Identification and Aflatoxin Production of Molds Isolated from Country Cured Hams

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Of 562 molds isolated from country cured hams, 403 isolates were of the genus *Penicillium*, 121 were *Aspergillus*, and 36 were *Cladosporium*, *Alternaria*, and other genera.

Country cured hams (6) are very popular in the southeastern United States. The surface of country cured hams often is covered by the growth of different species of mold, some of which might be considered desirable for improving the flavor; yet undesirable molds could cause spoilage of the product or could be pathogenic or toxigenic for man. Strains of *Aspergillus flavus* Link ex Fries and *A. parasiticus* Speare are able to produce aflatoxins (1, 14). One aflatoxin-producing strain was recovered from an Italian-type salami (3). Five strains of *A. flavus* were isolated from a single country cured ham by Strzelecki and co-workers (12). Four of these five strains produced aflatoxin. Experimentally, *A. flavus* and *A. parasiticus* were shown to be capable of producing aflatoxin on fresh beef, ham, and bacon (4, 5). Since the presence of *A. flavus* on hams conceivably could constitute a potential health hazard, it was considered of interest to note how often mold isolates capable of producing aflatoxin are found.

Samples from country cured hams were taken from different ham processors in the State of Georgia. Four hundred and fifty-five swabs were taken from 356 country cured hams from 11 ham processors. Each swab was inoculated on the following media: Czapek Dox agar, Czapek Dox agar plus 16% NaCl, malt agar, and potato dextrose agar (8). Penicillia were identified to genus only, and all species of aspergilli were identified by the methods outlined by Raper and Fennell (10). Production of aflatoxin by isolated strains of mold was determined by the screening method used by De Vogel et al. (7). Fluorescence under long-wavelength ultraviolet light was checked after 3 and 10 days of incubation at 27 C.

Isolates which showed blue or green fluorescence by the screening test were inoculated into 50 ml of YES broth (20% sucrose and 2% yeast extract) and onto sterile moist rice. Incubated broth and rice were incubated at 27 C for 7 to 12 days. After the mold cultures were extracted twice by shaking for 10 min with 75 ml of chloroform, the chloroform layer was collected with a separatory funnel, filtered, and evaporated to dryness at 40 C with a flash evaporator (12). The residue was dissolved with 5 ml of chloroform, and the mycotoxins were separated by using thin-layer chromatograms (TLC) coated with MN Silica Gel G-HR (Brinkmann Instr., Westburg, N.Y.). Chloroform-acetone (85:15 v/v) was used as the TLC developing solvent for aflatoxin.

Production of aflatoxin was determined visually by comparing the sample with aflatoxin standards (Southern Utilization Research and Development Laboratories, New Orleans, La.). From producing stains, the chloroform extract was rechromatographed on a preparative scale, and the suspect spot removed. The aflatoxin was eluted from the silica gel with chloroform and filtered, and the ultraviolet absorption spectra were obtained on a Perkin-Elmer model 202 spectrophotometer.

The method of Verrett et al. (13) was used for bioassay. Chloroform extracts of broth were evaporated to dryness and dissolved in 2 ml of sterile propylene glycol. A 0.03-ml amount of this solution was inoculated into the air sac of eggs by sterile syringe. A control was inoculated with the same amount of pure propylene glycol. The development of the embryo was observed after 4, 6, and 8 days. After 8 days, all eggs which failed to develop were discarded.

Of the 562 mold isolates taken from 356 country cured hams, 403 *Penicillium*, 121 *Aspergillus*, and 36 other mold isolates, mostly...
members of the genus Cladosporium or Alternaria, were identified (Table 1). The number of aspergilli depended on the age of the hams and on the amount of moisture in the storage room. All aspergilli were more abundant on the surface of hams aged for 12 months or longer than on 1- to 3-month-old hams. Under dry storage conditions, more aspergilli were isolated from 1- to 3-month-old hams, whereas under moist conditions these hams yielded more penicillia.

The largest number of aspergilli isolated were members of the A. glaucus group (Table 2). Twenty-one were A. repens, 18 were A. amstelodami, 3 were A. pseudoglaucus, and 2 were A. ruber. The A. versicolor group was the second most common, having 21 of A. versicolor and 13 of A. sydowi. These groups are of

### TABLE 1. Isolation of molds from country cured hams

| Ham processor | Age of hams (months) | No. of | Total molds |
|---------------|----------------------|--------|-------------|
|               | Hams     | Swabs   | Penicillium | Aspergillus | Other molds |             |
| 1             | 1-12     | 104     | 104         | 97          | 10          | 10          | 117         |
| 2             | 6-12     | 8       | 30          | 4           | 35          | 1           | 40          |
| 3             | 6-12     | 71      | 84          | 77          | 12          | 5           | 94          |
| 4             | 1        | 42      | 42          | 32          | 22          | 1           | 56          |
| 5             | 6        | 30      | 50          | 66          | 4           | 1           | 71          |
| 6             | 6        | 5       | 14          | 23          | —           | 3           | 26          |
| 7             | 3-6      | 14      | 14          | 24          | —           | —           | 24          |
| 8             | 1-2      | 27      | 27          | 5           | 5           | 3           | 13          |
| 9             | 1-2      | 40      | 40          | 55          | 1           | 1           | 57          |
| 10            | 6-12     | 12      | 40          | 19          | 23          | 1           | 43          |
| 11            | 12       | 3       | 10          | 1           | 10          | 10          | 21          |
| Total         |          | 356     | 455         | 403         | 123         | 36          | 562         |

### TABLE 2. Members of the genus Aspergillus isolated from country cured hams

| Groups and species of Aspergillus | No. of aspergilli isolated from hams of ham processor | No. identified |
|----------------------------------|-----------------------------------------------------|---------------|
|                                  | 1   | 2   | 3   | 4   | 5   | 6   | 7   | 8   | 9   | 10  | 11  |
| A. candidus                      |     |     |     |     |     |     |     |     |     |     | 1   |
| A. flavus                        |     |     |     |     |     |     |     |     |     |     | 3   |
| A. fumigatus                     |     |     |     |     |     |     |     |     |     |     | 2   |
| A. viride nutans                 |     |     |     |     |     |     |     |     |     |     | 1   |
| A. glaucus                       | 44  |     |     |     |     |     |     |     |     |     |     |
| A. amstelodami                   |     | 1   | 2   |     |     |     |     |     |     |     | 18  |
| A. pseudoglaucus                 |     |     |     |     |     |     |     |     |     |     | 3   |
| A. repens                        | 7   |     |     |     |     |     |     |     |     |     | 6   |
| A. ruber                         | 2   |     |     |     |     |     |     |     |     |     | 21  |
| A. nidulans                      |     |     |     |     |     |     |     |     |     |     |     |
| A. aurantiobrunneus              | 1   |     |     |     |     |     |     |     |     |     | 5   |
| A. ochraceus                     |     |     |     |     |     |     |     |     |     |     | 1   |
| A. restrictus                    |     |     |     |     |     |     |     |     |     |     | 31  |
| A. conicus                       |     |     |     |     |     |     |     |     |     |     | 4   |
| A. gracilis                      |     |     |     |     |     |     |     |     |     |     | 3   |
| A. penicilloides                 |     |     |     |     |     |     |     |     |     |     | 7   |
| A. restrictus                    |     |     |     |     |     |     |     |     |     |     | 17  |
| A. versicolor                    |     |     |     |     |     |     |     |     |     |     | 34  |
| A. sydowi                        |     | 4   |     |     |     |     |     |     |     |     | 13  |
| A. versicolor                    | 4   |     |     |     |     |     |     |     |     |     | 21  |
| Total                            | 10  | 35  | 12  | 23  | 4   |     |     |     | 5   | 23  | 121 |

* A total of 455 samplings; 104 samplings from processor 1, 30 from 2, 84 from 3, 42 from 4, 50 from 5, 14 from 6, 14 from 7, 27 from 8, 40 from 9, 40 from 10, and 10 from 11.

* Dashes indicate species not observed.
particular interest since previous work has indicated that certain strains of *A. sydowi* (15), *A. amstelodami* (9), and *A. ruber* (11) may be capable of producing toxic metabolites under some conditions, and since certain *A. versicolor* strains are known to produce sterigmatocystin.

Of 121 aspergilli isolated from 356 country cured hams of different ages and from different processors, only 3 were strains of *A. flavus*. Each of the three possessed distinct morphological and color characteristics. By comparing extracts of these strains with aflatoxin standards on TLC, it was found that only two were able to produce aflatoxin. Only aflatoxin B₁ was produced in detectable amounts by either strain. The absorption spectra of the eluted TLC spots for both strains showed the characteristic maxima of aflatoxin B₁ at 363 and 265 nm. The embryos of all 48 chick eggs inoculated with extracts of broth cultures of the two aflatoxin-producing strains of *A. flavus* failed to develop. In the same experiment, 20 of 24 control eggs hatched. These results, together with the screening test, TLC, and ultraviolet spectrophotometry, indicate that two of the three strains of *A. flavus* isolated from country cured hams produced aflatoxin B₁ when inoculated on experimental substrates.

Of 356 hams examined, only two yielded strains of *A. flavus* having the ability to produce aflatoxin. Previous work from this laboratory (Strzelecki et al., 1969) has shown that had these two toxigenic strains been provided with appropriate conditions for toxin production, they could have posed a real hazard. There still is no definite evidence to date that such hams contain harmful amounts of aflatoxin.

**LITERATURE CITED**

1. Allcroft, R. 1965. Aspects of aflatoxicosis in farm animals, p. 153–162. In G. N. Wogan (ed.), Symposium on mycotoxins in food stuffs. M.I.T. Press, Cambridge, Mass.
2. Ayres, J. C., D. A. Lillard, and L. Leistner. 1967. Mold ripened meat products. 20th Annu. Recipr. Meat Conf. June 14–16. p. 156–168.
3. Bullerman, L. B., and J. C. Ayres. 1968. Aflatoxin-producing potential of fungi isolated from cured and aged meats. Appl. Microbiol. 16:1945–1946.
4. Bullerman, L. B., P. A. Hartman, and J. C. Ayres. 1969. Aflatoxin production in meats. I. Stored meats. Appl. Microbiol. 18:714–717.
5. Bullerman, L. B., P. A. Hartman, and J. C. Ayres. 1969b. Aflatoxin production in meats. II. Aged dry salamis and aged country cured hams. Appl. Microbiol. 18:718–722.
6. Christian, A. J. 1964. Curing Georgia hams—country style. Animal Husbandry 5, Bulletin 627. Cooperative Extension Service, University of Georgia, Athens.
7. De Vogel, P., R. van Rhee, and B. Koelenmad. 1965. A rapid screening test for aflatoxin-synthesizing *Aspergillus* of the *flavus-oryzae* group. J. Appl. Bacteriol. 29:213.
8. Leistner, L., and J. C. Ayres. 1968. Molds and meats. Die Fleischwirtschaft 48:62–65.
9. Rabie, C. J., W. A. de Klerk, and M. Terblanche. 1964. Toxicity of *Aspergillus amstelodami* to poultry and rabbits. South African J. Agr. Sci. 7:341–346.
10. Raper, K. B., and D. L. Pennell. 1965. The genus *Aspergillus*. Williams and Wilkins Co., Baltimore.
11. Scott, De B. 1964. Toxigenic fungi isolated from cereal and legume product. Mycopathol. Mycol. Appl. 25 (suppl.):213–222.
12. Strzelecki, E., H. S. Lillard, and J. C. Ayres. 1969. Country cured ham as a possible source of aflatoxin. Appl. Microbiol. 18:938–939.
13. Verrett, M. J., J. P. Mariac, and J. McLaughlin. 1964. Use of the chicken embryo in the assay of aflatoxin toxicity. J. Ass. Offic. Anal. Chem. 47:1003–1006.
14. Wogan, G. N. 1966. Chemical nature and biological effects of the aflatoxin. Bacteriol. Rev. 30:460–470.
15. Woolley, D. W., J. Berger, W. H. Peterson, and H. Steenbock. 1939. Toxicity of *Aspergillus sydowi* and its correction. J. Nutr. 16:465–476.