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Aflatoxin Production via Cross-Feeding of Pathway Intermediates during Cofermentation of Aflatoxin Pathway-Blocked *Aspergillus parasiticus* Mutants

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Cofermentation of *Aspergillus parasiticus* strains (SRRC 163 and SRRC 2043) blocked at different steps in the aflatoxin B_1 (AFB_1) biosynthetic pathway in a synthetic liquid medium or on seeds (cottonseed, corn kernels, and peanuts) resulted in production of AFB_1. Strain SRRC 2043 accumulated *O*-methylsterigmatocystin (OMST), a late precursor in AFB_1 biosynthesis, whereas SRRC 163 accumulated averantin, an early precursor in the pathway. Strain SRRC 2043 secreted large amounts of OMST in culture relative to the amounts of several other pathway intermediates secreted into media (by other AFB_1 pathway-blocked strains). AFB_1 production occurred even when colonies of SRRC 163 and SRRC 2043 strains (producing no detectable AFB_1) were grown together on an agar medium while physically separated from each other by a filter membrane (0.22-μm pore size). In addition, when mycelia of strain SRRC 163 were added to culture filtrates (containing no mycelia but containing secreted OMST) of strain SRRC 2043, AFB_1 production occurred. The results suggested a chemical (rather than genetic) mechanism of complementation for AFB_1 production between AFB_1 pathway-blocked strains, since no mycelial contact was required between these strains for AFB_1 production. The mechanism for chemical complementation involves secretion of OMST by SRRC 2043 and subsequent absorption and conversion of OMST to AFB_1 by mycelia of strain SRRC 163.

Many of the biosynthetic intermediates in the aflatoxin B_1 (AFB_1) pathway have been identified (4, 7, 16, 17, 22, 23), and several of the enzymes catalyzing the biosynthetic steps in AFB_1 formation have been isolated (2, 6, 9, 10, 12, 13, 24, 25) and purified to homogeneity (5, 8, 14). The generally accepted pathway from the earliest known biosynthetic intermediate to the formation of AFB_1 is as follows: norsolorinic acid (NOR) → averantin (AVN) → averufanin → averufin → hydroxyversicolorone → versiconal hemiacetal acetate → versicolorin A (VER A) → sterigmatocystin → *O*-methylsterigmatocystin (OMST) → AFB_1. Some recent results have also indicated that versicolorin B may be the precursor of versicolorin A after versiconal hemiacetal acetate in AFB_1 synthesis (20, 24).

Mutant strains of *Aspergillus parasiticus* have been isolated that are blocked at different stages in the AFB_1 biosynthetic pathway and that accumulate different AFB_1 pathway intermediates, such as NOR, AVN, averufin, hydroxyversicolorone, VER A, or OMST (4, 7, 16, 17, 22, 23), in culture. These mutant strains, however, can convert in culture exogenously added aflatoxin precursors (beyond the point of the block) to AFB_1 (7, 16, 17, 19). Also, diploids of strains blocked at these different steps in AFB_1 biosynthesis will produce aflatoxin (3, 18), since in diploid form they possess all the genetic information necessary for synthesis of AFB_1. In addition, in an earlier study from this laboratory, it was observed that during cofermentation of two blocked strains of *A. parasiticus*, levels of OMST were greatly reduced in the culture with the simultaneous production of aflatoxins (11). The mechanism of this complementation (chemical or genetic) was not examined in detail in that study.

Chemical complementation (cross-feeding) for AFB_1 biosynthesis between blocked pathway mutants requires the secretion of biosynthetic pathway intermediates by one strain, their uptake by fungal mycelia of the other strain, and conversion of the intermediates to AFB_1 by enzymes catalyzing biosynthetic steps downstream from pathway blocks. Little is known about fungal transport mechanisms involved in secretion and/or uptake of metabolites in the AFB_1 pathway.

The purpose of the present investigation was to utilize selected AFB_1 pathway-blocked strains of *A. parasiticus* to learn more about the secretion and uptake of AFB_1 pathway intermediates produced in high levels in mycelia of these pathway-blocked strains. The present study was also designed to determine whether specific cross-feeding of pathway intermediates occurs between aflatoxigenic and/or nonaflatoxigenic strains during fungal strain interactions on various growth media, resulting in subsequent AFB_1 production.

MATERIALS AND METHODS

Cultures and culture conditions. Several AFB_1 pathway-blocked strains of *A. parasiticus* (SRRC 162, SRRC 163, SRRC 164, and SRRC 2043, which accumulate the AFB_1 pathway intermediates NOR, AVN, VER A, and OMST, respectively) and an AFB_1-producing strain of *A. parasiticus* (SRRC 143) were examined for their ability to secrete certain AFB_1 pathway metabolites into culture filtrates. Liquid cultures made with Adye and Mateles' medium (AM) (1) were initiated by the addition of spore solutions (200 μl) of fungal strains (10^8 spores per ml) to 100 ml of medium in 250-ml flasks. Spores were obtained from 1-week-old potato dextrose agar cultures of fungal strains (12). Cultures were incubated without shaking for 7 days or with shaking (150

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rpm) for 3 days at 29°C. Mycelial mats (nonshake cultures) or balls (shake cultures) were separated from culture filtrates and rinsed with deionized water over Miracloth (Calbiochem-Behring, La Jolla, Calif.) filters (any small fragments of residual mycelia in filtrates were removed by centrifugation at 5,000 × *g*), and mycelia and filtrates were retained for analysis of metabolites.

Secretion of AFB_1 pathway metabolites by fungal mycelia. Secretion of AFB_1 pathway intermediates and AFB_1 into AM medium or into a low-sugar resting medium (LSRM) (1) was quantitated by procedures described earlier for analysis of metabolites (7, 19). In some experiments, secondary metabolites were quantified separately in mycelia and culture filtrates. AFB_1 and OMST were separated by thin-layer chromatography with an ether-methanol-water (96:3:1) (vol/vol/vol) solvent system and quantified by scanning fluorimetry (fluorescence at 365 nm for AFB_1 and at 310 nm for OMST) (7). Separation of the metabolites, NOR, AVN, and VER A, by thin-layer chromatography with a toluene-ethyl acetate-acetate (50:30:4) (vol/vol/vol) solvent system was followed by scanning densitometry (UV absorption at 310 nm) (19) to quantify the compounds.

The relative rates of secretion of certain AFB_1 pathway metabolites by mycelia were also determined. Mycelia of the AFB_1-producing strain (SRRC 143) or the AFB_1 pathway-blocked strains (SRRC 163 and SRRC 2043) were harvested from AM shake cultures 3 days after inoculation (AFB_1 pathway metabolites had been produced by this time) (12) and washed with sterile deionized water by filtration on Miracloth to remove metabolites adhering externally to the mycelia. A portion of the damp mycelia (1.0 g) was added to 20 ml of LSRM in a 50-ml Erlenmeyer flask; quantities of AFB_1 pathway metabolites secreted into the medium (while shaking at 29°C) were determined, as described above, by removing 0.25-ml aliquots of filtrates (any residual mycelia in the aliquots were removed by centrifugation at 5,000 × *g* before the assay) at various periods and by analyzing filtrates for the metabolites.

Cofermentation of AFB_1 pathway-blocked strains of *A. parasiticus*. Production of AFB_1 was determined during cofermentation of two AFB_1 pathway-blocked strains of *A. parasiticus*; cofermentations were initiated by the same methods as those used for culturing individual strains in liquid medium (AM) (first section of Materials and Methods), except that in addition to inoculating AM medium, we also inoculated sterilized seeds with *A. parasiticus* spores. Seeds were prepared before inoculation as follows. Seeds were oven dried (50°C) for 3 days, weighed, and autoclaved for 15 min at 121°C, and the moisture content of the dried seeds was adjusted to 30% (by weight) with sterile deionized water. The *A. parasiticus* strains used in these experiments were SRRC 163 (blocked at the AVN → averufanin and VER A → sterigmatocystin biosynthetic steps) and SRRC 2043 (blocked at the OMST → AFB_1 biosynthetic step). An inoculum (200 μl of spore suspension) of each strain was added separately or together to either 100 ml of AM or to 5 g of selected seeds (peanuts, corn kernels, or cottonseed) and briefly shaken to distribute spore solutions in the media. Liquid (AM) and seed cultures were incubated for 5 and 7 days (without shaking), respectively, at 29°C. AFB_1 and OMST were then extracted and quantified from AM cultures as described above and from seed cultures according to reference 2a.

Tests for complementation for AFB_1 production without contact between mycelia of AFB_1 pathway-blocked mutants. The ability of mycelium-free filtrates of strain SRRC 2043 or SRRC 163 to complement for AFB_1 production was tested by the addition of the filtrates to deionized water-washed 3-day-old mycelia (from AM shake cultures) of strain SRRC 2043, SRRC 163, or SRRC 143. Filtrates were derived by incubating 1.0 g of 3-day-old mycelia of strain SRRC 2043 or SRRC 163 in 20 ml of LSRM for 6 h at 29°C with constant shaking (100 rpm). Mycelia were then removed by filtration (0.45-μm-pore-size polysulfone membranes; Gelman Sciences, Ann Arbor, Mich.), and the resulting mycelium-free filtrates containing the secreted AFB_1 pathway metabolites were quantitated (filtrates of strain SRRC 2043 contained 95 μg of OMST; AVN was not detected in filtrates of the AVN producer, strain SRRC 163). Mycelium-free filtrates subsequently were used for cross-feeding studies. Mycelia (1 g) of strain SRRC 163, SRRC 2043, or SRRC 143 were added to 20 ml of either strain SRRC 163 or strain SRRC 2043 culture filtrate and incubated for 15 h (29°C); at the end of the incubation period, metabolites were extracted from each culture and quantitated.

Another experiment was performed to test for chemical complementation for AFB_1 production without mycelial contact between strains. AM agar (2%) plates were prepared containing membrane barriers imbedded in the agar layer. Rectangular polyvinylidene difluoride filter membranes (5 by 3 cm; 0.22-μm pore size; Millipore Corp., Bedford, Mass.) were folded in half along their lengths at a 90-degree angle and placed into still-molten agar in the plates with one filter edge upright so that about 1 cm of the membrane would extend vertically above the surface of the agar. Plates were then allowed to cool before inoculation with 1 drop of conidial solution (10 μl; 10^8 spores per ml) of the two AFB_1 pathway-blocked strains, SRRC 163 and SRRC 2043, on opposite sides of the filter membrane. Separate plates were also inoculated individually with the above blocked pathway strains as well as the native AFB_1-producing strain (SRRC 143) on only one side of the membrane (no inoculum was placed on the other side of the membrane). Plate cultures were incubated for 10 days at 29°C until well-formed colonies (about 2 cm in diameter) and AFB_1 pathway metabolite production had resulted. At the end of the incubation period, colonies along with their underlying agar medium were removed from both sides of the filter membrane (when two strains were grown on the same plate) and extracted together to remove metabolites from the colonies and agar medium. When single strains were grown on a plate, the colony and a 1-cm-wide strip of agar medium on the opposite sides of the filter membranes (containing no mycelia) were removed and extracted separately. Extraction was done by shaking agar and colony pieces in 5 ml of a solution of acetone and water (50:50; vol/vol) for 30 min. Metabolites were then partitioned into 5 ml of $CHCl_3$ and recovered by drying the $CHCl_3$ under a stream of N_2. Metabolites were separated and quantified by the thin-layer chromatography procedure described above.

All experiments were repeated at least twice with three replicates for each experiment (the standard error of the mean is indicated in the tables). The range about the mean is shown for each datum point on the figure.

RESULTS

The abilities of mycelia of AFB_1 pathway-blocked strains and of a native AFB_1-producing strain of *A. parasiticus* to secrete AFB_1 pathway metabolites into AM media were quantitated (Table 1). Results indicated that most of the NOR, AVN, and VER A produced by pathway-blocked

TABLE 1. AFB_1 pathway metabolites produced in static cultures by five strains of *A. parasiticus*

Culture fraction	Fungal strain[a] and associated AFB_1 pathway metabolite(s) produced (μg[b])					
	SRRC 162		SRRC 163 (AVN)	SRRC 164 (VER A)	SRRC 2043 (OMST)	SRRC 143 (AFB_1)
	NOR	AFB_1				
Filtrate	0	148	146	201	1,086	866
Mycelia	6,808	102	2,112	3,124	1,286	585

[a] SRRC 162 is a leaky mutant that produces AFB_1 because the AFB_1 pathway is only partially blocked, but the mutant mainly accumulates the pathway intermediate NOR. SRRC 163, SRRC 164, and SRRC 2043 are AFB_1 pathway-blocked strains that accumulate the pathway intermediates AVN, VER A, and OMST, respectively, without producing aflatoxins. SRRC 143 is a wild-type AFB_1-producing strain of *A. parasiticus*. Traces of other AFB_1 pathway metabolites besides the ones accumulating (owing to the AFB_1 pathway block) were sometimes detected, but only the major metabolites accumulated by these fungal strains are reported here.

[b] Expressed as total micrograms of metabolite (in filtrate or mycelia) per 100 ml of culture medium. The lower detection limit was 0.1 μg for NOR, AVN, VER A, and OMST and 0.01 μg for AFB_1; values below the detection limits are reported as 0. The standard error about the mean (experiments were replicated twice with three replicates for each experiment) was never more than 20% in any single experiment.

TABLE 2. Production of AFB_1 and OMST by selected aflatoxin pathway-blocked strains of *A. parasiticus* growing alone or in cofermentation on different growth media

Growth medium	SRRC 163 alone[a]		SRRC 2043 alone[a]		SRRC 163 and 2043 in cofermentation[a]	
	AFB_1 (μg)	OMST (μg)	AFB_1 (μg)	OMST (μg)	AFB_1 (μg)	OMST (μg)
Peanuts[b]	0	0	0	295	203	392
Cottonseed[b]	0	0	0	377	435	236
Corn kernels[b]	0	0	0	311	39	149
Liquid medium[c]	0	0	0	2,040	181	1,810

[a] See Table 1, footnote *a*, for description of strains. Values are expressed as total micrograms of AFB_1 or OMST extracted per culture. The lower detection limit was 0.1 μg for OMST and 0.01 μg for AFB_1. The standard error about the mean (two experiments; three replicates each) was never more than 25% in any single experiment.

[b] Before inoculation, peanuts, corn kernels, and cottonseed were dried in a hot-air oven (50°C), preweighed, and autoclaved and their moisture was adjusted to 30% by the addition of sterile deionized water.

[c] A synthetic liquid medium (AM) (1).

strains (SRRC 162, SRRC 163, and SRRC 164, respectively) was retained in the fungal mycelia. In fact, SRRC 162 retained all detectable NOR within its mycelia. However, high amounts of OMST and AFB_1 were secreted into culture filtrates in AM cultures (Table 1).

The rates of secretion of certain metabolites by mycelia in LSRM were determined. OMST and AFB_1 were rapidly secreted from mycelia of strains SRRC 2043 and SRRC 143, respectively, when 72-h-old mycelia (containing AFB_1 pathway metabolites) were placed into LSRM (Fig. 1). Secretion of AVN by strain SRRC 163 was not detected in this experiment.

Cofermentation of two AFB_1 pathway-blocked strains of *A. parasiticus* (SRRC 163 and 2043) on peanuts, corn kernels, and cottonseed and in a synthetic liquid medium (AM) resulted in production of AFB_1 in the cultures (Table 2). OMST was detected in cultures inoculated with strain SRRC 2043 alone or in cultures inoculated with strains SRRC 2043 and SRRC 163 together. OMST and AFB_1 were not detected in cultures inoculated with strain SRRC 163 alone. Therefore, the production of AFB_1 was dependent on the growth of both blocked strains in cofermentation on the various substrates, since individual strains produced no AFB_1 in culture.

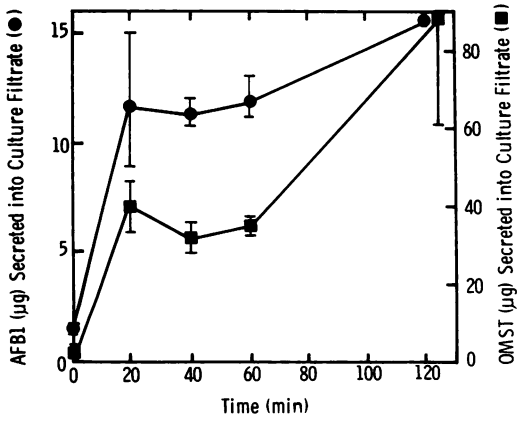

FIG. 1. OMST and AFB_1 secretion in LSRM by mycelia of strains SRRC 2043 and SRRC 143, respectively. AVN secretion by strain SRRC 163 was also examined, but no AVN was detected in filtrates. The ranges about the means (bars) are shown for each datum point.

Addition of mycelia of the AVN-accumulating strain (SRRC 163) to mycelium-free filtrates of the OMST-accumulating strain (SRRC 2043) resulted in AFB_1 production (Table 3). However, SRRC 2043 mycelia were unable to produce AFB_1 when supplied with the filtrates of SRRC 163. The addition of SRRC 143 (native AFB_1-producing strain) mycelia to filtrates from the AFB_1 pathway-blocked strains SRRC 2043 and SRRC 163 did not result in a significant increase in AFB_1 produced over that produced by SRRC 143 mycelia alone under similar conditions.

When colonies of AFB_1 pathway-blocked strains SRRC 163 and SRRC 2043 were grown together on an agar medium but separated by a membrane with a pore size small enough to prevent contact between the two strains, AFB_1 production occurred (Table 4). Over 80% of the AFB_1 was isolated from the agar medium supporting growth of strain SRRC 163 on one side of the membrane. Levels of AFB_1 produced by

TABLE 3. AFB_1 produced by mycelia of AFB_1 pathway-blocked or AFB_1-producing strains of *A. parasiticus* after incubation with mycelium-free culture filtrates

Source of mycelia[a]	AFB_1 produced (μg ± SE)		
	SRRC 2043 filtrate[b]	SRRC 163 filtrate[b]	Control (LSRM)[c]
SRRC 2043	0	0	0
SRRC 163	9.3 ± 1.1	0	0
SRRC 143	47.2 ± 7.2	51.9 ± 6.4	55.5 ± 10.4

[a] See Table 1, footnote *a*, for description of fungal strains.

[b] To obtain culture filtrates, 1 g of washed (see Materials and Methods) 72-h-old mycelia pregrown in liquid medium (1) were transferred into LSRM and then incubated for 6 h with constant shaking at 150 rpm at 29°C; mycelia were removed by filtration (0.45-μm-pore-size membrane), and filtrates containing secreted AFB_1 pathway metabolites were incubated in various combinations with mycelia of selected strains.

[c] Mycelia were added to LSRM containing no AFB_1 pathway metabolites as a control.

TABLE 4. AFB_1 and AFB_1 pathway metabolites produced by individual fungal colonies or physically separated colonies of the AFB_1 pathway-blocked strains of *A. parasiticus*

Strain[a]	Metabolite produced (μg ± SE)[b]					
	AVN		OMST		AFB_1	
	C	A	C	A	C	A
SRRC 163	128.7 ± 20.6	0	0	0	0	0
SRRC 2043	0	0	243.5 ± 31.2	3.6 ± 1.2	0	0
SRRC 163 + SRRC 2043[c]	146.5 ± 12.2	NA[d]	295.5 ± 24.6	NA	0.9 ± 0.3	NA
SRRC 143	0	0	0	0	56.9 ± 10.2	8.5 ± 2.2

[a] See Table 1, footnote *a*, for description of fungal strains.
[b] Individual colonies (C) and the adjacent agar medium (A), i.e., a 1-cm strip of agar medium on the other side of membrane filters (0.22-μm pore size; see Materials and Methdos) were removed from plates and extracted separately, and metabolites were quantitated.
[c] Fungal colonies of strains SRRC 163 and SRRC 2043 were grown in close proximity (≤2 mm apart) to one another but were separated by a filter membrane (0.22-μm pore size; see Materials and Methods). Colonies of both fungal strains were extracted together in this study, and metabolites were quantitated.
[d] NA, not analyzed.

colonies of strain SRRC 163 grown together with colonies of strain SRRC 2043 were low relative to levels of AFB_1 produced by the wild-type strain (SRRC 143). However, these low levels of AFB_1 were consistent with the low levels of AFB_1 precursor (OMST) diffusing toward SRRC 163 mycelia from the SRRC 2043 mycelia; only 2% of the total OMST produced by colonies of strain SRRC 2043 (growing alone) diffused across the membrane (Table 4). Strain SRRC 163, although producing AVN in colonies, secreted no detectable AVN into the agar medium.

DISCUSSION

The most likely mechanism to explain the observed AFB_1 production during cofermentation of AFB_1 nonproducing strains SRRC 163 and SRRC 2043 begins with the secretion of OMST by SRRC 2043 mycelia and then the uptake and conversion of the secreted OMST to AFB_1 by SRRC 163 mycelia. Strain SRRC 163 (unlike strain SRRC 2043) possesses a functional oxidoreductase (10, 12) capable of catalyzing OMST-to-AFB_1 conversion. Strain SRRC 2043, although incapable of performing the conversion of OMST to AFB_1, can synthesize and rapidly secrete OMST into the medium (Fig. 1). The facts that mycelia of strain SRRC 2043 are capable of secreting high amounts of OMST into the culture filtrate (present study) and that exogenously added OMST is absorbed efficiently by mycelia of the mutant strain SRRC 163 and converted to AFB_1 (7) indicate that when the two AFB_1 pathway-blocked strains (SRRC 163 and SRRC 2043) are placed together in cofermentation (this study), the above process probably leads to the complementation for AFB_1 production observed. The same process can be mimicked by supplying SRRC 163 mycelia with mycelium-free filtrates of strain SRRC 2043 (containing secreted OMST) (Table 3). These observations explain the observed cross-feeding between physically separated colonies of the two strains resulting in AFB_1 production (Table 4), in which complementation for AFB_1 production occurred even without physical contact between the two strains (Tables 3 and 4). A chemical mechanism of complementation is, therefore, indicated in all the cases presented here. However, the present study does not preclude the possibility that complementation under certain conditions could occur by hyphal fusion, anastomosis, and/or by a genetic mechanism (3, 18) requiring physical contact between strains.

Results in the present study demonstrated quantitatively that the mycelial mats in static cultures have a less than 7% capability for secretion of early AFB_1 pathway intermediates (NOR, AVN, and VER A) (Table 1), whereas greater than 50% of metabolites at or nearer the end of the AFB_1 pathway (OMST and AFB_1) are secreted by the fungal mycelia (Table 1 and Fig. 1). Earlier results from this laboratory (16, 17) suggested, qualitatively, that NOR and VER A pigments were contained in the nonsporulating mycelial balls obtained in shake cultures.

An appreciable number of dead cells may be present in mycelial balls larger than about 400 μm in diameter as has been suggested for other filamentous fungi (21). Mycelial mats produced in static cultures in the present study may also contain dead cells. Therefore, a possibility exists that metabolites (NOR, AVN, VER A, OMST, and AFB_1) might be released from dead cells, but even if dead fungal tissue was present, the strains still released relatively low amounts of early pathway metabolites (NOR, AVN, and VER A) and high amounts of late pathway metabolites (OMST and AFB_1) (Table 1). These results suggest that early aflatoxin pathway precursors are retained in sequestered domains within the cells, a phenomenon that has been observed in secondary metabolism of other filamentous fungi, particularly in the biosynthesis of antibiotics such as penicillin (15). In this way, the fungus could perform various intracellular enzymatic conversions involved in aflatoxin biosynthesis without premature release of the early pathway intermediates into the medium outside the fungal cell.

Differential release of AFB_1 pathway intermediates also could point to a differential mechanism in transport. It could be proposed that an active transport rather than a simple passive and nonspecific diffusion of these compounds from fungal cells may be involved in the secretion of OMST and AFB_1 from the mycelia, based on the chemical structures of these metabolites. This hypothesis is currently being investigated in our laboratory.

The biological importance of the chemical complementation mechanism observed in our laboratory as it applies to natural field contamination of seeds with AFB_1 is unknown. However, the laboratory results reported here point to certain conditions or restrictions that might limit the degree of field contamination obtained by the chemical complementation mechanism. First of all, under laboratory conditions, AFB_1 production by the complementation mechanism was relatively low compared with AFB_1 production by the native AFB_1-producing strain (SRRC 143), which possesses all the intracellular biosynthetic enzymes necessary for efficient AFB_1 production. However, the relatively low AFB_1 synthesis occurring during cofermentation by AFB_1 pathway-

blocked strains (compared with the native strain) may be due to inefficient cross-feeding of AFB_1 pathway metabolites occurring during the process of secretion and/or uptake of the metabolites by mycelia. Second, inconsistent results were obtained (data not shown) unless the moisture content of seed media was maintained at a minimum of 30%, suggesting that high moisture could be a critical requirement for consistent cross-feeding to occur in the laboratory or in the field. Third, results in the present study indicate that the addition of fungal filtrates containing the AFB_1 precursors (e.g., OMST) to a native AFB_1 producer did not increase the degree of AFB_1 production by the strain (Table 3); perhaps certain AFB_1 pathway enzymes of the native AFB_1 producer are already operating at their maximum rate and the addition of more enzyme substrate does not increase an already high AFB_1 production.

In conclusion, the present study demonstrated that under certain conditions, cross-feeding of metabolites of the AFB_1 pathway is possible between nonaflatoxigenic fungal strains, leading to AFB_1 synthesis. This complementation, however, appears to depend on the nature of the block in the complementing strains, since only late intermediates in the AFB_1 biosynthetic pathway are released into the surrounding medium.

It is known that native AFB_1 nonproducing strains of *A. parasiticus* that produce certain AFB_1 precursors exist in the field; the OMST-producing strain SRRC 2043 is a field isolate of *A. parasiticus* (7). Therefore, the possibility exists that the presence of high populations of such fungal strains in the field could contribute to the total AFB_1 contamination of field crops; the extent of this contribution, however, remains uncertain. This possibility has implications for the effectiveness of use of aflatoxin pathway-blocked strains released for biocontrol of aflatoxin contamination. It is conceivable that in a limited number of cases each blocked strain could complement other blocked strains. However, any contribution of the mutant strain in increasing toxin production by a wild-type strain would be extremely difficult to assess because of the apparently low aflatoxin production resulting from the cross-feeding relative to the high aflatoxin production by the wild-type strain.

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