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Mutagenic Activity of *Fusarium moniliforme* Isolates in the *Salmonella typhimurium* Assay

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A total of 33 isolates of *Fusarium moniliforme* from several food or feed crops were grown on sterile cracked corn, and chloroform-isopropanol extracts were assayed for mutagenic activity in the *Salmonella typhimurium*-microsome system by using tester strain TA98 or TA100 or both. Extracts of 21 (64%) of the isolates assayed against TA100 were mutagenic. Activities of seven of these extracts were increased markedly with incorporation of the liver homogenate (S-9) into the assay. Seven (33%) of the isolates assayed against TA98 were weakly active, with the liver homogenate having little effect on reversion rates.

Fusarium moniliforme occurs on many plants and is the cause of disease of several food and feed crops. The fungus has been isolated from 20 different crops in the United States and from over 42 crops worldwide (6, 16). *F. moniliforme* is considered one of the most important pathogens of corn in the world (9). Corn ear rot caused by this fungus is the most widespread disease of corn ears, and crop losses due to *F. moniliforme* ear rot have been reported as high as 56% in California (15). The incidence of corn stalk rot associated with this fungus was 21% in Minnesota in 1973 (8). *F. moniliforme* has also been implicated as a cause of stalk rot of sorghum, endosepsis of fig, crown rot of asparagus, scab of wheat and barley, "pokkah-boeng" or top rot of sugar cane, maize, and sorghum, and foot rot or stunting of rice (7). Crop losses due to bakanae of rice have been reported to be 4 to 15% in parts of Thailand and 20% in Hokkaido, Japan (12).

Although few chemical studies of toxins produced by *F. moniliforme* have been reported, the mold has been implicated in several mycotoxicoses of farm and laboratory animals. Mycotoxins isolated from *F. moniliforme* include the estrogen zearalenone (11) and the acute toxins fusariocin A (2) and moniliformin (17). Animals adversely affected by consumption of *F. moniliforme*-contaminated feed include horses and donkeys, chickens, pigs, rats, rabbits, and ducklings (9).

Results of our recent investigations of the mutagenic activity of extracts from several species of fungi have indicated that, although only a small percentage of the species examined exhibit this activity (4), extracts of several isolates of *F. moniliforme* were mutagenic. Reported here are the results of mutagen assays of extracts of *F. moniliforme* isolates obtained from a variety of sources.

MATERIALS AND METHODS

Fungi. *F. moniliforme* strains F-14 through F-1144 (Table 1) were generously provided by P. T. Spieth, Department of Genetics, University of California, Berkeley. All isolates were obtained from diseased plant material collected between 1974 and 1977. Single conidia were obtained from potato dextrose agar cultures and maintained at 22 to 25°C on potato dextrose agar slants. Transfers were made to fresh potato dextrose agar slants every 3 to 4 weeks.

Culture of fungi. Inoculum was prepared by washing spores and mycelium from potato dextrose agar slants of the fungus with 10 ml of sterile distilled water. A portion (1.10 ml) of this suspension was transferred to cotton-stoppered Fernbach flasks containing 40 g of cracked corn and 30 ml of distilled water. Before inoculation, flasks and substrate were sterilized by two 20-min autoclave treatments separated by a 12-h interval. Corn cultures were incubated for 2 weeks at 22 to 25°C under indirect lighting, followed by 1 week at 10 to 13°C without light, and finally 1 week at 22 to 25°C. The 1-week cold treatment was generally used because mutagen production by several isolates was maximized under these conditions.

Extraction of moldy substrates. Without removing the cotton plugs, 200 ml of a chloroform-isopropanol (1:1, vol/vol) solution was added to each flask by a method described previously (4). Mixtures were blended for 3 min and filtered through celite. The chloroform-isopropanol extract was dried (Na_2SO_4) and evaporated to 1 to 2 ml in vacuo at 40°C (bath temperature). Extracts were diluted to 3 ml with dimethyl sulfoxide and homogenized if necessary by the addition of 100% Tween 80 (J. T. Baker Chemical Co., Philipsburg, N.J.).

Mutagen assay. The bacterial tester strains *Salmonella typhimurium* TA100 and TA98, described previously (1), were obtained from B. N. Ames, De-

TABLE 1. *Mutagenic activity of F. moniliforme isolates grown on corn*

Strain	Host	Location	Assay with TA100						Assay with TA98						Mutagen producer[a]
			Plate incorporation assay				Disk assay		Plate incorporation assay				Disk assay		
			20 μl		200 μl				20 μl		200 μl				
			−S-9	+S-9	−S-9	+S-9	−S-9	+S-9	−S-9	+S-9	−S-9	+S-9	−S-9	+S-9	
F-14	Corn stalk	Central Calif.	320[b]	1,190	176	395	−	+							+
F-84	Sorghum	Central Calif.	280	1,820	480	568	+	+							+
F-99	Corn stalk	Central Calif.	158	178	7	11	−	−							−
F-102	Corn stalk	Central Calif.	171	226	0	0	−	−	35	63	0	0	−	−	−
F-104	Corn stalk	Central Calif.	183	142	89	129	−	−							−
F-106	Corn stalk	Central Calif.	169	184	46	24	−	−							−
F-107	Corn stalk	Central Calif.	152	173	165	124	−	−							−
F-594	Rice	N. Italy	168	294	171	134	−	+	85	31	194	105	−	−	+
F-670	Rice	S. Italy	140	164	110	120	−	−	46	31	62	24	−	−	−
F-678	Rice	S. Italy	146	149	195	93	−	−	53	32	46	44	−	−	−
F-739	Corn stalk	S. Italy	102	138	201	248	+	+	45	32	69	77	−	−	+
F-740	Corn stalk	S. Italy	96	131	608	646	±	+	50	31	34	42	+	−	+
F-743	Corn stalk	S. Italy	115	125	248	263	+	+	83	45	56	34	−	−	+
F-744	Corn stalk	S. Italy	137	169	118	98	±	+	61	27	90	35	+	−	+
F-747	Corn ear	S. Italy	150	97	279	670	+	+	55	51	78	118	±	−	+
F-919	Asparagus	N. Italy	102	117	90	88	−	−	50	38	36	22	−	−	−
F-1023	Corn ear	N. Italy	253	379	512	718	+	+	59	56	189	135	±	±	+
F-1025	Corn ear	N. Italy	85	108	217	191	+	+	68	41	187	138	−	−	+
F-1044	Rice	N. Italy	104	117	78	79	−	−	49	38	39	27	−	−	−
F-1112	Corn stalk	N. Italy	192	117	456	78	−	−	27	37	12	22	−	−	+
F-1125	Corn stalk	Greece	123	240	546	743	+	+	75	46	40	42	−	−	+
F-1127	Corn stalk	Greece	162	215	293	276	−	−	52	38	87	64	−	−	+
F-1128	Corn stalk	Greece	136	145	356	473	±	+	106	68	59	56	+	−	+
F-1131	Corn stalk	Greece	122	245	459	553	+	+	47	147	54	70	−	−	+
F-1144	Corn stalk	Minnesota	171	134	408	587	+	+	178	218	147	233	±	±	+
F-AJ-e-1	Corn ear	N. Calif.	142	810	208	316	−	+							+
F-AJ-e-2	Corn ear	N. Calif.	395	412	0	1,650	+	+							+
F-SX210	Seed corn	Minnesota	610	730	0	175	+	+							+
F-NCT12	Seed corn	Minnesota	168	178	0	0	−	−							−
F-SX397	Seed corn	Minnesota	156	173	142	164	−	−							−
F-M1	Corn ear	N. Calif.	142	136	96	1,216	−	+	42	58	38	45	−	−	+
F-ST	Corn ear	N. Calif.	298	151	712	1,224	+	+							+
F-TSC6	Corn ear	N. Calif.	143	149	152	168	−	−	31	39	46	25	−	−	−
Controls			140 ± 30	145 ± 25					36 ± 6	43 ± 9					

[a] Extracts were considered mutagenic if the reversion rate was greater than two times the background and/or a pronounced ring of revertant colonies was produced in the disk assay.

[b] Number of revertants.

partment of Biochemistry, University of California, Berkeley. Strain TA100 is reverted primarily by base substitution mutagens, and strain TA98 is reverted primarily by frame shift mutagens. Tester strain characteristics (presence of R factor plasmid and *rfa* character) were checked as suggested by Ames et al. (1). The hepatic S-9 mix was prepared with phenobarbital-treated male Sprague-Dawley rats (250 to 350 g). Appropriate plates were incubated with 100 μl of liver supernatant (S-9) per plate to test for microsome activation. Plates of *S. typhimurium* were prepared for the mutagen assay as previously described (1).

Fungal extracts were sterilized by filtration through an 0.45-μm filter and assayed by the plate incorporation method (20 or 200 μl of extract per plate, with and without S-9 mix) and the disk method (20 μl of extract applied to 6-mm sterile paper disks and placed on the agar surface). Equivalent volumes of solvents were added to control plates. Tester strain sensitivity and activity of the S-9 mix were checked with quercetin (3) (for TA100 and TA98, ~700 revertants per 30 μg with S-9 and ~300 revertants per 30 μg without S-

9) and sterigmatocystin (for TA100, ~400 revertants per 0.1 μg with S-9 and ~150 revertants per 0.1 μg without S-9; for TA98, ~190 revertants per 0.1 μg with S-9 and ~40 revertants per 0.1 μg without S-9).

RESULTS AND DISCUSSION

The results of mutagen assays of *F. moniliforme* isolates grown on sterile cracked corn are presented in Table 1. Of the 33 isolates assayed against *S. typhimurium* strain TA100, extracts of 21 isolates (64%) were active. Activity was observed in isolates from all geographical locations tested (California, Minnesota, Italy, Greece). Most (70%) of the isolates from corn seeds, stalks, and ears were mutagen producers, as was the isolate from sorghum. A low level of mutagenic activity was observed in the extract of one of the four rice isolates examined. Activity was not detected in the extract from the single isolate from asparagus. Pronounced antibacterial activity, which was not attributable to mutagenesis, was observed at the higher doses (200 μl) of isolates F-102 and F-NCT12.

Of the 22 isolates assayed against strain TA98, relatively weak mutagenic activities were observed in 7 (33%). Extracts from only strain F-1144 produced more than 200 revertant colonies per plate. Mutagenic activity against TA98 was not observed in the remaining 16 strains tested.

Although production of substances capable of inducing mutations in TA100 seems to be characteristic of most strains of *F. moniliforme*, several strains appear to produce compounds which may be classified into different groups. For example, extracts of seven strains (F-14, F-84, F-747, F-AJ-e-1, F-AJ-e-2, F-M1, F-ST) showed a marked increase in mutagenic activity with incorporation of the liver preparation (S-9) into the assay, whereas reversion rates of most of the other mutagenic extracts (F-594, F-739, F-740, F-743, F-744, F-1023, F-1125, F-1127, F-1128, F-1131, F-1144, F-SX210) were affected to only a minor degree or not at all by inclusion of the liver homogenate. Thus, at least two classes of mutagens active against TA100 are produced by *F. moniliforme*—those which require metabolic activation (S-9) and those which do not. In one case (F-1112), a pronounced decrease in activity was noted on incorporation of the liver homogenate, indicating metabolic deactivation of a mutagen.

Compounds responsible for the mutagenic activity of *F. moniliforme* do not appear to be certain known mycotoxins. Chemical assays (14) showed that the more active isolates (F-14, F-84, F-AJ-e-1, F-AJ-e-2, F-M1, F-ST) did not contain aflatoxins. Simple anthroquinone derivatives also do not appear to be responsible for the observed activity. Although compounds of this type are common fungal pigments (13), as a class of compounds they appear to be inactive against strain TA100 (5). Certain synthetic nitro- and amino-anthroquinone derivatives which are active against TA100 also show marked activity against TA98. Since only weak effects of extracts on reversion rates in TA98 were noted in our studies, it appears unlikely that nitrogen-containing anthroquinones account for the observed activity against TA100. Of course, more complex anthroquinones similar to rugulosin and luteoskyrin are not excluded on the basis of available evidence. Zearalenone, moniliformin, T-2 toxin, and other trichothecenes which are well-known *Fusarium* toxins do not induce mutations with TA100 (18) and therefore are not responsible for observed mutagenic activity. Isolation and characterization studies of active compounds are in progress.

To estimate the degree of hazard of mutagens produced by *F. moniliforme*, the observed mutagenic activities of some of the more active isolates were compared with the activities of known mutagens and carcinogens. Fungal extracts, as assayed, contained approximately 90% inactive corn oil, which was further diluted with dimethyl sulfoxide. Thus, only about 10% of the applied dose is likely to be fungal metabolites. As a result, of the 20 and 200 μl of extract applied to the plates, an estimated 2 and 20 μg, respectively, is fungal product. Nevertheless, if one assumes a roughly linear dose-response relationship, after the observed reversion rates indicated in Table 1 are adjusted for background revertants, the activities of crude extracts of F-SX210 (300 revertants per μg), F-AJ-e-2 (340 revertants per μg), and F-84 (840 revertants per μg) are comparable to the activity of pure benzo(a)pyrene (480 revertants per μg) (10). Although pure aflatoxin B_1 is a good deal more active (2,260 revertants per 0.1 μg) (10) than any of the crude extracts, the latter, of course, are mixtures of many substances, each of which may compromise only a small percentage of the total extract.

The observed mutagenic activity of *F. moniliforme* isolates grown on a common natural substrate, such as corn, may be significant in terms of human health. Most mammalian carcinogens are also mutagens, as determined by the *S. typhimurium* assay (10). Although a high correlation for mutagens as carcinogens has not yet been established, certainly highly mutagenic substances should be treated as carcinogens until proven otherwise. Thus, *F. moniliforme*, an established contaminant of many food and feed crops, may be a significant source of environmental mutagens and possibly carcinogens.

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