Potential Production of Sterigmatocystin on Country-Cured Ham

N. A. HALLS AND J. C. AYRES
Department of Food Science, University of Georgia, Athens, Georgia 30602

Received for publication 27 June 1973

In laboratory media, 10 of 16 isolates of Aspergillus versicolor from country-cured ham were capable of producing sterigmatocystin. Three of these isolates were tested and found to produce sterigmatocystin on country-cured ham after 14 days of incubation at 20 or 28 C.

Certain strains of Aspergillus versicolor produce a toxic metabolite named sterigmatocystin. It is toxic to mice (3), rats (7), monkeys (12), and ducklings (3), and is carcinogenic when injected (2) or fed (6, 8) to rats. Sterigmatocystin and cultures which are capable of its production have been recently detected in wheat (9) and coffee beans (5).

In a survey of the mycoflora of country-cured hams, Sutic et al. (11) found that molds identified within the A. versicolor group were the second most common aspergilli. The present investigation was undertaken to determine the capability of these isolates of A. versicolor to produce sterigmatocystin in laboratory media and to determine if country-cured ham could sustain sterigmatocystin formation.

Sterigmatocystin was extracted and separated by the method of Stack and Rodricks (10), and a derivative was formed by treatment with a 20% suspension of aluminum chloride in ethanol. By using a Turner model 110 fluorometer, determinations were made by comparing the yellow fluorescence of this derivative of sterigmatocystin samples with that of sterigmatocystin standards (Calbiochem, Los Angeles, Calif.). The lower limit of detection of sterigmatocystin by this method was 0.5 μg per slice of ham.

Spore suspensions of 16 isolates of A. versicolor from country-cured hams (11) were prepared by the method of Mateles and Adye (4), and the concentration of conidia was adjusted to 10⁴/ml. The isolates were screened for sterigmatocystin production in 3 laboratory media. These were: Czapek-Dox solution (Difco) supplemented with 0.5% yeast extract (CDY medium); yeast extract sucrose (YES) medium (20% sucrose, 2% yeast extract, pH 6.0); and citrate glucose phosphate (CGP) medium, wherein the following ingredients were dissolved in 1 liter of distilled water: glucose, 25 g; citric acid, 4.8 g; yeast extract, 0.5 g; Na₂HPO₄·7H₂O, 13.4 g; NH₄Cl, 2.5 g; MgSO₄·7H₂O, 0.5 g; ZnSO₄·7H₂O, 25 mg; FeCl₃·6H₂O, 25 mg; CuSO₄·5H₂O, 4 mg; Na₂MoO₄, 2H₂O, 2.5 mg; and MnSO₄·H₂O, 0.6 mg; pH 4.8. CDY and CGP media were dispensed in 25-ml amounts, and YES medium was dispensed in 50-ml amounts in 250-ml Erlenmeyer flasks; the media were sterilized by autoclaving at 121 C for 15 min. Each flask was inoculated with 10⁴ spores and incubated at 28 C for 7, 10, or 14 days.

Results of the screening (Table 1) indicate that, when given suitable conditions, a large number of isolates of A. versicolor can produce sterigmatocystin, but that its production may be suppressed in media such as CDY or YES which are commonly used to screen toxigenic molds.

Three isolates, which had produced sterigmatocystin in all three laboratory media, were used to inoculate country-cured ham. Slices (0.5 to 1.0 cm thick and weighing about 25 g) were cut from the center section of a fully cured and aged ham, surface sterilized by dipping in 1% NaOCl for 1 min, rinsed with sterile water, and blotted dry on sterile cheese cloth. The slices were inoculated by swabbing with 2 ml of a suspension containing 5 × 10⁸ spores/ml of A. versicolor, and thus a load of 10⁸ spores per slice of ham was obtained. Each inoculated slice was hung in a sterile 1-pint (470 ml) Mason jar, equipped with a Mason lid modified in the manner described by Bullerman et al. (1), and incubated at 20 or 28 C.

At 20 and 28 C, the ham supported growth of all 3 isolates with which it had been inoculated; no mold growth was apparent on uninoculated...
slices of ham. After 7 days of incubation, sterigmatocystin was detected in only 1 of 6 ham slices incubated at 20°C, and in none of those incubated at 28°C (Table 2). However, after 14 days, sterigmatocystin was detectable in all but 1 slice of ham.

Country-cured hams may be aged for 6 months to 2 years (1) in conditions wherein temperature and humidity control may be primitive. It is during this period that mold growth occurs on the ham. This investigation has shown that a large proportion of isolates of *A. versicolor* from country-cured ham are capable of producing sterigmatocystin and that country-cured ham, at temperatures which may prevail during aging, is a suitable substrate for production of this toxin. Although there is no direct evidence that country-cured hams contain sterigmatocystin, they may provide an environment wherein a toxigenic mold such as *A. versicolor* could present a health hazard to the consumer.

This investigation was supported by Food and Drug Administration grant FD-00155.

We thank J. R. Rehberg for technical assistance.

### LITERATURE CITED

1. Bullerman, L. B., P. A. Hartman, and J. C. Ayres. 1969. Aflatoxin production in meats. II. Aged dry salamis and aged country cured hams. Appl. Microbiol. 18:718-722.

2. Dickens, F., H. E. H. Jones, and H. B. Wayneforth. 1966. Oral, subcutaneous and intratracheal administration of carcinogenic lactones and related substances; the intratracheal administration of cigarette tar in the rat. Brit. J. Cancer 20:134-144.

3. Lillehoj, E. B., and A. Ciegler. 1968. Biological activity of sterigmatocystin. Mycopathol. Mycol. Appl. 35:373-376.

4. Mateles, R. I., and J. C. Adye. 1965. Production of aflatoxins in submerged culture. Appl. Microbiol. 13:208-211.

5. Purchase, I. F. H., and M. E. Pretorius. 1973. Sterigmatocystin in coffee beans. J. Ass. Offic. Anal. Chem. 56:225-226.

6. Purchase, I. F. H., and J. J. van der Watt. 1968. Carcinogenicity of sterigmatocystin. Food Cosmet. Toxicol. 6:555-556.

7. Purchase, I. F. H., and J. J. van der Watt. 1969. Acute toxicity of sterigmatocystin to rats. Food Cosmet. Toxicol. 7:135-139.

8. Purchase, I. F. H., and J. J. van der Watt. 1970. Carcinogenicity of sterigmatocystin. Food Cosmet. Toxicol. 8:289-295.

9. Scott, P. M., W. van Walbeek, B. Kennedy, and D. Anyeti. 1972. Mycotoxins (ochratoxin A, citrinin and sterigmatocystin) and toxicogenic fungi in grains and other agricultural products. J. Agr. Food Chem. 20:1103-1109.

10. Stack, J. E., and J. V. Rodricks. 1971. Method for analysis and chemical confirmation of sterigmatocystin. J. Ass. Offic. Anal. Chem. 54:86-90.

11. Sutic, M., J. C. Ayres, and P. E. Koehler. 1972. Identification and aflatoxin production of molds isolated from country cured hams. Appl. Microbiol. 23:656-658.

12. van der Watt, J. J., and I. F. H. Purchase. 1970. The acute toxicity of retorine, aflatoxin and sterigmatocystin in vervet monkeys. Brit. J. Exp. Pathol. 51:185-190.

### TABLE 1. Production of sterigmatocystin by 16 isolates of *A. versicolor* from country-cured ham

| Incubation (days) | CDY medium | YES medium | CGP medium |
|------------------|------------|------------|------------|
| 7                | 5          | NT*        | 10         |
| 10               | 7          | 4          | 10         |
| 14               | NT*        | 5          | NT*        |

* NT, Not tested.

### TABLE 2. Production of sterigmatocystin on country-cured ham

| Organism          | Sterigmatocystin (µg) produced per slice of ham* |
|-------------------|-----------------------------------------------|
|                   | 20°C 7 days | 14 days | 28°C 7 days | 14 days |
| *A. versicolor* XVII/24 | 12 | <0.5 | 17 |
|                   | <0.5 | 7 | <0.5 | 20 |
| *A. versicolor* XVII/27 | 5 | <0.5 | 10 |
|                   | <0.5 | 8 | <0.5 | 12 |
| *A. versicolor* XVII/32 | 5 | <0.5 | 6 |
|                   | <0.5 | 4 | <0.5 | 16 |
| Uninoculated      | <0.5 | <0.5 | <0.5 | <0.5 |

* Mean of two replicate assays.