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New Species of *Aspergillus* Producing Sterigmatocystin

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A number of species belonging to the genus *Aspergillus* were evaluated for their toxicity to ducklings and the ability to produce sterigmatocystin. Three new species capable of producing sterigmatocystin were found, namely, *Aspergillus aurantio-brunneus*, *Aspergillus quadrilineatus*, and *Aspergillus ustus*. All three were toxic to ducklings. The production of sterigmatocystin by *Aspergillus rugulosus* was confirmed, and the toxicity of *Aspergillus stellatus* and *Aspergillus multicolor* is described.

Sterigmatocystin is a carcinogenic compound that has been shown to affect various species of experimental animals (19). It was first isolated from *Aspergillus versicolor* (Vuillemin) Tiraboschi (3, 5), and has been shown to be produced also by an isolate of *A. rugulosus* (1). The list of known producers was extended to *A. nidulans* (Eidam) Winter and an unidentified species of *Bipolaris* by Holzapfel et al. (7). The latter was later shown to be *Bipolaris sorokiniana* (Saccardo in Sorok) Shoem, also known as *Drechslera sorokiniana* (Saccardo) Subram & Jain (9). Recently, Schroeder and Kelton (13) reported that a large number of isolates (57) of *A. flavus* Link and 20 isolates of *A. parasiticus* Speare also produce sterigmatocystin. They extended the list of known producers to *A. chevalieri*, *A. ruber*, and *A. amstelodami*.

Sterigmatocystin and aflatoxin have long been considered related, and sterigmatocystin has been proposed as an intermediate in several schemes for aflatoxin biosynthesis (2). The conversion of sterigmatocystin into aflatoxin by mycelium of *A. parasiticus* (8) and the enzymatic conversion of sterigmatocystin into aflatoxin B_1 by cell-free extracts of *A. parasiticus* have been demonstrated (17).

The fungi capable of producing sterigmatocystin have been isolated from a wide variety of foodstuffs. *A. versicolor*, which produces the highest yields, has been found in grains (20), bread (11), wheat flour (6), grape juice (16), meat, and cheese (10), and the toxin has been detected in wheat (14). Schroeder and Kelton (13) found that the heavy producers of sterigmatocystin, *A. versicolor*, *A. nidulans*, and *A. rugulosus*, did not produce aflatoxin. These belong to the *A. nidulans* and *A. versicolor* groups of the aspergilli. Those belonging to the *A. glaucus* group (*A. chevalieri*, *A. ruber*, *A. amstelodami*) produced small quantities of sterigmatocystin and aflatoxin and the ability was transient. With all isolates of *A. flavus* and *A. parasiticus*, these authors found that those isolates that produced aflatoxin also accumulated sterigmatocystin, *A. flavus* much more so than *A. parasiticus*.

In view of the fairly large number of species capable of producing sterigmatocystin and in view of these species being encountered in various foodstuffs, it was decided to evaluate other species in both the *A. nidulans* and *A. versicolor* groups for their ability to produce sterigmatocystin.

MATERIALS AND METHODS

The following isolates of aspergilli belonging to the *A. versicolor*, *A. nidulans*, and *A. flavus* groups were tested: *A. asperescens* Stolk, CBS 110.51; *A. aurantio-brunneus* (Atkins, Hindson, & Russell) Raper & Fennell, CBS 465.65; *A. caespitosus* Raper & Thom, CBS 246.73; *A. janus* Raper & Thom, CBS 118.45; *A. quadrilineatus* Thom & Raper, CBS 235.65; *A. multicolor* Sappa, CBS 133.54; *A. recurvatus* Raper & Fennell, CBS 496.65; *A. sydowii* (Bain & Sartory) Thom & Church, 593.65; *A. stellatus* Curzi, CBS 598.65; *A. unguis* (Emile-Weil & Gaudin) Thom & Raper, CBS 595.65; *A. rugulosus* NRRL 206 (a); *A. rugulosus* NRRL 207 (b); *A. rugulosus* NRRL 209 (c); *A. rugulosus* NRRL 210 (d); *A. rugulosus* NRRL 216 (e); *A. rugulosus* NRRL 1241 (f); *A. rugulosus* NRRL 4581 (g); *A. rugulosus* As-RL-8 (h); *A. flavus* S-70-64C (a); *A. flavus* p-70-41b (b); and *A. flavus* Pep-72-2e (c). An isolate of *A. ustus* (Bainier) Thom & Church, designated MRC 96, was isolated as a contaminant from a liquid culture of *B. sorokiniana* and was also included in the study.

Culture techniques. Inocula were prepared by growing the fungi on 30 ml of potato dextrose agar in 250-ml Erlenmeyer flasks, which were incubated at 25°C for 10 days. Spore suspensions were used to inoculate whole yellow corn in 2-liter fruit jars. The corn (400 g of corn and 400 ml of water) was previously autoclaved for 1 h on 2 consecutive days at 121°C and, after inoculation, incubated at 25°C for 21

days. The material was dried, milled to a fine meal, and stored at 4°C until used.

Toxicity tests. The moldy meal was incorporated into a commercial chicken mash on a 50% weight basis. Control feed consisted of 50% mash to which was added 50% yellow corn meal obtained by autoclaving corn for 1 h on 2 consecutive days. One-day-old Pekin ducklings were used and were fed ad libitum for 14 days. Weights of survivors were recorded.

Chemical analysis. Qualitative and quantitative determinations of sterigmatocystin were made by thin-layer chromatography according to Stack and Rodricks (18). Sterigmatocystin was identified by its R_f and brick-red fluorescence under long-wave ultraviolet irradiation. Confirmation of identity was done by preparation of the acetate and hemiacetal derivatives. Plates were sprayed with $AlCl_3$ to enhance fluorescence.

Aflatoxin estimations were made by thin-layer chromatography according to the method of Dantzman and Stoloff (4). All determinations were done in duplicate.

RESULTS

The results in Table 1 show that two species in the *A. nidulans* group, namely, *A. quadrilineatus* and *A. aurantio-brunneus*, are capable of producing sterigmatocystin. The isolate of *A. ustus* also produced sterigmatocystin, but in low quantity. Six of the isolates of *A. rugulosus* produced sterigmatocystin in appreciable quantities.

The three *A. flavus* isolates all produced aflatoxin, two in very low quantities. None of these could be shown to produce sterigmatocystin. None of the other species produced any aflatoxin. Results in Table 2 show that only *A. asperescens* was nontoxic.

DISCUSSION

Semeniuk et al. (15) evaluated isolates of all the above species of *Aspergillus* except *A. stellatus*. They found *A. janus* to be markedly toxic and isolates of *A. sydowii* and *A. ustus* to be moderately toxic to chickens. *A. asperescens* was found to be mildly toxic to chickens and moderately toxic to mice. *A. caespitosus*, *A. rugulosus*, and *A. flavus* were found to be mildly toxic to mice. Three of the isolates of *A. rugulosus* evaluated in their study (NRRL 206, NRRL 207, and NRRL 209) and found to be nontoxic to chickens were included in our tests. All three, as well as most of the other isolates of *A. rugulosus*, proved to be toxic. Chemical analysis showed that all except the least toxic isolate produced sterigmatocystin. The differences between their results and ours most probably lie in the different periods of incubation used. Semeniuk et al. (15) grew their cultures for 5 to 7 days, whereas ours were incubated for 21 days. Other factors, such as differences in growth substrates and different test animals, may also be involved.

Schroeder et al. (13) reported that different

TABLE 1. *Estimation of sterigmatocystin and aflatoxin production by different species of Aspergillus*

Aspergillus group	Species	Sterigmatocystin (mg/kg)	Aflatoxin (mg/kg)
A. versicolor	*A. asperescens*	0	0
	A. caespitosus	0	0
	A. janus	0	0
	A. sydowii	0	0
	A. aurantio-brunneus	66	0
	A. quadrilineatus	98	0
	A. multicolor	0	0
	A. recurvatus	0	0
	A. stellatus	0	0
	A. unguis	0	0
A. nindulans	*A. rugulosus* (a)	80	0
	A. rugulosus (b)	92	0
	A. rugulosus (c)	412	0
	A. rugulosus (d)	166	0
	A. rugulosus (e)	0	0
	A. rugulosus (f)	83	0
	A. rugulosus (g)	154	0
	A. rugulosus (h)	0	0
A. ustus	*A. ustus*	15	0
A. flavus	*A. flavus* (a)	0	8.0
	A. flavus (b)	0	<1.0
	A. flavus (c)	0	<1.0

TABLE 2. *Toxicity of different species and isolates of Aspergillus to ducklings*

Species	No. died/ no. tested	Avg days to death	Avg wt of survivors (g)
A. asperescens	0/8		208
A. aurantio-brunneus	7/8	7	70
A. caespitosus	8/8	6	
A. janus	3/8	7	166
A. quadrilineatus	5/8	9	79
A. multicolor	7/8	7	76
A. recurvatus	4/8	6	155
A. sydowii	4/8	6	133
A. stellatus	8/8	6	
A. unguis	5/8	6	71
A. ustus	4/4	5	
A. rugulosus (a)	4/4	6	
A. rugulosus (b)	4/4	5	
A. rugulosus (c)	4/4	8	
A. rugulosus (d)	4/4	7	
A. rugulosus (e)	3/4	5	70
A. rugulosus (f)	4/4	6	
A. rugulosus (g)	4/4	8	
A. rugulosus (h)	0/4		133
A. flavus (a)	2/4	12	132
A. flavus (b)	4/4	8	
A. flavus (c)	4/4	5	
Control	0/4		221

isolates of *A. parasiticus* and *A. flavus* that produced aflatoxin lost this ability after a while. We encountered the same phenomenon with *A. ustus*. This isolate was used in a number of experiments, and >10 positive determinations for sterigmatocystin were made. This isolate eventually completely lost the ability to produce sterigmatocystin. The other two new species added to the list of sterigmatocystin producers, *A. aurantio-brunneus* and *A. quadrilineatus*, were also both toxic to ducklings. In the case of *A. ustus*, the amount of sterigmatocystin produced was low and the toxicity could have been partly due to the presence of other toxins, such as ausdiol, which were not tested for. None of the other toxic species or isolates produced sterigmatocystin or aflatoxin (except *A. flavus*, which produced aflatoxin), and no tests were carried out for other mycotoxins. In the case of *A. caespitosus*, the toxins involved are probably verruculogen or fumitremorgin B (12), as the ducklings showed mild tremorgenic effects.

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