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Formation of Aflatoxins by *Aspergillus ostianus* Wehmer

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Aflatoxins are a group of four toxic metabolites produced by certain strains of a limited number of fungal species, *Aspergillus flavus*, *A. parasiticus*, and *Penicillium puberulum* (R. D. Hartley, B. F. Nesbitt, and J. O'Kelly, Nature **198**:1056, 1963; R. C. Codner, K. Sargeant, and R. Yeo, Biotechnol. Bioeng. **5**:185, 1963; F. A. Hodges et al., Science **145**:1439, 1964). The detection of aflatoxins in isolates of several other species of *Aspergillus* and *Penicillium* has recently been reported by M. M. Kulik and C. E. Holaday (Mycopathol. Mycol. Appl. **30**:137, 1966).

We have now found that a member of the *A. ochraceus* group, *A. ostianus* Wehmer, also produces aflatoxins. The strain was isolated from a Japanese dried fish product (katsuobushi), and identified by one of us (J. F.) according to the methods defined by K. B. Raper and D. I. Fennell (*The Genus Aspergillus*, The Williams and Wilkins Co., Baltimore, 1965). It has been deposited at the Northern Regional Research Laboratory, U.S. Department of Agriculture, Peoria, Ill., under accession number NRRL A-15156.

The fungus was grown at 25 C in 12 Fernbach flasks, each containing 200 ml of Mycological Broth (Difco) to which 0.5% yeast extract was added. After 7 days, the cultures were extracted with chloroform, and the combined extracts were chromatographed on a column of silica gel (W. A. Pons, Jr., et al., J. Assoc. Offic. Anal. Chemists **49**:554, 1966). After the initial wash of the column with ether, the aflatoxins, as well as red and yellow pigments, were eluted with chloroform containing 3% methanol. The presence of aflatoxins B_1 and G_1 in the eluate was shown by thin-layer chromatography on 250-μ layers of Silica Gel G-HR. Six solvent systems were employed as follows: chloroform-acetone (9:1), chloroform-methanol (93:7), benzene-ethanol-water (46:35:19, top layer), ethyl acetate saturated with water, benzene-methanol-acetic acid (24:2:1), and toluene-ethyl acetate-formic acid (5:4:1). Each system indicated the presence of fluorescent substances identical with internal standards of aflatoxins B_1 and G_1. The amounts of aflatoxins B_1 and G_1 were estimated (J. Assoc. Offic. Anal. Chemists **49**:229, 1966) as 1 mg each per liter of culture medium.

The identities of the compounds were confirmed by formation of acid derivatives on a submicrogram scale (P. J. Andrellos and G. R. Reid, J. Assoc. Offic. Agr. Chemists **47**:801, 1964) from the respective aflatoxin that had been obtained by preparative thin-layer chromatography of a small portion of the eluate from the silica gel column. The remainder of the eluate was rechromatographed on a column of neutral Florisil (M. R. Heusinkveld, C. C. Shera, and F. J. Baur, J. Assoc. Offic. Agr. Chemists **48**:448, 1965). Interfering pigments were eluted with 2 and 6% acetic acid in chloroform, whereupon the aflatoxins were eluted with acetone and separated by repeated thick-layer chromatography on Silica Gel G. The ultraviolet-absorption spectra of the isolated aflatoxins B_1 and G_1 were in accordance with those in the literature (T. Asao et al., J. Am. Chem. Soc. **87**:882, 1965). Quantitation of the aflatoxins by their ultraviolet spectra agreed with recoveries estimated by thin-layer chromatography. An infrared spectrum of the aflatoxin B_1 was the same as that of the authentic compound.

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