APPLIED AND ENVIRONMENTAL MICROBIOLOGY, May 1999, p. 2264–2266 Vol. 65, No. 5
0099-2240/99/$04.00+0

Divergence of West African and North American Communities of *Aspergillus* Section *Flavi*

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Received 28 September 1998/Accepted 26 February 1999

West African *Aspergillus flavus* S isolates differed from North American isolates. Both produced aflatoxin B_1. However, 40 and 100% of West African isolates also produced aflatoxin G_1 in NH_4 medium and urea medium, respectively. No North American S strain isolate produced aflatoxin G_1. This geographical and physiological divergence may influence aflatoxin management.

Aflatoxins are fungal metabolites that suppress animal immune systems, are probable human carcinogens, and frequently contaminate foods and feeds (15, 17). Diverse communities of aflatoxin-producing fungi occupy soils where aflatoxin contamination is common (2). Aflatoxin producers are asexual fungi belonging to *Aspergillus* section *Flavi* (9). These fungi vary in genetic, morphological, and physiological traits (3, 4). North American communities of section *Flavi* differ by region in both species composition and aflatoxin-producing potential (8).

The most common aflatoxin-producing species, *Aspergillus flavus*, can be divided into two strains. The S strain produces numerous small sclerotia (average diameter, <400 μm) and high levels of aflatoxins. The L strain produces fewer, larger sclerotia and, on average, less aflatoxin (4, 13, 18). S strain isolates have been referred to as atypical (18), microsclerotium producing (14), and *Aspergillus flavus* var. *parvisclerotigenus* (19). Within the S strain, some isolates, termed S_B, produce only B aflatoxins, while others, termed S_{BG}, produce both B and G aflatoxins (12–14, 18). Molecular phylogenetics suggests that S_B isolates are closely related to the *A. flavus* type culture and other L strain isolates (12).

In the United States, S strain incidence within section *Flavi* communities ranges from less than 5% to more than 90% (8). S strain isolates from Indonesia, Thailand, the Philippines, and Africa are also known (13, 14, 18). From North America, only S_B isolates have been reported (4, 8). However, in Thailand both S_B and S_{BG} isolates occur (18). The only S strain isolates previously reported from Africa are two S_{BG} isolates collected three decades ago in Nigeria (13). Our objectives in this study were, firstly, to compare S strain communities in West Africa with those previously characterized in North America and, secondly, to compare ammonium- and urea-based media for assessing the S strain phenotype.

We recovered 67 S strain isolates from 15 agricultural soils collected during 1994 and 1995 in the Republic of Benin by the dilution plate technique on modified rose bengal agar (5). Isolates were evaluated for aflatoxin-producing ability (8) in a medium containing 3 g of $(NH_4)_2SO_4$/liter (27 mM) as the sole N source (1). Initial screens (limit of detection, 1 μg of aflatoxin B_1/fermentation) of African isolates detected both S_B (40 isolates) and S_{BG} (27 isolates) phenotypes. All 374 S strain isolates from North America previously examined in the same medium had the S_B phenotype (4, 8). The African S_B isolates produced less aflatoxin B_1 than was reported for North American S_B isolates. Most (59%) North American S_B isolates produced >100 μg of aflatoxin B_1 per 70-ml fermentation (8), whereas just 2% of African S_B isolates produced that quantity. Two percent of North American S_B isolates produced <0.5 μg of aflatoxin B_1 per fermentation, whereas 15% of African S_B isolates produced <0.5 μg.

African S_B isolates were reevaluated in medium with 3 g of urea/liter (50 mM) substituted for NH_4. All 24 isolates examined had the S_{BG} phenotype in urea. To determine if the two phenotypes were dependent on the nitrogen source or concentration, four representatives of each phenotype were assayed in independent tests in medium with either 45 or 100 mM N supplied as either $(NH_4)_2SO_4$ or urea (Table 1). Cultures were fermented on a rotary shaker (30°C, 150 rpm, 5 days) in 250-ml flasks containing 70 ml of medium seeded with approximately 5,000 conidia (8). Media were adjusted to pH 4.7 prior to autoclaving. After fermentation, the final filtrate pH was measured prior to the addition of 70 ml of acetone. Mycelia were caught on Whatman no. 4 filter paper during filtration, dried (55°C, 3 days), and weighed. Filtrates were diluted to 50% with water and were extracted twice with 25 ml of methylene chloride. Extracts were passed through anhydrous sodium sulfate, combined, and evaporated to dryness, and the residue was dissolved in 4 ml of methylene chloride (8). Residues were concentrated or diluted as appropriate, applied with aflatoxin standards to thin-layer chromatography plates, and developed with ether–methanol–water (96:3:1). Aflatoxins were quantified with fluorescence densitometry (8, 16).

Increasing the NH_4 concentration from 45 to 100 mM did not significantly increase aflatoxin production by any of the eight isolates examined (Table 1). However, African S strain isolates produced at least 7-fold more aflatoxin B_1 and at least 58-fold more aflatoxin G_1 with urea than with NH_4. Most North American isolates produced statistically similar quantities of aflatoxin with NH_4 and urea. However, one North American isolate (LA 2-5) produced significantly less aflatoxin with urea. The growth of African isolates was not inhibited by NH_4. Indeed, African isolates produced greater biomass than North American isolates in NH_4 medium, and frequently the mass produced by African isolates in NH_4 medium was similar to that in the molar equivalent urea medium (Table 1). All isolates modified the pH of the ammonium medium similarly.

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TABLE 1. Production of aflatoxins B_1 and G_1 by representative West African and North American S strain isolates in liquid media containing either ammonium or urea as the sole nitrogen source

Test	Origin	Isolate	N concn (mM)	Result in NH_4 or urea medium[a]							
				Aflatoxin B_1 (μg)		Aflatoxin G_1 (μg)		Final pH		Mass (g)	
				NH_4	Urea	NH_4	Urea	NH_4	Urea	NH_4	Urea
1	Benin	BN008R	45	6 B	940 A	0 B	270 A	3.2 B	3.1 B	790 B	700 B
			100	16 B	1,400 A	0.04 B	1,900 A	2.3 C	3.6 A	830 B	1,000 A
2	Benin	BN038G	45	400 E	1,900 D	6 E	240 D	2.4 E	3.4 DE	850 D	770 E
			100	370 E	2,000 D	4 E	290 D	2.4 DE	3.9 E	730 E	870 D
3	Benin	BN026G	45	35 G	240 F	0.9 G	71 F	2.3 H	3.3 G	970 F	620 G
			100	13 G	180 F	0.7 G	69 F	2.4 H	4.0 F	990 F	760 FG
4	Benin	BN040B	45	30 J	890 I	1 J	210 I	2.2 K	3.3 J	990 I	690 J
			100	28 J	1,900 I	1 J	930 I	2.4 K	3.9 I	910 I	920 I
5	United States	YV2-19	45	620 L	1,400 L	0	0	2.5 M	3.9 L	310 M	580 M
			100	780 L	1,600 L	0	0	2.5 M	4.1 L	310 M	890 L
6	United States	LA2-5	45	940 N	220 O	0	0	2.4 P	3.3 O	580 P	950 O
			100	900 N	380 O	0	0	2.5 P	4.6 N	450 P	1,100 N
7	United States	MS22-41	45	830 Q	940 Q	0	0	2.5 S	3.4 R	300 S	680 R
			100	620 Q	1,200 Q	0	0	3.0 RS	4.2 Q	260 S	960 Q
8	United States	AL3-22	45	680 T	590 T	0	0	2.7 V	3.5 U	250 V	640 U
			100	1,100 T	820 T	0	0	2.5 V	4.0 T	360 V	870 T

[a] Results are averages of three replicates. Values for a variable within a test followed by a common letter are not significantly different ($P = 0.05$) according to Fisher's least significant difference test. Each Benin isolate originated from a separate agricultural field. Isolates YV2-19, LA2-5, MS22-41, and AL3-22 originated from agricultural fields in Arizona, Louisiana, Mississippi, and Alabama, respectively.

However, African isolates reduced the pH of the urea medium slightly more than North American isolates.

In two additional tests, African and North American isolates were compared in 50 mM urea medium. For each test 20 African and 20 North American isolates were evaluated as described above in a single fermentation. Different isolates were used in each test, and test 2 included A-11612 and A-11611 from Nigeria (13). All African isolates had the S_{BG} phenotype, and all North American isolates had the S_B phenotype. More than 50% of African isolates and more than 10% of North American isolates produced >1 mg of aflatoxin B_1/fermentation (Fig. 1), and more than 90% of African isolates

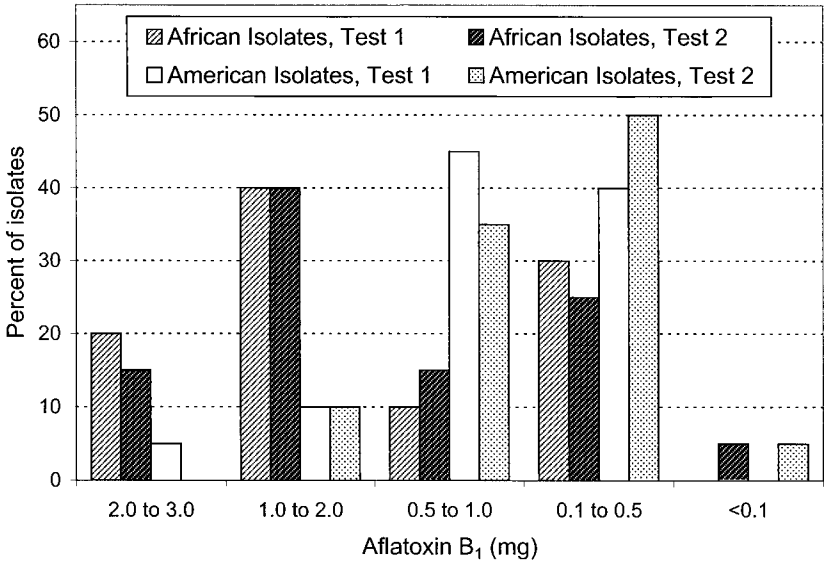

FIG. 1. Percentage of *A. flavus* S strain isolates producing various quantities of aflatoxin B_1 in a chemically defined medium containing urea as the sole nitrogen source. Results of two tests are shown. Each test evaluated production by 20 West African and 20 North American isolates. Values are expressed in milligrams of aflatoxin B_1 per 70-ml fermentation.

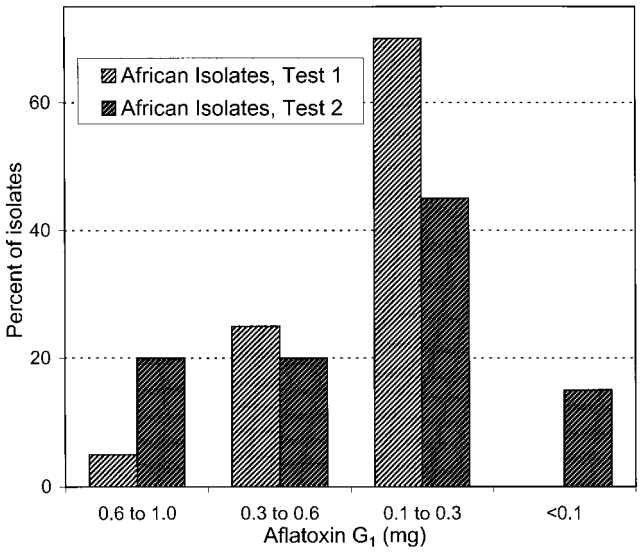

FIG. 2. Percentage of *A. flavus* S strain isolates producing various quantities of aflatoxin G_1 in a chemically defined medium containing urea as the sole nitrogen source. Each test evaluated production by 20 West African and 20 North American isolates. No North American isolate produced aflatoxin G_1. Values are expressed in milligrams of aflatoxin G_1 per 70-ml fermentation.

produced between 0.1 and 1.0 mg of aflatoxin G_1 (Fig. 2).

The urea tests suggest that the S_B phenotypes detected during initial screens of African isolates resulted from a reduced capacity of ammonium to support aflatoxin production and not from an innate inability of the African isolates to produce aflatoxin G_1. No African isolate expressed the S_B phenotype in urea. In North America, S_B isolates predominate, and no S_{BG} isolates have been detected among the several hundred S strain isolates checked on NH_4 medium (4, 8) or among the >50 isolates checked either on other media (10, 13) or on crops (4, 11). The present results demonstrate that communities of section *Flavi* in North America are different from those in West Africa. The causes of the divergence are unclear. S_{BG} isolates may be ancestral to S_B isolates (12), and the S_B phenotype may have arisen outside of Africa. Thus, S_B isolates might never have become established in West Africa. However, it is also possible that as yet unidentified selective forces active in West Africa and/or North America are responsible for observed differences in community composition.

The occurrence of aflatoxins in food creates international concern (9). Both the North American S_B and the West African S_{BG} isolates have great aflatoxin-producing potential. The aflatoxin-producing potentials of *A. flavus* communities can impact crop contamination (6, 8). Therefore, incidences of both S strain types should be of interest. S_B isolates can be major contributors to the contamination of cottonseed with aflatoxin (7). However, the extent to which S_{BG} isolates contribute to aflatoxin contamination in West Africa, and elsewhere, is unknown. Adapting aflatoxin management strategies from North America for use in Africa requires consideration of different cultures and agronomic systems. The divergence in fungal communities described here is an additional factor to consider.

We thank Darlene Downey, Kerrilee Kobbeman, and Nicole Hurban for technical assistance.

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