A Research Note

Temperature Limits for Production of Aflatoxin by Twenty-five Isolates of Aspergillus flavus and Aspergillus parasiticus

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ABSTRACT

Aflatoxicogenic isolates of Aspergillus were tested for their aflatoxin production after 8 weeks of growth on wort agar medium at high (41, 46 ± 1 C) and low (2, 7 ± 0.5 C) temperatures. Controls were grown at approximately 22 C, a temperature known to be favorable for aflatoxin production. There were two replications of each treatment. All replicate cultures of the 25 isolates grown at 22 C were positive for aflatoxin. Aflatoxins were not detected in wort agar cultures were incubated at other test temperatures. It appears that both Aspergillus flavus and Aspergillus parasiticus will not produce aflatoxins when grown at < 7.5 C or at ≥ 40 C.

Our earlier investigations (6) demonstrated that production of aflatoxins, using only two Aspergillus flavus isolates, was limited by temperatures of < 7.5 C or/and ≥ 40 C. Other investigators, in general working with one or a limited number of A. flavus or A. parasiticus isolates, have not conclusively demonstrated similar limiting temperatures. Rabie and Smalley (4) reported that small amounts of aflatoxin G1 were produced after 12 days at 42 C; however, no B1 was detected and there was no chemical or bioassay confirmation of the G1 aflatoxin. Diener and Davis (1, 2) reported a minimal temperature for production in peanuts of 13 ± 1 C after 21 days at a relative humidity of 97-99%, although damaged kernels developed aflatoxin at 12 C. They also claimed a maximum temperature for aflatoxin production of 41.5 ± 1.5 C. This is in contrast to our studies (6) demonstrating increasing production at 13 C at 3, 6, and 12 weeks after inoculation. Sorenson et al. (10) studied production of aflatoxin in the temperature range of 8 to 37 C and reported that no aflatoxin was produced at 8 C. However, their tabulated data show that a small amount (less than 0.01 µg/g of rice) of aflatoxin B1 and G1 was recovered after 21 days at 8 C. Walbeck et al. (11) studied five strains of A. flavus at 7.5 and 10 C and reported aflatoxin production at both temperatures. Temperatures negative for aflatoxin production were not determined. Schroeder and Hein (7) used four isolates in their investigation but studied aflatoxin production only at temperatures from 10 to 40 C and only for a 10-day period. They reported that small amounts of aflatoxin were produced at the two temperature extremes. More recently, studies by Shih and Marth (8, 9) appear to demonstrate production of small amounts of aflatoxins at 45 C. However, this seems questionable as there were no studies which would serve to eliminate the possibility that the inoculum contributed aflatoxins to the experimental flasks. This possibility is further re-enforced by their data showing no significant differences between treatments examined at 3, 5, and 7 days. In 1976, Northolt et al. (3) reported that aflatoxin B1 was produced by A. parasiticus (NRRL-2999) at temperatures as low as 13 C and as high as 32 C.

On the assumption that different species and also strains of species, particularly from widely differing localities, may vary considerably in some of their physiological responses to environmental conditions, it appeared necessary to determine the limiting temperatures for aflatoxin production of a large number of isolates from various geographical and substrate sources.

MATERIALS AND METHODS

Twenty-five aflatoxin-producing A. flavus, A. parasiticus, or undetermined species of the A. flavus group (5) were selected. Isolates used in this study, from the Division of Microbiology's Culture Collection, were M-1, 3, 30, 52, 66, 112, 141, 185, 198, 201, 219, 224, 226, 260, 268, 270, 276, 320, 326, 332, 344, 346, 347, 363, and 892. They were isolated from samples of peanut, rice, seeds, walnut, corn, cottonseed, grass seed, ink, pond water, cocoa, and strawberry from various localities in the United States and Brazil.

To ascertain (a) what level of aflatoxin would be detected in extractions from flasks containing wort agar inoculated with spores of the highest aflatoxin-producing strain of Aspergillus, and (b) that the inoculum did not contribute any aflatoxin to the flasks after germination of spores and growth of Aspergillus for 20 h, a series of flasks was examined at intervals starting at 20 h after inoculation. At each extraction period three flasks were examined.

*One author (E.H.M.) agreed to the validity of these interpretations of their data (personal communication).
spores of each isolate were inoculated into 50 ml of wort agar in each of ten 200-ml Erlenmeyer flasks. Approximately 20 h after inoculation, four flasks of each isolate were transferred to incubators (Precision Scientific Co., Chicago, Ill.) and held for 8 weeks at temperatures of 2 and of 7 ± 0.5°C; similarly, four flasks of each isolate were kept in Eclonap Gravity Circulator Humidified Incubators (American Instrument Co., Silver Spring, Md.) at 41 and 46 ± 1°C. Two flasks of each isolate (controls) were kept at ambient laboratory temperature of about 22°C, which is within a favorable range for aflatoxin production.

Since no growth occurred at 2 and 46°C, cultures were extracted by flooding the surface of the agar with 50 ml of CHCl₃, as were most of those grown at 7°C. Cultures grown at 41°C and at laboratory temperature (controls) were extracted with two 50-ml portions of CHCl₃. Only cultures of isolate M-363 grew at 7°C. These were also extracted with a total of 100 ml of CHCl₃ because both flasks contained a white mycelial growth about 12 mm in diameter.

All CHCl₃ extracts were filtered through a S&S No. 588 filter paper, concentrated, and dried over steam. The presence or absence of any aflatoxins was determined by spotting samples onto thin-layer glass plates coated to about 0.25-mm thickness with Silica Gel G-HR. Authentic aflatoxins B₁ and G₁ were spotted as standards. Except for samples containing large quantities of aflatoxins, all dried extracts were diluted to only 0.5 ml with CHCl₃ for spotting. Plates were developed with a solvent system of 7% methanol in CHCl₃ in an insulated chamber lined with blotting paper and were examined over long-wave ultraviolet light.

RESULTS AND DISCUSSION

In the experiment to ascertain what level of aflatoxin would be recovered, no aflatoxins were detected through 48 h. This demonstrated that, at 20 h after inoculation, there was no aflatoxin present in flasks subsequently grown at the various temperatures of the experiment. All three replicate flasks were positive for aflatoxins B₁ and G₁ when extracted 50 h after inoculation. The lowest detection level per 50 ml of wort agar for aflatoxin B₁ was 0.017 μg and for aflatoxin G₁, 0.05 μg.

All replications of all 25 A. flavus/parasiticus isolates grown for 8 weeks at 2, 7, 41, and 46°C failed to produce any detectable aflatoxin. All replications of the 25 isolates grown at the control temperature of 22°C produced aflatoxin, ranging from a low production of 0.19 μg/50 ml wort agar (M-219) to a high production of 930 μg/50 ml wort agar (M-363), thereby demonstrating the ability of the isolates to produce aflatoxins when grown at favorable temperatures. All isolates kept at 41°C produced convoluted mycelial mats with sparse or no spore production. Growth of the controls was normal with abundant spore production.

It would appear from these and other experiments (1-4, 6-11) that neither A. flavus nor A. parasiticus, when grown on wort agar or various commodities, will produce aflatoxins either at ≤ 7.5°C or at ≥ 40°C. From this it seems probable that aflatoxin-free commodities stored at these temperatures should remain free of these toxic substances even when moisture conditions are favorable for growth of A. flavus and A. parasiticus.

REFERENCES