Mutant of Aspergillus flavus Producing More Aflatoxin B₂ than B₁

K. E. PAPA

Department of Plant Pathology and Plant Genetics, University of Georgia, Athens, Georgia 30602

Received for publication 9 June 1976

A mutant of Aspergillus flavus having a high and relatively stable aflatoxin B₂/B₁ ratio was recovered after treatment with nitrosoguanidine.

Significant differences in aflatoxin B₁ and B₂ levels have been demonstrated among induced mutants of Aspergillus flavus (1). In spite of the variability that can be induced in aflatoxin levels, aflatoxin B₂, the dihydronic derivative of B₁, generally accumulates in smaller quantities than B₁. Schroeder and Carlton (3) reported an isolate of A. flavus obtained from black pepper which produced only B₂ but whose production declined rapidly on subculturing. The present study yielded a relatively stable mutant of A. flavus accumulating larger quantities of B₂ than B₁.

A culture of A. flavus isolated from pecan meats and producing approximately 2,000 μg of aflatoxin B₁ per g of dry mycelial weight, very little B₂, and no G toxins was provided by R. T. Hanlin (2). Auxotrophic color mutants were obtained by replica plating after exposure of conidia to ultraviolet light (1). A tan, biotin- and pyridoxin-requiring mutant not significantly different from the parental culture in aflatoxin production was subsequently treated with nitrosoguanidine in an attempt to produce aflatoxin variants. Conidia were suspended in a 0.02% solution of nitrosoguanidine for 1 h and agitated by means of a magnetic stirrer. An 1-ml portion of treated spore suspension was added to 1 liter of cooled (45°C) complete medium consisting of Czapek-Dox broth and 1.5% agar supplemented with 0.75% malt extract and 0.25% yeast extract, mixed thoroughly, and poured into petri dishes. The viability of spores after treatment ranged from 30 to 40%.

Aflatoxin determinations were made on 54 isolates grown on 2% yeast extract and 20% sucrose (YES medium). Fifty milliliters of the YES medium in a 250-ml Erlenmeyer flask was inoculated with spores from plates 7 to 10 days old, incubated for 7 days at 27°C, and stored frozen. The extraction and assay procedures were similar to those of Lillard et al. (2). Aflatoxins were identified by comparison with an aflatoxin standard obtained from the Southern Utilization Research and Development Laboratory, United States Department of Agriculture, New Orleans, La. Thin-layer chromatographic plates (EM precoated) were spotted with standard and appropriately diluted extract. Quantification of aflatoxin was by direct measurement of fluorescence using a Turner model 111 fluorimeter. The extracted mycelial mats were filtered, dried overnight at 70°C, and weighed. Aflatoxin was expressed as μg/g of dry mycelial weight.

A mutant producing higher levels of aflatoxin B₂ than B₁ was recovered. This mutant was subcultured and assayed on 21 different dates over a 10-month period. The B₁ levels ranged form 348 to 1,209 μg with a mean of 627 ± 49 (standard error) μg/g of dry mycelial weight. Levels of B₂ ranged from 2,054 to 11,106 μg with a mean of 4,818 ± 486 μg/g of dry mycelial weight. For each assay the level of aflatoxin B₂ exceeded the level of B₁, with a mean B₂/B₁ ratio equal to 7.7. With the exception of two extremely high readings, which probably reflect environmental influences or experimental error, B₁ and B₂ quantities remained relatively stable. These quantities are considerably more uniform than those obtained for other mutant and wild-type strains (1).

Additional studies of this mutant strain, including parasexual crosses with other isolates, should provide information concerning the interconversion of aflatoxins B₁ and B₂.

This investigation was supported by grant FD 00746 from the United States Food and Drug Administration. Appreciation is expressed to Becky Garza for her capable technical assistance and to L. L. Foudin for helpful suggestions.

LITERATURE CITED