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Variants of *Aspergillus alutaceus* var. *alutaceus* (Formerly *Aspergillus ochraceus*) with Altered Ochratoxin A Production

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The present studies, using *Aspergillus alutaceus* var. *alutaceus* Berkeley et Curtis (formerly *A. ochraceus* Wilhelm) NRRL 3174 along with three other wild-type strains, were undertaken in an attempt to understand the effects of irradiation and other treatments on mycotoxin production in grain. Bedford barley was inoculated with spores of NRRL 3174, gamma irradiated, and incubated at 28°C and 25% moisture. After 10 days of incubation, two colony types, ochre (parental) and yellow (variant), were isolated from the grain. Further culturing of the yellow variant resulted in the spontaneous appearance of a white variant that exhibited greatly enhanced fluorescence under UV light. In subsequent work, we have also isolated variants producing a soluble red pigment. In addition, in model experiments involving irradiation (1 kGy) of pure cultures, induction frequencies ranging between 2 and 4% (survival basis) were observed for the yellow and red variants. Inoculation of these variants into wheat and incubation for 14 days at 28°C and 32% moisture resulted in ochratoxin A production in the relative amounts of 0.09:1:4.6:9.3 for the red, ochre (parental), yellow, and white variants, respectively. Additional characteristics of these isolates are described. Confirmation that the white high-ochratoxin-A-producing variants were derived from the parental strain was demonstrated by obtaining revertant sectors in monoclonal cultures of the variants.

Ochratoxin A is a low-molecular-weight secondary metabolite produced by several species of the genus *Aspergillus* and by *Penicillium verrucosum* and is a potent nephrotoxin and hepatotoxin (3, 15). Since the 1960 epidemic of turkey X disease (2) in Britain, considerable research has been done on aflatoxin biosynthesis by related species *Aspergillus flavus* and *Aspergillus parasiticus*, but genetic control and regulation of fungal secondary metabolism are still poorly understood. In the last 2 decades, a number of variant strains that were blocked in specific steps of the biosynthetic pathway for aflatoxin have been isolated and used to elucidate parts of the pathway (1, 5, 8–10, 14). With isolated exceptions (13), most of the mutants of these organisms found to date have exhibited decreased levels of aflatoxin production compared with levels produced by the parental or wild-type strain, indicating that the wild type may be producing near-maximum amounts of mycotoxin (1, 5, 14, 18).

Considerably less work has been done on the regulation of ochratoxin production in those species of *Aspergillus* and *Penicillium* that produce these mycotoxins. The availability of variants with altered toxigenicity would provide invaluable tools for establishing the control as well as the pathway involved in ochratoxin biosynthesis. The parasexual cycle as demonstrated by Papa (11) for *Aspergillus* spp. and the use of labeled precursors with such variants should facilitate this type of research.

Aspergillus alutaceus var. *alutaceus* Berkeley et Curtis (1875), formerly *Aspergillus ochraceus* Wilhelm (1877), NRRL 3174 produces ochratoxin A. We report here four strains with altered toxigenicity derived from this fungus after gamma irradiation. One strain produces much less ochratoxin A than the parent, while the remaining three produce more toxin. The availability of these variants of differing toxigenic potential should aid in unraveling the phenomenon of control of toxin production by toxigenic fungi and its perturbation by a variety of physical and chemical agents.

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MATERIALS AND METHODS

Media and chemicals. Dehydrated potato dextrose agar (PDA), Czapek agar (CA), malt extract agar, and yeast extract (YE) were products of Difco Laboratories, Detroit, Mich. Tween 80 was obtained from Koch-Light Laboratories Ltd., Colmbrook, Buckinghamshire, England. Sodium chloride, tartaric acid, and chloroform, certified American Chemical Society grades, were obtained from Fisher Scientific Co., Fair Lawn, N.J. Sucrose was commercial grade. CA-YE with 20% (wt/vol) sucrose (CY20S) was prepared from the ingredients listed above.

Parent strain. *A. ochraceus* Wilhelm ATCC 22947 (=NRRL 3174) was obtained from the American Type Culture Collection, Rockville, Md. The culture was maintained by regular transfer onto slants of PDA containing 0.1% yeast extract and 2% sodium chloride (PDA-YE-NaCl). This parental strain was given the accession number 001 in our laboratory. The name *A. alutaceus* var. *alutaceus* Berkeley et Curtis was assigned to this organism because it is an earlier name suggested by Subramanian (16) that was later endorsed by Samson and Gams (12). Three additional wild-type strains of *A. alutaceus* (ATCC 18412, ATCC 18642, and ATCC 60532) were also obtained from the American Type Culture Collection.

Variant strain production. Spores from 7-day-old cultures of the parent strain grown at 28°C on PDA-YE-NaCl slants were harvested in sterile 0.1% Tween 80, filtered through four layers of sterile cheesecloth, and washed in fresh Tween 80 (0.1%). The spores were counted in a hemacytometer and added to barley (variety Bedford) at a concentration of 10^6 spores per g. The moisture content of the barley was adjusted to 25% (dry weight basis). The inoculated grain was then irradiated in a ^{60}Co irradiator (Gamma Cell 220; AECL)

to a dose of 1.0 kGy at a dose rate of 198 Gy/min. The irradiated grain and a comparable unirradiated but inoculated control were incubated at 28°C and 98% relative humidity. After 10 days of incubation, 25 seeds (approximately 1 g) of each lot were shaken with 10 ml of sterile distilled water containing sterile sand, and serial dilutions of the supernatant were plated in PDA-YE-NaCl medium that had been acidified with 1.6 ml of sterile 10% tartaric acid per 100 ml of medium. After incubation, the colonies were examined for any characteristics that differed from those of the parental strain. This protocol was later repeated except that autoclaved barley (20 min at 121°C) was used as the substrate to ensure that any isolated variants were derived from the parental strain and were not contaminants.

Ochratoxin A production. Ochratoxin A production by the parent and the three variant strains of *A. alutaceus* var. *alutaceus* was tested in liquid culture and in wheat. All strains were grown for 7 days on PDA-YE-NaCl slants, and the spores were harvested in sterile Tween 80 and inoculated at the rate of 10^6 spores per flask for both liquid and wheat substrates. Liquid-culture production was as described previously (4). Six flasks (500 ml each) of wheat (60 g) that had been adjusted to a moisture content of 32% (dry weight) were autoclaved (20 min at 121°C), cooled, and inoculated with spores of each strain. Similarly, six flasks (500 ml each), each containing 100 ml of sterile liquid medium, were inoculated with each strain. All flasks were incubated at 28°C. At 7, 14, and 21 days of incubation, two flasks per strain of both the liquid and wheat cultures were analyzed separately for ochratoxin A content. When a variant was isolated from sterilized barley that had been inoculated with spores of the parent strain and irradiated, it along with the parent and three other ochratoxin A-producing wild-type strains obtained from ATCC (ATCC 18412, ATCC 18642, and ATCC 60532) were tested for ochratoxin A production, as described above, after 7 and 14 days of incubation.

Ochratoxin A analysis. The presence of ochratoxin A was determined by a method described previously (4) that consisted of reversed-phase thin-layer chromatography cleanup followed by analysis by high-pressure liquid chromatography on a Beckman Ultrasphere ODS 5-μm column (4.6 mm by 25 cm). Eluting solvent consisted of 30% H_2O (pH 2.1; adjusted with phosphoric acid) and 70% methanol-isopropanol (90:10). Hewlett-Packard fluorescence detector 1046A in conjunction with Hewlett Packard integrator 3390A was used. The pump, controller, and oven were products of LKB-Produkter AB. All solvents used were high-pressure liquid chromatography grade.

Strain characterization. Propagation and growth rate (colony diameter) determination for the parental and variant strains was done on PDA and PDA-YE-NaCl media at 28°C in the dark and at 23°C with continuous fluorescent lighting. Growth rate determinations were also done on CA, malt extract agar, and CY20S media at both temperatures. Average conidial sizes were determined on 7-day-old cultures grown on PDA-YE-NaCl at 23°C. Conidia were measured on a Zeiss Ultraphot microscope at × 1,000 magnification. The nuclear state of the conidia was determined after they were stained with acridine orange. After being fixed for 3 min in methanol, the conidia were stained in a solution of acridine (100 mg/l in Sorensen's 0.067 M phosphate buffer) for 3 min and washed three times for 3 min each time in the 0.067 M buffer. Wet-mount slides (in buffer) were observed in an Olympus BHS microscope with a reflected-light fluorescence attachment. The DNA-containing region could easily be identified by the bright-green fluorescence. The nuclei of the conidia were counted blind in duplicate samples, and the results were combined.

Scanning electron microscopy. Conidia grown on agar medium or autoclaved barley were prepared for scanning electron microscopy by using the simplified method of Kozakiewicz (7). Conidia were applied to standard aluminium stubs, coated with gold (Gold Sputter unit; Balzer Union), and then examined directly in the microscope (ISI model DS130). Photographs were taken using Ilford 120 film.

Reversion of white variants to parental phenotype. Confirmation that the high-ochratoxin-A-producing variants were derived from the parental strain was done by demonstrating that sectors exhibiting parental characteristics were formed in colonies that originated from single conidia of the variant strains. Dilution plating of conidia of strains 006 and 076 was done on PDA-YE-NaCl. The plates were incubated at 23°C for 18 h and examined under the microscope at ×40 magnification. Plates with fewer than 15 germinating conidia were selected, and well-separated colonies, in which the germ tubes grew from a single conidium, were marked. The plates were incubated for 5 days at 23°C. Conidia from the marked colonies were dispersed in 0.1% Tween 80 and used to inoculate plates of PDA-YE-NaCl. These were incubated at 23 and 28°C for 8 days, and the colonies were examined for sectors with ochre spores and a darker color on the reverse. In total, 140 monoclonal isolates of each strain were plated and incubated at each temperature.

The red-pigment-producing variant (007) could not be tested in this way because the colony phenotypes were similar, and any revertant sectors to the parental (no red pigment) phenotype would be masked by the diffusing red pigment from the variant.

RESULTS

Isolation of variant strains. Dilution plating of the barley sample that had been inoculated with spores of the parental strain (001), irradiated to 1.0 kGy and incubated for 10 days at 28°C to allow for regrowth of surviving progeny, resulted in two types of colonies. Some of the colonies exhibited light-yellow spores, while the remainder showed the ochre spores characteristic of the parent. One colony with yellow spores was propagated under the accession number 005. This strain displayed greater fluorescence under UV light than the parent strain, suggesting greater production of ochratoxin. In addition, when this isolate was propagated in petri dishes in PDA-YE-NaCl medium, a sector with even greater fluorescence and creamy white spores was observed. An isolate of this sector was propagated under the accession number 006. All the colonies of the unirradiated control had ochre spores. To establish conclusively that such variants were derived from the parental strain and did not represent grain contaminants, the experiment was repeated on autoclaved barley substrate. An isolate similar to 006 was derived and propagated under the number 076.

In another experiment, the frequency of occurrence of these types of variants following exposure of water-suspended spores of the parental strain to 1.0 kGy of gamma irradiation was determined by counting the numbers of colonies with different spore colors (Table 1). In addition to the colonies with yellow and white spores, some colonies produced a red pigment that diffused into the medium. One such isolate was designated 007. Some of the colonies with the lighter spore color displayed greater fluorescence under UV light than the parental types (Table 2).

Strain characteristics. On PDA or PDA-YE-NaCl, parent

TABLE 1. Characteristics of parental and variant strains of *A. alutaceus* var. *alutaceus* grown on PDA-YE-NaCl for 7 days

Strain	Color		Soluble red pigment	Mean colony diam (mm)		Mean conidial size (μm)
	Conidia	Sclerotia		23°C	28°C	
001 (parent)	Ochre	Lavender	−	62	85	3.0
005	Light yellow	Pale lavender	−	58	80	3.7
006	Yellowish-white	Ivory	−	57	79	4.2
007	Ochre	Lavender	+	63	81	2.9
076	Yellowish-white	Ivory	−	60	81	3.6

TABLE 2. Conversion of *A. alutaceus* var. *alutaceus* to lighter and darker spore color variants without and with gamma radiation[a]

Treatment	Dilution	Total no. of colonies	Lighter colonies		Darker colonies	
			No.	Frequency (%)	No.	Frequency (%)
No irradiation	10^5	176	0	<0.3	0	<0.3
		153	0		0	
1.0 kGy of gamma irradiation	10^2	197	5	2.7	6	3.7
		209	6		9	

[a] Surviving fraction (s/s_0) for irradiated sample was 0.0012. (s/s_0 = no. of survivors/no. irradiated = $2.03 \times 10^4/1.65 \times 10^7 = 0.0012$.)

strain 001 and red-pigment-producing strain 007 were indistinguishable as to spore color, sclerotial color, and growth characteristics at 28°C in the dark and 23°C in the light. Strain 007 consistently produced a dark-red pigment on PDA, PDA-YE-NaCl, and malt extract agar. No red pigment was produced on CY20S agar. Both strains (007 and 001) produced dark-lavender sclerotia at 28°C but no sclerotia at all, or only very few, at 23°C. Both strains produced more conidia at 23 than at 28°C. The conidial heads of both strains were radiate when young and split into columns with age. This characteristic was particularly evident on grain substrates. Strains 005, 006, and 076 did not exhibit significant conidial-head splitting. The strain characteristics of the parent and of variant strains grown on PDA-YE-NaCl are given in Table 1. Growth rates on CA, malt extract agar, and CY20S of the parent and the variants were not significantly

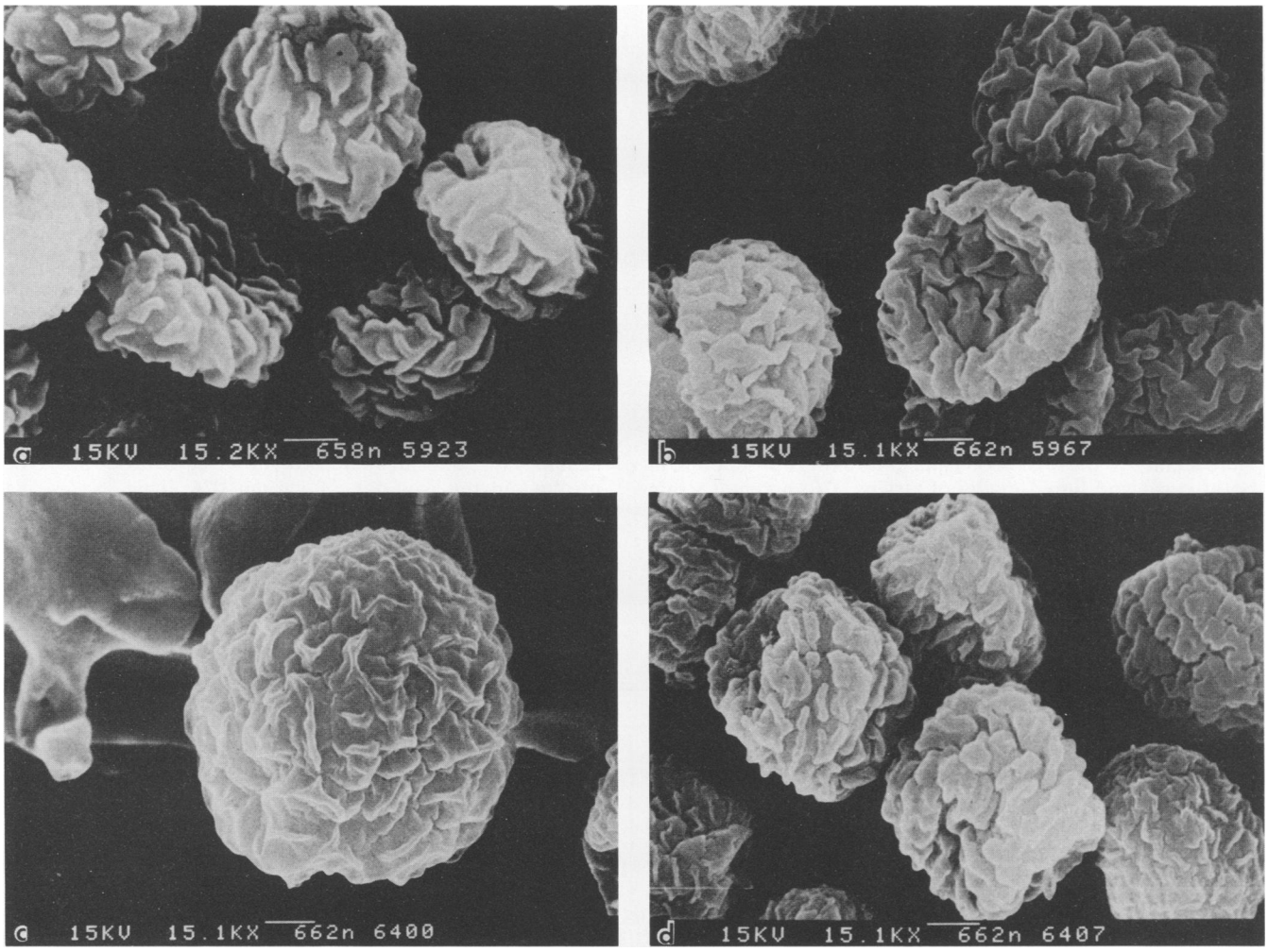

FIG. 1. Scanning electron micrographs of *A. alutaceus* var. *alutaceus* conidia of parent strain 001 (a) and of yellow (strain 005) (b), white (strain 006) (c), and red (strain 007) (d) variants.

TABLE 3. Ochratoxin A production by parent strain and two variants of *A. alutaceus* var. *alutaceus* in liquid and wheat media[a]

Strain	Medium	Production of ochratoxin A (μg/g or ml of substrate)[b]		
		7 days	14 days	21 days
Parent (001)	Liquid	82.4 ± 5.9	63.3 ± 7.5	71.4 ± 4.9
	Wheat	159.9 ± 18.3	381.7 ± 2.9	366.5 ± 8.3
Yellow (005)	Liquid	127.1 ± 6.8	94.0 ± 5.9	91.9 ± 4.2
	Wheat	370.1 ± 26.4	1,253.1 ± 45.1	1,348.8 ± 60.9
White (006)	Liquid	611.2 ± 90.4	378.2 ± 52.6	628.9 ± 95.1
	Wheat	1,709.8 ± 150.2	1,344.0 ± 74.6	2,339.5 ± 14.2

[a] Experiment 1.
[b] Weight/weight (dry basis) for wheat substrates; weight/volume for liquid media.

different between the strains on each medium. Growth was slowest on CA and fastest on CY20S. Although there was growth of 005, 006, and 076 on CA, there was no sporulation by these strains. Parent strain 001 and variant 007 sporulated on CA. All strains sporulated on PDA-YE-NaCl.

Mean conidial diameters are shown in Table 1. Conidia of strains 005, 006, and 076 were more variable in size than those of strains 001 and 007. Examination of conidia by fluorescence microscopy after acridine orange staining demonstrated that the majority of the conidia were mononucleated with 0, 0, 4, 0.5, and 0.2% of the conidia being binucleate in strains 001, 005, 006, 007, and 076, respectively. In strains exhibiting variable conidial size, larger conidia were associated with larger nuclei.

When the spore architecture technique of Kozakiewicz (7) is used, all four strains have a similar surface ornamentation, although the conidia of strains 005 and 006 are larger than those of 001 and 007 (Fig. 1).

Ochratoxin A production. The initial observation that increased fluorescence in the yellow- and white-spore colonies could be due to greater ochratoxin A production by these strains was confirmed by the experiment shown in Table 3. That this characteristic remained constant over repeated transfers is shown in Table 4. This latter experiment was done several months later than the one shown in Table 3 and also tested ochratoxin A production by strain 007. It should be noted that this red-pigment-producing strain produced very little of the mycotoxin. Variant strain 076 was derived from parent strain 001 under aseptic conditions. The cultural and growth characteristics of this variant were similar to those of 006. The ochratoxin A production in both wheat and liquid media of strains 076, parental strain 001, and three additional wild-type strains is shown in Table 5. Strain 076 was more toxigenic than the parent or any of the wild-type strains in both substrates.

Reversion to parental phenotype. Of 140 monoclonal isolates of strains 006 and 076 that were centrally inoculated on 10-cm-diameter petri dishes and incubated at 23 and 28°C, one colony of strain 006 incubated at 28°C exhibited a single sector with parental characteristics. Strain 076 exhibited characteristics of the parent in one sector of a colony grown at 23°C and one sector of a colony grown at 28°C.

DISCUSSION

The first studies in our laboratory involved the production of ochratoxin A by *A. alutaceus* var. *alutaceus* NRRL 3174 in nonsterilized grain. The possibility therefore exists that the strains with increased toxigenicity that were isolated in those experiments were derived from the grain flora rather than the introduced inoculum. This was considered unlikely, however, for the following reasons: (i) toxigenic strains of *A. alutaceus* are not common on stored grains in western Canada, (ii) repeated attempts to isolate *A. alutaceus* from the same lot of uninoculated grain have been unsuccessful, and (iii) the data in Table 2 indicate that irradiation of conidia of a pure culture of the parental strain resulted in variants exhibiting a lighter spore color and increased fluorescence similar to those of strains 005 and 006. The growth characteristics of the variants on the various media are compatible with the hypothesis that they were derived from the parent. The fact that strains 005, 006, and 076 did not sporulate on CA may indicate that these are auxotrophs requiring certain nutrients for sporulation.

Later experiments in which sterilized barley was inoculated with conidia of the parental strain, irradiated with gamma rays, and incubated to allow regrowth of survivors resulted in the isolation of variant 076. This variant was similar to 006 and also produced increased levels of ochratoxin A. Confirmation that these strains were not contaminants of the original parental culture was achieved when revertants with parental characteristics were obtained.

TABLE 4. Ochratoxin A production by parent strain and three variants of *A. alutaceus* var. *alutaceus* in liquid and wheat media[a]

Strain	Medium	Production of ochratoxin A (μg/g or ml of substrate)[b]		
		7 days	14 days	21 days
Red (007)	Liquid	3.6 ± 0.7	1.5 ± 0.9	2.4 ± 0.4
	Wheat	17.6 ± 3.8	21.7 ± 2.6	33.3 ± 1.2
Parent (001)	Liquid	121.4 ± 9.4	110.8 ± 4.7	93.1 ± 22.9
	Wheat	209.9 ± 2.3	248.4 ± 34.3	254.9 ± 1.9
Yellow (005)	Liquid	350.5 ± 64.1	327.3 ± 27.1	184.5 ± 21.0
	Wheat	986.5 ± 9.5	1,132.7 ± 75.8	1,316.2 ± 65.4
White (006)	Liquid	962.7 ± 104.1	725.9 ± 39.9	652.8 ± 42.9
	Wheat	2,272.0 ± 49.3	3,217.2 ± 188.9	2,589.8 ± 50.8

[a] Experiment 2, performed 3 months after experiment 1.
[b] Weight/weight (dry basis) for wheat substrates; weight/volume for liquid media.

TABLE 5. Ochratoxin A production by four wild-type strains of *A. alutaceus* var. *alutaceus* and variant white strain 076[a]

Strain	Medium	Production of ochratoxin A (μg/g or ml of substrate)[b]	
		7 days	14 days
001 (ATCC 22947)	Liquid	12.1 ± 0.4	17.5 ± 2.3
	Wheat	69.3 ± 4.8	132.8 ± 9.6
ATCC 18412	Liquid	0.3 ± 0.02	1.2 ± 0.1
	Wheat	3.5 ± 1.2	0.8 ± 0.1
ATCC 18642	Liquid	3.4 ± 0.4	4.1 ± 0.4
	Wheat	1.4 ± 0.1	27.8 ± 4.0
ATCC 60532	Liquid	5.1 ± 0.7	8.2 ± 0.5
	Wheat	114.1 ± 6.2	184.4 ± 7.1
Variant 076	Liquid	276.3 ± 21.4	636.7 ± 20.1
	Wheat	510.5 ± 25.0	875.9 ± 31.0

[a] Strain 076 was isolated from autoclaved barley that was inoculated with strain 001 and irradiated with 1.0 kGy of gamma radiation.

[b] Weight/weight (dry basis) for wheat substrates; weight/volume for liquid media.

In later work characterizing the induction of these types of variants, preliminary results indicate that variants very similar to those described here can be induced by treating inoculated grain with gaseous fumigating agents (data not shown). These studies will be published at a later date.

It appears that there is an inverse relationship between the darkness of colony color of the five strains and their ochratoxin A production. This may be an indication that colored precursors of the mycotoxin are accumulating within the mycelium of the less-productive strains, analagous to what has been seen in *A. flavus* and *A. parasiticus* mutants by other workers (1, 5, 9, 10). Color mutants of *A. flavus* var. *columnaris* with enhanced protease and amylase activity have been obtained after treatment with UV irradiation (6). These workers found a concomitant conversion of some of these molds from nontoxic to toxic. Nontoxic revertants were also obtained. They suggested "that this loss may derive from altered expression of the relevant genes rather than the loss of the genes . . . . It is unlikely that a single mutagenic event could have deleted all the genes necessary for the relevant biosynthetic pathway and that a succeeding one could have restored them." A similar mechanism may be involved in our system. Consequently we have chosen to call the derived strains variants, reserving the term mutants for those strains that are known to result from changes to the genome.

Reversion of these radiation-induced variant strains has not been studied in any detail. The effects of nutritional, physical, and chemical factors on reversion rate could suggest whether these altered strains were the result of genetic or epigenetic events. Formation of disomic strains would be consistent with the data. Such strains can result in increased mycotoxin levels due to gene dosage effects. Revertants would be the result of the subsequent loss of the extra chromosome. Upshall et al. (17) described increased penicillin production by disomic strains of *A. nidulans*. However, it is equally likely that such strains are the result of altered gene expression. Whatever the case, it is clear from the data in Tables 2 and 3 that these variants remain stable for months with respect to their characteristic of ochratoxin A production in damp grain. That being the case, they hold the promise of being useful tools for establishing the pathway as well as the control mechanisms involved in ochratoxin A production by *A. alutaceus* var. *alutaceus*.

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REFERENCES

1. **Bennett, J. W.** 1979. Aflatoxins and anthraquinones from diploids of *Aspergillus parasiticus*. J. Gen. Microbiol. **113**:127–136.
2. **Blount, W. P.** 1961. Turkey X disease. J. Br. Turkey Fed. **19**:55–58, 61–71.
3. **Dwivedi, P., and R. B. Burns.** 1984. Pathology of ochratoxicosis A in young broiler chicks. Res. Vet. Sci. **36**:92–103.
4. **Frohlich, A. A., R. R. Marquardt, and A. Bernatsky.** 1988. Quantitation of ochratoxin A: use of reverse phase thin-layer chromatography for sample cleanup followed by liquid chromatography or direct fluorescence measurement. J. Off. Anal. Chem. **71**:949–953.
5. **Henderberg, A., J. W. Bennett, and L. S. Lee.** 1988. Biosynthetic origin of aflatoxin G_1: confirmation of sterigmatocystin and lack of confirmation of aflatoxin B_1 as precursors. J. Gen. Microbiol. **134**:661–667.
6. **Kalayanamitr, A., A. Bhumeratana, T. W. Flegel, T. Glinsukon, and A. Shinmyo.** 1987. Occurrence of toxicity among protease, amylase, and colour mutants of a nontoxic soy sauce koji mold. Appl. Environ. Microbiol. **53**:1980–1982.
7. **Kozakiewicz, Z.** 1989. *Aspergillus* species on stored products. Mycological papers, no. 161. CAB International Mycological Institute, Ferrylane, Kew, United Kingdom.
8. **Lee, L. S.** 1989. Metabolic percursor regulation of aflatoxin formation in toxigenic and non-toxigenic strains of *Aspergillus flavus*. Mycopathologia **107**:127–130.
9. **Lee, L. S., J. W. Bennett, A. F. Cucullu, and J. B. Stanley.** 1975. Synthesis of versicolorin A by a mutant of *A. parasiticus* deficient in aflatoxin production. J. Agric. Food Chem. **23**:1132–1134.
10. **McCormick, S. P., D. Bhatnagar, and L. S. Lee.** 1987. Averufanin is an aflatoxin B_1 precursor between averantin and averufin in the biosynthetic pathway. Appl. Environ. Microbiol. **53**:14–16.
11. **Papa, K. E.** 1978. The parasexual cycle in *Aspergillus parasiticus*. Mycologia **70**:766–773.
12. **Samson, R. A., and W. Gams.** 1985. Typification of the species *Aspergillus* and associated teleomorphs, p. 31–54. *In* R. A. Samson and J. I. Pitt (ed.), Advances in *Penicillium* and *Aspergillus* systematics. Plenum Press, New York.
13. **Schindler, A. F., A. N. Abadie, and R. E. Simpson.** 1980. Enhanced aflatoxin production by *Aspergillus flavus* and *Aspergillus parasiticus* after gamma irradiation of the spore inoculum. J. Food Prot. **43**:7–9.
14. **Singh, R., and D. P. H. Hsieh.** 1977. Aflatoxin biosynthetic pathway: elucidation by using blocked mutants of *Aspergillus parasiticus*. Arch. Biochem. Biophys. **178**:285–292.
15. **Sreemannarayana, O., R. R. Marquardt, A. A. Frohlich, D. Abramson, and G. D. Phillips.** 1989. Organ weights, liver constituents, and serum components in growing chicks fed ochratoxin A. Arch. Environ. Contam. Toxicol. **18**:404–410.
16. **Subramanian, C. V.** 1971. Hyphomycetes. An account of Indian species, except Cercosporae. Indian Council for Agricultural Research, New Delhi.
17. **Upshall, A., B. Giddings, S. C. Teow, and I. D. Mortimore.** 1979. Novel methods of genetic analysis in fungi, p. 197–204. *In* O. K. Sebek and A. I. Laskin (ed.), Genetics of industrial microorganisms. Proceedings of the 3rd International Symposium, Madison, Wis., 4 to 9 June 1978. American Society for Microbiology, Washington, D.C.
18. **Yabe, K., H. Nakamura, Y. Ando, N. Terakado, H. Nakajima, and T. Hamasaki.** 1988. Isolation and characterization of *Aspergillus parasiticus* mutants with impaired aflatoxin production by a novel tip culture method. Appl. Environ. Microbiol. **54**:2096–2100.