crookwellense* from different geographic locations after 14 days at 25 °C

Geographical location	Culture collection isolate numbers		Source	Toxins[a] μg/kg			
	NRRL[b]			F-2[d]	NIV[e]	DAS[f]	FUS[g]
Australia	13336	R[c]3090	Debris, Pasture	ND[h]	ND	ND	110
	13340	2153	Soil, Pasture	0.5	1	ND	190
	13352	2770	Soil, Pasture	0.1	1	ND	250
	13359	3582	Soil, Pasture	0.5	0.5	ND	90
	13361	3611	Soil	2.1	ND	ND	150
	28090	KF[i]701	Potato	ND	ND	0.7	150
Poland	28091	707	Potato	0.04	ND	1.1	ND
	28097	752	Potato	0.42	ND	ND	800
	28098	754	Potato	2.1	ND	ND	300
	28099	755	Potato	2	ND	ND	140
	28101	725	Potato	1.05	ND	ND	160
United States	13364	R-3933[j]	Turf grass	1.2	0.5	ND	450
	26164	4006	Turf grass	ND	ND	ND	ND
	26161	2201	Corn stalk	1.4	ND	ND	ND
	28142	2305	Corn	ND	ND	ND	ND
	26163	3968	Potato	ND	ND	ND	ND
Canada	28143	5179	Red clover roots	ND	ND	ND	ND
	28144	7161	Wheat crown	1.4	ND	ND	ND

[a] Each number is the average of these samples.
[b] Northern Regional Research Culture Collection.
[c] Pennsylvania State University, Culture Collection.
[d] Zearalenone.
[e] Nivalenol.
[f] Diacetoxyscirponol.
[g] Fusarin-C.
[h] Not detected.
[i] Institute of Food Technology Culture Collection, Agricultural Universityo f Poznan, Poland.
[j] Fusarenone and 15-acetylfusarenone X ere detected (<2 μg/g) only in strain R-3933.

to 30–33% moisture content before autoclaving) in 500-ml Erlenmeyer flasks. Each isolate was grown in triplicate and incubated at 25° for 14 days. The culture material was then dried in a forced-air oven at ambient temperature for 18 hours, milled and subjected to chemical analyses. Control uninoculated corn was prepared as above, but not inoculated.

Chemical analysis

Milled cultured material [30 g] from each isolate was blended with 90% aqueous methanol [150 ml] for 3 min in a Waring blendor. The CH_3OH extract was filtered and 100 ml was placed on a roto-evaporator to remove CH_3OH; the remaining water layer [ca. 80 ml] was partitioned with equal volume of ethyl acetate three times each. The combined ethyl acetate extracts were dried over anhydrous sodium sulfate, filtered and evaporated to dryness. The remaining residue was analyzed for the trichothecenes, and zearalenone, zearalenol, and fusarin C by normal-phase and C-18 reverse-phase thin-layer chromatography (TLC) as previously described [3]. The identity of metabolites detected by TLC were confirmed by GC/MS after conversion to their trimethylsilyl ether derivatives [8].

Results and discussion

Metabolites produced by geographic isolates of *F. crookwellense* on corn are presented in Table 2. Four of the five Australian isolates obtained from pasture and soil produced zearalenone at levels from 0.5 to 2.1 μg per g of corn; three isolates produced nivalenol (0.5 to 1 μg/g) and all produced high amounts of fusarin C (90 to 250 μg/g). Of the North American strains, two of the five originating from the United States produced zearalenone at levels of 1.2 to 1.4 μg/g, and one of the two Canadian strains produced an average of 1.2 μg/g zearalenone. The North American strain R-3933 produced nivalenol (0.5 μg/g), fusarenone X (<2 μg/g), 15-acetoxy fusarenone X (<2 μg/g), and fusarin C. Five out of the six *F. crookwellense* strains originating from potato tuber from central Poland produced fusarin C in amounts from 140–800 μg per g of culture material and 0.42 to 2.1 μg/g) zearalenone. In addition, two of the Polish strains produced an average of 0.7 and 1.1 μg/g diacetoxyscirpenol. The majority of the strains originating from Australia and Poland elaborated zearalenone as well as fusarin C. Only one strain originating from the United States produced fusarin C which was not produced by the Canadian strains.

Nivalenol, a type B trichothecene (contains a keto group on carbon 8), was produced by three out of the five Australian strains. Diacetoxyscirpenol (type A trichothecene, no keto group on carbon 8) was elaborated by two of the six Polish strains. Recently we reported that *F. crookwellense* KF 748 isolated from potato tuber from Poland produced type B trichothecenes (fusarenone X and nivalenol) [3]. Only one out of the five North American strain produced nivalenol. No zearalenols were detected in the corn fermented with any *F. crookwellense* isolates. The results indicate the ability of *F. crookwellense* to biosynthesize mycotoxin metabolites is dependent upon substrate as well as geographic location.

Acknowledgements

We thank Darcy L. Shackelford for GC/MS assistance.

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