Improved Yield of Aflatoxin by Incremental Increases of Temperature

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Increasing the initial temperature of the rice fermentation of Aspergillus parasiticus NRRL 2999 from 15 to 21 C after 24 h of incubation and then to 28 C after 48 h resulted in about a fourfold increase in total aflatoxin over the usual fermentation which is held constant at 28 C for 6 days. The percentage of aflatoxin B1, the most toxic component, in the total aflatoxin was also increased.

The method of Shotwell et al. (7) for the production of aflatoxin offers several advantages, particularly for experiments requiring the continual feeding of aflatoxin to animals. The rice substrate is cheap, the fermentation has no interference in the common analytical methods, the toxic kernels are easily ground to a fine though not dusty powder which mixes easily and thoroughly into a variety of diets, and the yield is good enough so that usually the amount of toxic rice powder added to the diet is too small to alter the nutrient quality. Other advantages are the ease of extraction of the aflatoxin and its purity. Nevertheless, the quantities of aflatoxin consumed during feeding experiments are so large (880 mg during a typical dose response experiment with chickens [9]) that any improvement in yield represents appreciable savings in effort and expense.

The present investigation was prompted by the observation of an unusually high yield of aflatoxin from a fermentation exposed accidentally during early incubation to subnormal temperatures as the result of an electric power shortage. A more systematic inquiry into the effect of temperature alterations during the fermentation resulted in a modification which gives about a fourfold increase in the yield of aflatoxin over that obtained with the method of Shotwell et al. (7).

Except for the variation in temperature, the rice fermentations were done by the method of Shotwell et al. (7) in the flasks described by Smith and Hamilton (8). The contents of at least 40 flasks were combined for each determination. The results reported are the means of four replicate experiments. The replicate means were subjected to an analysis of variance in which an F-ratio was calculated, and because a significant ratio was obtained, the least significant difference between treatment means was determined (1). The total aflatoxin content of the fermented rice was determined by the method of Nabney and Nesbitt (4) with the modification of Wiseman et al. (10). The flasks were incubated on platform shakers in rooms where the temperature was controlled within 0.5 C of the stated temperature. The percentages of aflatoxin B1, B2, G1, and G2 were determined colorimetrically (4) after separation on thin-layer chromatograms (7).

The effect on aflatoxin yield of varying the temperature of the fermentation during the initial 48 h of incubation is shown in Table 1. When the temperature was held at 15 C for 48 h before raising it to the normal 28 C for the remainder of the 6-day incubation period, the yield was increased highly significantly (P < 0.01) to 0.77 mg/g as compared to a yield of 0.46 mg/g when the temperature was maintained at 28 C throughout the fermentation. When the initial temperature of 15 C was increased to 21 C after 24 h and increased to the normal 28 C at the end of 48 h, the yield was increased still further (in a highly significant fashion; P < 0.001) to 1.85 mg/g. When the initial temperature of 15 C was raised to 28 C after 24 h and then to 32 C at 48 h, the yield was decreased highly significantly (P < 0.01) from the control value to 0.07 mg/g.

The percentages of aflatoxins B1, B2, G1, and G2 in the total aflatoxin are shown in Table 1 for the control fermentation in which the temperature was constant and for the fermentation conditions which gave the highest total yield. The percentages of 71, 9, 16, and 4 for B1, B2,
TABLE 1. Effect of incremental increases of temperature on aflatoxin production by Aspergillus parasiticus NRRL 2999

| Expt | Temperature (°C) | Aflatoxin yield* |
|------|-----------------|------------------|
|      | 1*  | 2   | 3  | Total (mg/g) | B1 (%) | B2 (%) | G1 (%) | G2 (%) |
| I    | 28  | 28  | 28 | 0.46        | 71     | 9      | 16     | 4      |
| II   | 15  | 15  | 28 | 0.77        | 87     | 9      | 16     | 4      |
| III  | 15  | 21  | 28 | 1.85        | 88     | 9      | 2      | 1      |
| IV   | 15  | 28  | 32 | 0.07        | 71     | 9      | 16     | 4      |

* Temperature 1 was for the first 24 h of incubation, temperature 2 was for the second 24 h, and temperature 3 was for the remaining 4 days of incubation.

Each value is the mean of four independent experiments.

G1, and G2, respectively, in the control fermentation agree closely with those obtained by Shotwell et al. (7). When the temperature was raised from an initial 15 C to 21 C after 24 h and then to 28 C after 48 h, the percentages were altered to 88, 2, 9, and 1, respectively.

The enhancement of the B:G ratios of the toxins by changing the temperature agrees with earlier observations. Schroeder and Hein (6), Diener and Davis (3), and Schindler et al. (5) found increased production of aflatoxin B in relation to aflatoxin G when the fermentation was done at constant elevated temperatures. Schroeder and Hein (6) obtained evidence that the diminution of aflatoxin G relative to B at elevated temperatures was the result of accelerated catabolism of G at higher temperatures. Our data, in which the final temperature was the same in the two experiments, suggest instead that an enhanced production of aflatoxin B occurred in our experiments.

This improved yield of aflatoxin by the use of incremental increases of temperature during fermentation has permitted a considerable savings in the time and effort needed for the production of aflatoxin B for feeding trials. An additional benefit has been the enhanced percentage of B1 aflatoxin in the total aflatoxin since aflatoxin B1 is recognized as the most toxic of the components (2).

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