Factors Affecting the Production of Sterigmatocystin in Semisynthetic Media

N. A. HALLS* AND J. C. AYRES
Department of Food Science, University of Georgia, Athens, Georgia 30602
Received for publication 4 June 1975

Production of sterigmatocystin by Aspergillus versicolor was stimulated by inorganic phosphate when used in conjunction with citric acid cycle compounds.

This study was undertaken to investigate some factors which might affect the production of sterigmatocystin by Aspergillus versicolor. Sterigmatocystin was initially isolated from A. versicolor (Vuillemin) Tiraboschi and characterized (1–3) as a xanthone group attached to a furofuran moiety. Its toxic and carcinogenic properties are similar to those of aflatoxin but less potent. There are few quantitative data on in vitro production of sterigmatocystin.

The sterigmatocystin-producing mold used in this study was A. versicolor XVII/32 isolated from country cured ham (7). Spore suspensions were prepared by the method of Mateles and Adye (4), and the concentration of conidia was adjusted to 10⁹/ml.

The following basal medium of glucose mineral salts (GMS) was used in this investigation: glucose, 25 g; yeast extract, 0.5 g; NH₄Cl, 2.5 g; MgSO₄·7H₂O, 0.5 g; ZnSO₄·7H₂O, 25 mg; FeCl₃·6H₂O, 4 mg; Na₂MoO₄·2H₂O, 2.5 mg; MnSO₄·H₂O, 0.6 mg. After dissolving in 500 ml of distilled water, the medium was made up to 1 liter by adding a suspension of CaCO₃ in water (1%, wt/vol) or 0.05 M solutions of citric acid cycle compounds.

These solutions were prepared from the sodium salts of phthalate, acetate, citrate, α-ketoglutarate, succinate, fumarate, or malate. To investigate the effect of phosphate, dibasic sodium phosphate was added to these media at a final concentration of 0.05 M. The pH of each of these media was adjusted with HCl to 4.8, and then the media were dispensed in 25-ml amounts in 250-ml Erlenmeyer flasks and sterilized by autoclaving at 121 C for 15 min. Each flask was inoculated with 10⁶ spores and incubated without shaking at 28 C. Sterigmatocystin was extracted and separated by the method of Stack and Rodricks (6), and its concentration was determined using a Turner model 110 fluorometer (lower limit of detection, 0.5 mg).

Growth of molds was estimated as dry weight of mycelium.

The accumulation of acid and products that occurred when unbuffered GMS was used rapidly curtailed growth of A. versicolor. Results of growth overall in the variously buffered GMS media (Table 1) indicated that the greatest increase in growth took place in the first 4 days of incubation and that phosphate was stimulatory. Sterigmatocystin was assayed after 4, 7, 10, and 14 days of incubation. None was recovered from any of the media (Fig. 1) until after day 4. The highest levels of sterigmatocystin were recovered after 10 days of incubation. There was little degradation or synthesis between days 10 and 14.

In media buffered with CaCO₃ or phthalate, sterigmatocystin production was low and phosphate had little effect on the amounts of mycoxin recovered. Succinate, malate, fumarate, α-ketoglutarate, and citrate enhanced sterigmatocystin production, whereas acetate did not. In all of these substrates, except those containing succinate, much higher amounts of sterigmatocystin were recovered when phosphate was added. However, on the medium containing succinate, phosphate had no additive stimulatory effect.

The physiological interrelationships of structurally similar mycotoxins are not well understood. Reddy et al. (5) reported that phosphate levels comparable to those used in this investig-

Table 1. Growth of A. versicolor in stationary flasks of GMS medium incubated at 28 C

| Medium          | Mean dry wt (mg)* |
|-----------------|-------------------|
|                 | 4t 7 10 14        |
| GMS             | 108 129 145 173   |
| GMS with added phosphate (0.05 M) | 154 188 201 212 |

* Plus or minus 30 to 60 mg.

† Incubation time (days).

* Present address: Department of Pharmacy, The University, Manchester M19 3PL, England.
Fig. 1. Production of sterigmatocystin. 25 ml of GMS media. (A) GMS buffered with CaCO₃ (○); CaCO₃ plus phosphate (●); buffered with phthalate (□); phthalate plus phosphate (▲). (B) GMS buffered with acetate (□); acetate plus phosphate (●); buffered with citrate (○); citrate plus phosphate (▲). (C) GMS buffered with α-ketoglutarate (□); α-ketoglutarate plus phosphate (●); buffered with succinate (○); succinate plus phosphate (▲). (D) GMS medium buffered with fumarate (□); fumarate plus phosphate (●); buffered with malate (○); malate plus phosphate (▲).

This investigation drastically reduced the yield of aflatoxin B₁ produced by cultures of A. parasiticus. Yet this study has shown that phosphate, when used in compounds associated with the citric acid cycle, may stimulate the production of sterigmatocystin by A. versicolor.

This investigation was supported by grant FD-00155-07 from the Office of Research Grants, Food and Drug Administration.

LITERATURE CITED
1. Birkinshaw, J. H., and I. M. M. Hammady. 1957. Metabolic products of Aspergillus versicolor (Vuillemin) Tiraboschi. Biochem. J. 65:162–166.
2. Bullock, E. J. C. Roberts, and J. G. Underwood. 1962. Studies on mycological chemistry. Part XI. The structure of isoosterigmatocystin and an amended structure for sterigmatocystin. J. Chem. Soc. (1962):4179–4183.
3. Davies, J. E., D. Kirkaldy, and J. C. Roberts. 1960. Studies on mycological chemistry. Part VII. Sterigmatocystin, a metabolite of Aspergillus versicolor (Vuillemin) Tiraboschi. J. Chem. Soc. (1960):2169–2178.
4. Mateles, R. I., and J. C. Adye. 1965. Production of aflatoxins in submerged culture. Appl. Microbiol. 13:208–211.
5. Reddy, T. V., L. Viswanathan, and T. A. Venkitesubramanian. 1971. High aflatoxin production on a chemically defined medium. Appl. Microbiol. 22:329–336.
6. Stack, M., and J. V. Rodricks. 1971. Method for analysis and chemical confirmation of sterigmatocystin. J. Assoc. Off. Anal. Chem. 54:86–90.
7. Sutic, M., J. C. Ayres, and P. E. Koehler. 1972. Identification and aflatoxin production of molds isolated from country cured hams. Appl. Microbiol. 23:6656–658.