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Lack of Mutagenicity to *Salmonella typhimurium* of Some *Fusarium* Mycotoxins

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The mutagenicity of eight *Fusarium* toxins (mono-, di-, and triacetoxyscirpenol, T-2 toxin, deoxynivalenol, 3-acetyl-deoxynivalenol, zearalenone, and moniliformin) and of two positive controls (aflatoxin B_1 and sterigmatocystin) to histidine-requiring strains TA 98, 100, 1535, and 1537 of *Salmonella typhimurium* was tested both with and without metabolic activation. Both aflatoxin B_1 and sterigmatocystin, but none of the eight *Fusarium* toxins, were mutagenic to *S. typhimurium*. The lack of mutagenic activity of T-2 toxin and diacetoxyscirpenol supports the negative results that have been obtained with in vivo carcinogenicity tests. The negative mutagenicity of the four other 12,13-epoxytrichothecenes tested, and of zearalenone and moniliformin, could not be correlated with in vivo tests because published accounts of their chronic toxicity were not available.

Several carcinogenic mycotoxins including aflatoxin B_1 and sterigmatocystin have been shown to be mutagenic to histidine-requiring strains of *Salmonella typhimurium* (4, 5, 13). Little information is, however, available on the mutagenicity of the mycotoxins produced by *Fusarium* species. Ueno et al. (19) found that nivalenol and fusarenon-X induced respiratory-deficient mutants in yeast cells, but of six *Fusarium* toxins (butenolide, fusarenon-X, fusaric acid, moniliformin, T-2 toxin, and zearalenone), the only one to exhibit deoxyribonucleic acid-attacking ability with the recombination-deficient mutant of *Bacillus subtilis* M45 (*rec*$^-$) was zearalenone and, similarly, its derivative zearalenol-b (18). As far as can be determined, the only *Fusarium* toxins that have been tested for mutagenicity to *S. typhimurium* (strains TA 98 and 100) are fusarenon-X, diacetoxyscirpenol, and T-2 toxin (13, 17). In these tests negative results were obtained for diacetoxyscirpenol (13) and T-2 toxin (17), but conflicting results were reported for fusarenon-X.

This paper reports on the lack of mutagenic activity of eight *Fusarium* mycotoxins for histidine-requiring strains of *S. typhimurium*.

MATERIALS AND METHODS

Mycotoxins. Zearalenone and aflatoxin B_1 were obtained from Makor Chemicals, Jerusalem, Israel; T-2 toxin was from H. R. Burmeister, Northern Regional Research Laboratory, Peoria, Ill.; moniliformin and sterigmatocystin were from M. Steyn, South African Medical Research Council, Tygerberg, South Africa; mono-, di-, and triacetoxyscirpenol were from P. S. Steyn, Council for Scientific and Industrial Research, Pretoria, South Africa; and 3-acetyldeoxynivalenol was from T. Yoshizawa, Kagawa University, Miki-Tyo, Kakawa-Ken, Japan. Deoxynivalenol was prepared from the 3-acetyldeoxynivalenol by alkaline hydrolysis with methanolic ammonium hydroxide (20).

Bacterial strains. *S. typhimurium* strains TA 98, TA 100 (11), TA 1535, and TA 1537 (5) were kindly supplied by B. N. Ames, University of California, Berkeley. Overnight shake cultures (37°C) in nutrient broth (Difco) were used at concentrations of 0.1 ml/plate.

Mutagenicity test. Liver homogenate fractions (S-9 mix) were prepared according to the method of Ames et al. (4). Induction of liver enzymes was accomplished by a single intraperitoneal injection of male Wistar-derived rats with the polychlorinated biphenyl Aroclor 1254 (Monsanto) (6). The protein concentration of the microsomal fraction (S-9) was 30.6 mg/ml, as determined by the method of Lowry et al. (10), and it was incorporated into the S-9 mix at a ratio of 0.08 ml/ml.

The mutagenicity assay was carried out as described by Ames et al. (4). In tests with metabolic activation, 0.5 ml of S-9 mix per plate was added. S-9 mix without microsomal fraction was used in tests without metabolic activation. The mycotoxins were tested at the concentrations given in Table 1. The concentrations were determined by the availability of the various toxins as well as by preliminary trials. With the exception of moniliformin, which was dissolved in sterile distilled water, all of the mycotoxins were dissolved in dimethyl sulfoxide and incorporated in the assays at a concentration of 0.1 ml/plate.

RESULTS AND DISCUSSION

It is clear from Table 1 that both the positive controls, aflatoxin B_1 and sterigmatocystin, were

TABLE 1. *Reversion of S. typhimurium tester strains with Fusarium toxins*

Mycotoxin	Concn (μg/plate)	Histidine revertants/plate								Mutagenicity[a]
		+S-9				−S-9				
		TA98	TA100	TA1535	TA1537	TA98	TA100	TA1535	TA1537	
Monoacetoxyscirpenol	0	13	27	8	7	5	44	9	3	
	0.25	15	22	12	9	5	31	10	6	
	2.5	17	27	13	7	8	37	11	5	−
	25	17	26	15	10	6	43	21	4	
	250	12	25	11	7	6	43	14	3	
Diacetoxyscirpenol	0	30	44	18	6	13	49	29	5	
	0.25	29	36	19	6	24	57	32	3	
	2.5	23	56	20	9	23	60	24	12	−
	25	25	42	17	5	27	62	25	8	
	250	24	47	21	8	13	61	26	5	
Triacetoxyscirpenol	0	26	29	23	18	7	28	14	7	
	0.25	46	42	18	13	19	47	13	9	
	2.5	28	34	26	14	10	43	11	8	−
	25	33	37	22	13	28	46	22	8	
	250	25	48	29	15	11	35	11	7	
T-2 toxin	0	21	21	13	5	7	32	11	7	
	0.1	12	21	12	7	10	30	13	7	
	1	18	17	4	0	8	25	12	9	−
	10	17	17	9	10	6	26	10	3	
	100	21	15	0	6	13	30	13	5	
Deoxynivalenol	0	30	14	8	9	9	18	15	4	
	0.4	45	14	8	10	8	20	13	11	
	4	28	10	9	12	9	21	13	8	−
	40	26	19	14	6	9	22	18	10	
	400	33	19	12	10	8	18	11	12	
3-Acetyldeoxynivalenol	0	12	14	13	8	10	20	8	1	
	0.4	35	12	13	9	12	16	11	5	
	4	29	13	13	12	9	18	9	6	−
	40	15	19	8	12	14	13	6	5	
	400	27	11	8	12	14	20	9	6	
Moniliformin	0	24	18	9	10	6	29	5	4	
	0.25	28	27	7	6	8	24	11	4	
	2.5	26	26	11	6	13	33	9	7	−
	25	21	37	10	11	12	26	8	4	
	250	21	28	8	4	12	28	8	6	
Zearalenone	0	32	53	37	11	13	57	41	8	
	0.4	35	40	42	7	21	69	31	10	
	4	33	31	41	10	16	79	48	4	−
	40	58	35	51	14	22	60	39	8	
	400	53	34	33	9	18	35	28	10	
Aflatoxin B_1 (control)	0	22	39	14	6	14	32	15	9	
	0.1	471	475	18	22	17	50	18	13	
	0.3	385	1293	47	47	20	74	18	6	+
	0.7	316	118	14	6	30	161	14	11	
	1	256	67	12	6	13	254	12	10	
Sterigmatocystin (control)	0	37	87	21	25	12	72	29	14	
	1	102	116	18	17	18	122	31	32	+
	3	49	124	30	22	30	97	36	8	
	7	47	106	25	21	22	87	32	21	
	10	41	511	13	27	25	97	29	18	

[a] Positive mutagenicity was based on the presence of a positive dose response and a yield of at least three times as many revertant colonies over the spontaneous mutation rate.

mutagenic for *S. typhimurium* after metabolic activation and, in the case of aflatoxin B_1, also without metabolic activation. None of the eight *Fusarium* toxins tested gave a positive mutagenicity test based on the presence of a positive dose-response relationship and a yield of at least three times as many revertant colonies over the spontaneous mutation rate, either with or without metabolic activation.

It is difficult to equate the results of mutagenicity tests of *Fusarium* toxins with in vivo carcinogenicity tests because surprisingly little information is available on the chronic toxicity of either culture material or pure toxic metabolites of *Fusarium* species. A very low incidence of malignant tumors in various organs was observed in long-term feeding studies in mice and rats with rice cultures of *F. nivale* Fn 2b and *F. graminearum* NRRL 2830 (7, 14). A statistically significant increase in lung adenomas has been observed in mice dosed for 12 months with aqueous extracts of barley cultures of *F. sporotrichioides* no. 63 (1-3). Application of ethanol extracts of wheat cultures of *F. sporotrichioides* and *F. poae* to the skin, esophagus, and stomach of rodents had local cytotoxic effects, which were followed by regeneration and basal cell hyperplasia of the squamous epithelium (15), and long-term application of these extracts resulted in unspecified chronic lesions in a variety of organs, including the digestive tract, brain, and sex organs (R. Schoental, A. Z. Joffe, and B. Yagen, Br. J. Cancer **34**: 310, 1976). The carcinogen(s) supposedly present in the culture material or extracts was not chemically identified in any of the reports cited above.

In long-term feeding studies with crystalline T-2 toxin, Marasas et al. (12) found that this compound did not cause neoplasia or hyperplasia in either rainbow trout or rats. Mice treated topically with T-2 toxin as initiator and croton oil as cocarcinogen failed to develop any papillomas, but a few papillomas developed on mice treated with 7,12-dimethylbenz[a]anthracene followed by application of T-2 toxin (12). Lindenfelser et al. (9) also found that T-2 toxin, as well as diacetoxyscirpenol, did not act as a tumor initiator but promoted the development of statistically nonsignificant numbers of papillomas on 7,12-dimethylbenz(a)anthracene-initiated mice. The negative results obtained with T-2 toxin in in vivo carcinogenicity tests (9, 12) are supported by the negative results in the *rec* assay with *B. subtilis* (18) as well as by the lack of mutagenicity to *S. typhimirium* found by Ueno (17) and us. Similarly, the negative mutagenicity of diacetoxyscirpenol reported here and by Nagao et al. (13) is in agreement with the negative results obtained with in vivo carcinogenicity tests (9, 16). In long-term feeding studies with fusarenon-X in rats and mice, a very low incidence of malignant tumors was found in various organs (7, 14). Conflicting results have been reported regarding the mutagenicity of this compound to *S. typhimurium*. Nagao et al. (13) found fusarenon-X to be mutagenic without metabolic activation, but Ueno (17) reported negative results both with and without metabolic activation. Four other 12,13-epoxytrichothecenes (mono- and triacetoxyscirpenol, deoxynivalenol, and 3-acetyldeoxynivalenol) which had not previously been tested for carcinogenicity in experimental animals were found to be nonmutagenic in the present investigation.

The available evidence from in vivo and/or mutagenicity testing indicates that with the possible exception of fusarenon-X, none of the other 12,13-epoxytrichothecenes examined so far are likely to be carcinogenic.

The situation with regard to zearalenone is unresolved because a positive effect in the test for deoxyribonucleic acid-attaching ability with *B. subtilis* has been reported (18), whereas we found a lack of mutagenicity to *S. typhimurium*. Unfortunately, no published accounts of the chronic effects of zearalenone in experimental animals could be traced in the literature. It seems that in vivo carcinogenicity tests as well as further studies on the mutagenicity of zearalenone are required.

Although moniliformin is exceedingly toxic (8), the lack of deoxyribonucleic acid-attacking ability in *B. subtilis* (18) and of mutagenicity to *S. typhimurium* indicates that it is probably not carcinogenic. Final evaluation of the carcinogenic potential of moniliformin will, however, have to await the results of chronic exposure studies in animals.

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