Country Cured Ham as a Possible Source of Aflatoxin

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Of 10 fungi isolated from a heavily molded country cured ham, 4 were identified as toxigenic strains of Aspergillus flavus.

The potential health hazard of aflatoxin in foods has been frequently discussed since the metabolite was first discovered. Strains of Aspergillus flavus Link ex Fries and Aspergillus parasiticus Speare produce this toxic and carcinogenic metabolite (5). These molds are isolated from cereals, grains, and ground-nuts and many of the isolates are able to produce aflatoxin. Insofar as is known, there is only one report of the isolation of a toxigenic mold strain from cured meats, i.e., that cited by Bullerman and Ayres (2) of a toxigenic strain of A. flavus from an Italian type salami.

Aspergilli and penicillia were isolated from four heavily molded country cured hams, and, in the present study, on one of these hams five strains of A. flavus, two strains of A. ochraceus Wilhelm, one strain of A. tamarii Kita, and two strains of Penicillium miczynski Zaleski were recovered and tested for toxigenicity. The average moisture value of 10 samples from this ham was 33%. The predominance of aspergilli in country cured ham, as was indicated in earlier work (1), is explained by the low moisture content.

The molds were grown in 250- or 500-ml Erlenmeyer flasks and screened for production of aflatoxin by inoculating 10⁶ spores into 50 ml of yeast extract containing 20% sucrose (YES), incubating at room temperature for 7 days, and then extracting the mold culture twice with 50 ml of chloroform. The mixture was shaken on a gyratory shaker for 30 min and filtered; the chloroform layer was collected with a separatory funnel and then evaporated to dryness with a flash evaporator. The residue was cooled and resuspended in exactly 5 ml of chloroform. The chloroform extract was resolved on thin-layer chromatograms (20 by 20 cm; 0.25-mm thickness of Silica Gel G-HR) with 1:9 (v/v) acetone-chloroform as a developing solvent. The concentration of the various spots was determined visually by comparing with aflatoxin standards obtained from the Southern Utilization Research and Development Laboratory, USDA, New Orleans, La. Of the 10 isolates, only 4 of the 5 A. flavus produced aflatoxin. Aflatoxins G₁ and G₂ were not detected in cultures of any of the four positive strains, and total B₁ and B₂ content ranged from 49 to 85 μg/50 ml (YES). Extracts were considered to be negative if 3 μlitters of the concentrated sample showed no fluorescent compounds. The detection of aflatoxin B in the absence of G has also been reported by Diener and Davis (3). These authors found that among the toxin-producing isolates of A. flavus from agricultural commodities, 90% produced only aflatoxin B.

Though the Rf values or patterns of the fluorescent compounds were identical to those of standard aflatoxin, further confirmation of their identity was obtained by using bio-assay techniques. Extracts were purified by preparative thin-layer chromatography. The method of Verrett et al. (6) was used to determine toxicity to the chick embryo. Inoculation of 0.025 μg per fertile egg prior to incubation resulted in death of the embryo. Administration of the extract to 1-day-old duckings per os resulted in bile-duct cell proliferation typical of aflatoxin B₁.

Toxigenic strains of A. flavus were detected in cured hams; had these toxigenic molds been provided with appropriate conditions for toxin production, they could have produced a real hazard to foods. Although aspergilli are common flora on aged and cured meats, the identification of toxigenic strains of A. flavus has not previously been reported from this source.

The two strains of A. ochraceus were screened
for ochratoxin production by the method of Eppley (4). Thin-layer chromatograms of the unknown samples were compared to extracts from a known ochratoxin producer and ochratoxin standard provided by A. D. Campbell, Federal Food and Drug Administration, Washington, D.C. Both strains were found negative for ochratoxin production.

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LITERATURE CITED