APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Oct. 1983, p. 948–950
0099-2240/83/100948-03$02.00/0

Vol. 46, No. 4

Comparison of Cytotoxicity and Thin-Layer Chromatography Methods for Detection of Mycotoxins

JEAN ROBB[1]* AND MARY NORVAL[2]

Department of Microbiology, East of Scotland College of Agriculture,[1] and Department of Bacteriology, University of Edinburgh Medical School,[2] Edinburgh, Scotland

Received 11 April 1983/Accepted 14 July 1983

Thirty-three standard mycotoxins were assayed by thin-layer chromatography and by cytotoxicity in HEp-2 and Chang cells. Various levels of detection were found. The cytotoxicity test was significantly more sensitive than thin-layer chromatography for the trichothecenes and should be useful for screening extracts from animal feedstuffs for the presence of unknown mycotoxins.

It has become increasingly obvious in recent years that outbreaks of disease may occur in farm animals in Britain owing to the ingestion of various mycotoxins in feedstuffs. In 1960, an episode of aflatoxicosis (Turkey X disease) occurred when many thousands of turkeys died after eating rations containing groundnut meal contaminated by *Aspergillus flavus* which had produced aflatoxin (reviewed in reference 1). More recently, toxins elaborated by various species of *Fusarium* in the maize and wheat components of the feed were implicated in an outbreak of suboptimal growth in broiler chickens (8). It is thus of importance to be able to screen various animal feedstuffs for the presence of mycotoxins and to assay for these compounds in outbreaks of disease of unknown etiology. A multimycotoxin-screening procedure has been described by Patterson and Roberts (7) and involves extraction of the feedstuff with organic solvents followed by thin-layer chromatography (TLC) and identification by comparison with mycotoxin standards. This screen is therefore confined to the identification of known toxins and, in addition, is relatively insensitive for the detection of some compounds.

Various biological methods have been described for the assay of mycotoxins, ranging from whole animals such as brine shrimp (3) and chicken embryos (5) to in vitro tissue culture systems such as human embryo fibroblasts (2), rabbit reticulocytes (4), and HeLa cells (9). The range of mycotoxins which may be detected by a given assay has been rarely characterized; also, the relative sensitivities of the various systems have not been compared. Therefore, it was thought useful to develop a standard biological assay which could be carried out routinely with ease and to compare the sensitivity and range of mycotoxins detected with a conventional assay employing TLC.

There were 33 standard preparations of mycotoxins available which had been tested for purity and identity by nuclear magnetic resonance spectroscopy. The sources of these were aflatoxin G_1, cytochalasin B and D, scopoletin, and T-2 toxin from Sigma Chemical Co.; aflatoxins B_1, B_2, G_2, and M_1, citrinin, diacetoxyscirpenol, HT-2 toxin, luteoskyrin, 5-methoxysterigmatocystin, *O*-methyl sterigmatocystin, ochratoxin A, patulin, penicillic acid, PR toxin, rubratoxin B, sterigmatocystin, T-2 tetraol, and zearalenone from Makor Chemicals, Israel; roridin, T-2 triol, verrucarin A, and verrucarol from Cambridge Research Biochemicals Ltd.; 4-acetoxy-3,15-scirpendiol, 15-acetoxy-3,4-scirpendiol, scirpentriol, triacetoxyscirpene isolated from *Fusarium sulphurium* (10), and moniliformin isolated from *Fusarium monoliforme*, all kindly donated by P. S. Steyn; and deoxynivalenol kindly donated by M. Shepherd. Each was dissolved in acetone at 1 mg/ml, and 10-fold serial dilutions were made in acetone. Controls contained acetone alone.

For the TLC, plates (Merck art. 5554 DC-Alufolien Kieselgel 60F 254 and art. 5553 DC-Alufolien Kieselgel 60) were spotted with 5μl samples of each standard or dilution of standard, run in toluene-ethyl acetate-formic acid (60:30:10), and sprayed with 50% aqueous H_2SO_4 when necessary, before viewing in a UV cabinet (shortwave, 254 nm; longwave, 366 nm). R_f values are noted in Table 1. The lowest dilution at which spots could be detected by eye was noted.

For the biological assays, two cell lines were used, HEp-2 cells and Chang cells, both human epithelial type, the latter of liver origin. These were maintained on Earles-based Eagles medium supplemented with 5% newborn calf serum, 200 IU of penicillin per ml, and 200 μg of streptomycin per ml, and subcultured by tryp-

TABLE 1. Lowest levels at which mycotoxins were detected in various tests

Mycotoxin	Nanograms of mycotoxin			R_f values
	Cytotoxicity		TLC	
	HEp-2	Chang		
4-Acetoxy-3,15-scirpendiol	100	10	50	0.14
15-Acetoxy-3,4-scirpendiol	100	10	500	0.09
Aflatoxin B_1	100	100	0.5	0.17
Aflatoxin B_2	100	100	0.5	0.13
Aflatoxin G_1	ND[a]	ND	0.5	0.14
Aflatoxin G_2	1,000	100	0.5	0.11
Aflatoxin M_1	10	10	0.5	0.0
Citrinin	10	100	0.05	0.42
Cytochalasin B	ND	ND	500	0.3
Cytochalasin D	10	10	50	0.17
Deoxynivalenol	100	100	500	0.0
Diacetoxyscirpenol	1	10	500	0.23
HT-2 toxin	10	1	500	0.18
Luteoskyrin	ND	ND	500	0.38
5-Methoxysterigmatocystin	Not done	ND	5,000	0.66
O-Methyl sterigmatocystin	100	10	5,000	0.32
Moniliformin	ND	ND	5,000	0.0
Ochratoxin A	ND	ND	0.05	0.45
Patulin	100	100	500	0.32
Penicillic acid	1,000	1	50	0.35
PR toxin	1	100	50	0.44
Roridin A	0.0001	0.1	500	0.26
Rubratoxin B	ND	ND	ND	0.22
Scirpentriol	100	10	50	0.04
Scopoletin	ND	ND	0.5	0.27
Sterigmatocystin	100	10	5,000	0.56
T-2 tetraol	10	100	500	0.04
T-2 toxin	10	0.01	500	0.26
Triacetoxyscirpene	10	10	500	0.38
T-2 triol	100	100	500	0.1
Verrucarin A	0.1	0.1	5,000	0.35
Verrucarol	1,000	1,000	50	0.15
Zearalenone	100	100	50	0.55

[a] ND, Not detected at 1,000 ng.

sinization when confluent. In the test, they were cultured on cover slips in 1 ml of medium until semiconfluent. One microliter of each standard or dilution of standard was added, and after 48 h of incubation at 37°C, the cover slips were removed, the cells were fixed in ethanol, stained with Giemsa stain, and examined microscopically to assess the cytotoxic effect, if any. The lowest dilution at which a cytocidal effect was apparent was noted, i.e., marked inhibition of growth, cell rounding, and detachment from the surface. Each observation was made in triplicate, and cytotoxic levels were found to vary by a maximum of 1 dilution. The results are given in Table 1.

It may be seen that there was great variation in the level at which the standard mycotoxins were detected in the assay systems used, from no effect at 1,000 ng to visible spots at 0.05 ng in the TLC for citrinin and ochratoxin A, and cytotoxicity at 0.0001 ng for roridin A in HEp-2 cells. Although the TLC method detected almost all of the compounds used, it was not as sensitive as the cytotoxic assay for at least 13 of the 33 mycotoxins tested. This difference in sensitivity was most striking for the trichothecenes, for example, T-2 toxin and verrucarin. The cytotoxic test did not detect eight compounds with either Chang or HEp-2 cells, and generally there was some difference in the level of sensitivity between the two lines. Lompe and Milczewski (6) described the effect of 16 mycotoxins on three human and two porcine cell lines and found various levels of sensitivity, suggesting the use of more than one line of different origin in screening. The level of sensitivity of HEp-2 cells to roridin A was higher than expected but was reproducible in three separate series of dilutions and cytotoxic tests.

Thus, when screening for mycotoxins in such materials as animal feedstuffs, it would be advantageous to use a cytotoxic assay system in

addition to TLC. Apart from the increased sensitivity for the trichothecene group, the cytotoxic method has a good chance of picking up unknown toxins which may be present in samples of feedstuff associated with outbreaks of disease and for which there are no standards available.

We thank T. J. Simpson for supplying some of the mycotoxins used in this work and for helpful discussions.

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