NOTES

Accumulation of Only Aflatoxin B₂ by a Strain of Aspergillus flavus

H. W. SCHROEDER AND W. W. CARLTON

Agricultural Research Service, U.S. Department of Agriculture, College Station, Texas 77840, and School of Veterinary Science and Medicine, Purdue University, Lafayette, Indiana 47907

Received for publication 24 August 1972

A strain of Aspergillus flavus isolated from ground black pepper produced only aflatoxin B₂ on several natural substrates.

Strains of aflatoxin-producing species of the Aspergillus flavus group of fungi are known to vary greatly in ability to produce and accumulate compounds of the aflatoxin series. To our knowledge, there has been no previous report of a strain capable of accumulating large quantities of only the dihydro derivative of either of the two major toxins, B₁, and G₁.

We have isolated, from ground black pepper, a unique strain of A. flavus Link that accumulates only aflatoxin B₂ on several natural substrates. This culture carries the code number Pep-70-1h in our culture collection. Fermentations were conducted on slurries of ground peanut (6% ground seed to water, w/v) in 250-ml Erlenmeyer flasks or 2,800-ml Fernbach flasks at 25°C for 7 days. The compound was identified initially by comparison with authentic aflatoxin B₂ by thin-layer chromatography. Later the material was separated from most impurities by silica gel column chromatography and crystallized successively from chloroform. The recrystallized compound was confirmed as aflatoxin B₂ by comparing its mass spectrum with the spectrum of authentic B₂ (1).

The toxicity of crude chloroform extracts from these fermentations was established by the duckling bioassay. The concentration of aflatoxin B₂ in extracts of the cultural filtrate and extracts of the mycelial mats were estimated by thin-layer chromatography. Male Pekin ducks, received within 36 hr of hatching and weighing about 40 g, were dosed intrasophageally with three concentrations of the toxins from each of the two sources. The toxins were administered daily at concentrations of 100, 50, and 25 μg per duck in 0.25 ml of dimethylsulfoxide (DMSO). Control ducks were dosed with same amount of DMSO. Ducks were necropsied when found dead or when terminated. Portions of the liver were fixed in 10% buffered Formalin, processed for paraffin sectioning, and stained with hematoxylin and eosin.

The lethal effects of the extracts and the degeneration observed in the livers of necropsied ducklings were typical of aflatoxicosis in the duckling. Biliary hyperplasia accompanied by hepatic cell degeneration occurred in the livers of most of the test ducks. In the ducks given 100 and 50 μg per day, proliferated biliary cells were mainly periportal in location (Fig. 1), but were distributed within lobules as well in ducks given 25 μg/day. The dark-staining, oval biliary cells occurred as thin cords or columns of cells and as solid clumps which occasionally surrounded and isolated hepatocytes (Fig. 2). Degenerative changes and vacuole formation, suggestive of fatty change, were especially prominent in hepatocytes in close proximity to the proliferated biliary cells. The severity of the hepatic changes between ducks administered the extract of the culture filtrate or the extract of the fungal mat did not differ significantly. Livers of the control ducks administered DMSO were histologically normal.

Yields of aflatoxin B₂ were greater on ground peanut slurry than on similarly prepared media from rice, sorghum, pecan, or cottonseed. When originally isolated, the strain produced aflatoxin B₂ at the rate of 900 μg/g of ground peanut. With repeated subculturing, production declined rapidly. The cultures now produce ca. 200 μg/g of ground peanut. At no time during the period covered by these experi-
Fig. 1. Low-power photomicrograph of liver of Pekin duck killed when moribund after receiving two daily doses of 100 µg of aflatoxin B₁. Extensive periportal biliary cell hyperplasia is accompanied by severe parenchymal cell degeneration and necrosis. Hematoxylin and eosin. x56.

Fig. 2. Medium-power photomicrograph of liver of Pekin duck killed after receiving 10 daily doses of 25 µg of aflatoxin B₁. The proliferated biliary cells are arranged in cords which surround and isolate hepatocytes which are vacuolated and pale-stained. Hematoxylin and eosin. x350.
ments has this strain produced aflatoxins B₁, G₁, or G₂. With this strain, pure aflatoxin B₂ can be produced easily in large quantities. Further study of this fungus might provide additional information about the various steps in fungal metabolic cycles leading to the production and accumulation of aflatoxins.

We thank W. F. Haddon, Western Regional Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Albany, Calif., for the mass spectral analysis of the compound. These studies were conducted in cooperation with the Department of Plant Sciences, Texas A&M University, College Station, Texas. The bioassay was supported by cooperative agreement 12-14-100-9091(51), Market Quality Research Division, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, Md. A culture of Pep-70-1h has been deposited with the American Type Culture Collection with the accession number ATCC24109.

LITERATURE CITED

1. Haddon, W. F., M. Wiley, and A. C. Waiss, Jr. 1971. Aflatoxin detection by thin-layer chromatography—mass spectrometry. Ann. Chem. 43:268–270.