Effect of Aflatoxin B_1 and Rubratoxin B on Bacteriophage and Rabbit Cornea Cells

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Mycotoxins, secondary fungal metabolites, first gained world-wide recognition in 1961, when aflatoxin was reported as the chemical agent responsible for turkey "X" disease in England (BLOUNT, 1961). Since this report, at least 30 toxic fungal metabolites have been chemically characterized. In addition to toxic manifestations, a few mycotoxins, such as aflatoxin B_1 are carcinogenic to a variety of animal species. (WILSON and HAYES, 1973). Other mycotoxins, for example, rubratoxin B, are potent embryocides and teratogens (HOOD, INNES and HAYES, 1973).

Methods currently employed for detection and quantitation of mycotoxins are of two general types: physiochemical and biological. Each has been widely used and both are necessary for effective control and expansion of knowledge in the field. Current physiochemical methods are capable of detecting in agricultural products aflatoxin B_1 at a few parts per billion (ppb) but only parts per million (ppm) in the case of rubratoxin B (HAYES and McCAIN, 1975). Biological assays for aflatoxin B_1 have utilized a variety of systems including cell cultures (LEGATOR, 1971) whereas bioassays for rubratoxin B have been limited to a protozoan (HAYES, MELTON and SMITH, 1974; WYATT and TOWNSEND, 1974). The present study reports a sensitivity assay for aflatoxin B_1 and rubratoxin B using a cell culture system which is inexpensive, easy to maintain and which requires a short incubation period. The effect of both compounds on bacteriophages also is reported.

MATERIALS AND METHODS

Aflatoxin B_1 was purchased from Calbiochem, Los Angeles, CA. Rubratoxin B was prepared by the method of HAYES and WILSON (1968). Compound purity was established by melting point, thin-layer chromatography and infrared and mass spectra. Mycotoxins were stored

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in the dark until used.

Bacteriophages (DNA coliphages PK, P2tsp, P2ts13 and P2ts40) and rabbit cornea cells were obtained from Drs. E. W. Six and J. Rodriguez, respectively, University of Iowa, Iowa City, IO. Escherichia coli T_1 was from the culture collection of the University of Alabama.

DNA coliphages and E. coli T_1 bacteriophage were tested at various times for toxin sensitivity by the total plaque-forming unit (PFU) method in samples cleared by centrifugation and by the single-step growth curve procedure of EISENSTARK (1967). A semisynthetic medium (L-Broth bacto-tryphone, 10 g; yeast extract, 5 g; glucose, 1 g; NaCl, 10 g; 1 N NaOH, 2.5 ml; glass-distilled water, 1000 ml) incubated at 37° C with or without agar was used for the bacteriophage experiments. Toxins in 0.1 ml acetone were added to the medium and the solvent was removed under vacuum before inoculation.

Rabbit cornea cells were maintained in bottles containing SRI-14 medium supplemented with 5% fetal bovine serum at 37° C in a humidified CO_2 incubator (DIXON, DULMADGE and SCHABEL, 1966). After a monlayer of cells developed, the medium was changed and the appropriate toxin concentration was added aseptically. Rubratoxin B and aflatoxin B_1 were dissolved in 0.05 M phosphate buffer, pH 7.1, and in 95% ethanol, respectively. Neither solvent was toxic to rabbit cornea cells.

RESULTS AND DISCUSSION

No change in PFU number of the bacteriophages tested was observed at 25 µg aflatoxin B_1 or 100 µg rubratoxin B per ml of medium. Further, the single-step growth curves (same concentration) indicated no interference with DNA coliphage or E. coli T_1 bacteriophage replication (data not presented). Results obtained with aflatoxin B_1 differed from earlier reports. Whereas JEMMALI (1969) reported that a Streptococcus lactis phage incubated with aflatoxin before exposure to the sensitive bacterium inhibited the infective process and LILLEHOJ and CIEGLER (1969) reported the ability of aflatoxin B_1 to induce lysis using lysogenic and indicator strains of Bacillus megaterium, the replication cycle of virulent DNA coliphages and E. coli T_1 was not inhibited by either toxin under the conditions for our experiment.

Rabbit cornea cells, however, were adversely affected (Table 1). Aflatoxin (5 µg/ml of medium) produced within 24 hr a cytopathic effect. By 72 hr

100% of the cells were abnormally shaped in the presence of 20 µg aflatoxin B_1 per ml of growth medium, whereas 75 µg rubratoxin B per ml of growth medium were required to affect 100% of the cells. Cells exposed to rubratoxin did not round up and become dense as did aflatoxin-treated cells but rather were large, irregularly shaped and packed peripherally with tiny inclusions which were refractile and spherical. These inclusions gave the appearance of air bubbles or lipid droplets. In addition, rubratoxin-treated cells contained large vacuoles.

TABLE 1.

Effect of Aflatoxin B_1 and Rubratoxin B on Rabbit Cornea Cells Grown in Tissue Culture

Aflatoxin B_1 (µg/ml)	% Abnormal Cells (Hour)		
	24	48	72
0	0	0	0
1	0	10	50
5	10	50	50
15	10	50	75
20	50	75	100
25	25	75	100
Rubratoxin B (µg/ml)			
0	0	0	0
5	0	25	50
25	10	25	50
50	10	25	75
75	10	25	100
100	25	50	100

Although aflatoxin B_1 affects a variety of cells grown in culture, only a single report exists in the literature indicating that rubratoxin B possess such properties (UMEDA, SAITO and SAITO, 1970). Elongation of the M phase with chromosomal damage resulted after HeLa cells were exposed to rubratoxin. Our data indicated that rabbit cornea cells grown in culture also were adversely affected by rubratoxin B and, in addition, were a sensitive indicator for both of these mycotoxins.

The minimal concentration of aflatoxin B_1 and rubratoxin B required to kill 50% of the cells was 1 µg and 5 µg per ml of tissue culture medium, respectively. This biological assay is 5 times more sensitive for rubratoxin than the corresponding physiochemical method. Since P. rubrum and A. flavus have been isolated from the same incriminated feeds, this assay not only offers a sensitive test for rubratoxin B but provides a means to confirm rubratoxin B in samples contaminated with aflatoxin B_1 because of the different morphological changes induced in the cell by each compound.

SUMMARY

Rabbit cornea cells exhibited a sensitivity to 1 µg aflatoxin B_1 and 5 µg rubratoxin B per ml of growth medium. No changes were observed in the bacteriophages tested in the presence of 25 µg aflatoxin B_1 or 100 µg rubratoxin B per ml of medium by the plaque-forming unit method or single-step growth curves.

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