Aflatoxin Production by a Variant of *Aspergillus oryzae* (NRRL Strain 1988) on Cowpeas (*Vigna sinensis*)

Abstract. *Aflatoxin B_1, B_2, G_1, and G_2 are produced when a variant of* Aspergillus oryzae *(NRRL strain 1988) is grown on cowpeas or rice. The present study indicates that a strain of* Aspergillus oryzae *approved for use in food processing is variable and the resulting variant, unlike the parent strain, has a propensity to produce aflatoxin.*

Aspergillus oryzae is widely used for preparation of koji, a starter in the production of soy sauce, and miso, a fermented soybean paste used in Japanese cooking (annual use, 900,000 metric tons) (*1*). Miso is prepared by first inoculating rice with *A. oryzae*, a combination called koji-rice, which produces an enzyme source for subsequent admixture with soybeans (*2*). The process is analogous to that in which malt is used in brewing. The subsequent soybean-koji-rice fermentation is conducted at 27°C for 50 hours. Since the widespread use of this fungus in food processing was known, the organism was thoroughly investigated for production of toxin. Hesseltine (*3*) tested 52 strains of *A. oryzae* known to be in commercial use and found no aflatoxin production. He also checked NRRL strain 1988 for aflatoxin production using wheat, corn, rice, and millet as substrates, and obtained negative results. Matssura (*4*) did an even broader study including the testing of 128 samples of miso, 28 samples of koji-rice, and 238 strains of industrial koji inoculum (*A. oryzae*), but he also found no aflatoxin production. Mislivec *et al.* (*5*) examined this fungus on a variety of substrates and found no aflatoxins. Other substrates that were used include sterile peanuts (*6*), corn, oats, rye, rice, and soybeans (*7*). From this total effort it was concluded that *A. oryzae* was not an aflatoxin producer.

However, when we inoculated cowpeas (*Vigna sinensis*) with *A. oryzae* (NRRL strain 1988) significant quantities of aflatoxin B_1, B_2, G_1, and G_2 were produced. These results are surprising because this microorganism had been tested extensively for production of aflatoxin and none had been found.

It has long been known that the substrate affects aflatoxin yields (*7*) and that there are variations in this yield even on the same substrate (*8*). Growth conditions, including time, temperature, *p*H, and substrate moisture level, also affect the production of this toxin (*9*).

The cowpeas for the diet were prepared by soaking them (2 kg) overnight at 25°C; they were dehulled by hand and then cooked for 12 minutes at 121°C. After draining, the peas were spread out for inoculation in a previously sterilized hood. The inoculum was prepared from cultures of *A. oryzae* (NRRL strain 1988) grown on potato-dextrose agar slants and incubated for 7 days at 25°C. Spores were harvested by adding 0.8 ml of sterile distilled water to each slant, followed by dislodgment with a sterile needle. The spore crop from two agar slants was sufficient for 100 g of cowpeas. Inoculated, partially dried cowpeas were packed tightly into petri dishes and incubated for 42 hours at 33°C and 50 percent relative humidity. Fermented cowpeas were then dried in an air oven for 12 hours at 50°C and ground in a Wiley mill through a 20-mesh screen.

We fed male weanling Sprague-Dawley rats (ARS—Sprague-Dawley, Madison, Wis.) diets containing 23 percent fermented cowpeas that had been dried after fermentation. (We used 23 percent cowpeas in order to provide a diet containing 10 percent protein.) The remainder of the diet was prepared according to the method of the Association of Official Agricultural Chemists (AOAC) (*10*) and was composed of cornstarch, salt mixture, vitamin mixture, cottonseed oil, and nonnutritive cellulosic fiber.

Diets were fed to rats in groups of six. All six animals on the fermented cowpea diet died within 7 days. The other three groups grew well, including a control fed on casein and others fed on unfermented cooked peas and peas inoculated with *Rhizopus oligosporus*. Growth curves for the test animals are shown in Fig. 1.

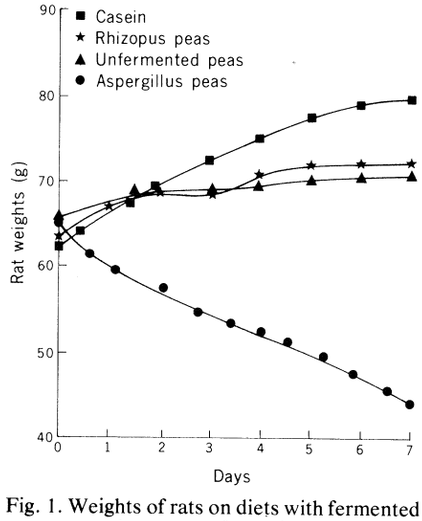

Fig. 1. Weights of rats on diets with fermented cowpeas as the source of protein.

Subsequent analysis of the cowpea powder by the AOAC method (*10*), in which thin-layer chromatography (TLC) is used, showed the presence of B_1, B_2, G_1, and G_2 aflatoxins. Confirmation of this was obtained by further analysis of the substance from the B_1 spot on the TLC plate according to the AOAC suggested method (*10*), employing the acetate derivative.

The culture of *A. oryzae* originally obtained from the Northern Regional Research Laboratory (their strain NRRL 1988) produced substantial levels of aflatoxin on cowpeas (Table 1), although a second culture from this laboratory (now the Northern Utilization Research and Development Division) showed no detectable aflatoxin production. A third culture of NRRL strain 1988, obtained from the American Type Culture Collection (ATCC 9362), also produced aflatoxin on cowpeas but at lower levels than the original NRRL strain 1988 (Table 1). The inconsistent behavior of different isolates of *A. orzyae* NRRL strain 1988 indicates the existence of subtle differences in or variants of this organism. Independent examinations of the original NRRL strain 1988 were performed by the Department of Plant Pathology, Rutgers University, and the Department of Food Science, University of Georgia. Independent experiments at the University of Georgia showed that *A. oryzae* from our source (NRRL) produced aflatoxin (*11*). These

Table 1. Aflatoxin production by a strain of *Aspergillus oryzae* on cowpeas. *Aspergillus oryzae*, NRRL strain 1988, was obtained from the Northern Utilization Research and Development Division 4 April 1974. This strain produced aflatoxin on rice (expressed as grams per kilogram: B_1, 930; B_2, 89; G_1, 647; and G_2, 143). The culture grew with difficulty on soybeans. However, a second culture obtained from the same laboratory 14 June 1974 did not produce aflatoxin on cowpeas or rice. Data are the average of five analyses. We also obtained *A. oryzae*, NRRL strain 1988, from the American Type Culture Collection (ATCC 9362) in June 1974. These data are also the average value of five analyses.

Type of aflatoxin	Aflatoxin (g/kg) from *A. oryzae*	
	Strain 1988 from NRRL	Strain 1988 from ATCC (ATCC 9362)
B_1	9400 ± 200	150 ± 40
B_2	1500 ± 200	50 ± 10
G_1	6600 ± 350	125 ± 10
G_2	1400 ± 400	100 ± 30

investigators also observed aflatoxin production by an isolate of strain 1988 obtained from other sources but were unable to repeat the finding. The culture possessed the gross colony appearance, the morphology and size of the conidial structures, and the cultural response on Czapek's medium characteristic of *A. oryzae*. However, 11-day-old cultures of this microorganism exhibited a deep green color and spore dimensions and patterns that are not characteristic of *A. oryzae*.

In summary, it has been shown that aflatoxin accumulation results from the growth of a variant strain of *A. oryzae* (NRRL strain 1988) on cowpeas. Aflatoxin was also produced by this organism growing on rice, but not on soybean. These findings lead us to conclude that this strain is a variable one. While NRRL 1988 does not produce aflatoxin during fermentation of soy sauce, it appears to have the capability of toxin production on other substrates. The use of this strain of *Aspergillus oryzae* for food fermentation and production of enzymes as food processing aids should be reexamined in light of these findings.

NABIL EL-HAG
ROY E. MORSE

Department of Food Science, Cook College, Rutgers—The State University of New Jersey, New Brunswick 08903

References and Notes

1. W. Hesseltine, *Mycologia* **57**, 149 (1965).
2. B. Lockwood, *Soybean Dig.* **7**, 19 (1947).
3. W. Hesseltine, L. Shotwell, J. Ellis, D. Stubblefield, *Bacteriol. Rev.* **30**, 795 (1966).
4. S. Matssura, M. Manabe, T. Sato, in *Proceedings of the First U.S.-Japan Conference on Natural Resources* (U.S.-Japan Cooperative Program on Natural Resources and Department of the Interior, 1968), p. 48.
5. B. Mislivec, H. Hunter, J. Tuite, *Appl. Microbiol.* **16**, 1053 (1968).
6. H. Delongh, R. O. Vles, R. DeVogel, *Mycotoxins in Foodstuffs*, G. Wogan, Ed. (MIT Press, Cambridge, Mass., 1965), p. 235.
7. H. Armbrecht, A. Hodges, R. Smith, A. Nelson, *J. Assoc. Off. Agric. Chem.* **46**, 805 (1963).
8. C. Codner, K. Sargeant, R. Yeo, *Biotechnol. Bioeng.* **5**, 185 (1963).
9. L. A. Goldblatt, *Aflatoxin* (Academic Press, New York, 1969), pp. 13–44.
10. Association of Official Agricultural Chemists, *Official Methods of Analysis* (Association of Official Agricultural Chemists, Washington, D.C., ed. 11, 1970), p. 246.
11. T. Hamsa, personal communication.
12. We thank N. F. Haard, Rutgers, for his invaluable cooperation and support; S. Hagan, Agricultural Research Service, U.S. Department of Agriculture, for her assistance with aflatoxin assays; W. Hesseltine of Northern Utilization Research and Development Division, U.S. Department of Agriculture, Peoria, Ill., for his cooperation, interest, and for supplying cultures of NRRL strain 1988; J. Peterson, Rutgers, for his identification of the variant cultures of *Aspergillus oryzae*; and to J. Ayres and T. Hamsa, University of Georgia, for their identification of the microorganism. This is a paper of the Journal Series, New Jersey Agricultural Experiment Station, Cook College, Rutgers—The State University of New Jersey, New Brunswick.

21 January 1976; revised 8 March 1976

Trypanosomatid Flagellate in the Phloem of Diseased Coconut Palms

Abstract. *Ultrastructural observations of the phloem of coconut palms affected by "hartrot" disease in Suriname have revealed the presence of the plant-infecting flagellate* Phytomonas *in mature sieve tubes. The occurrence of these flagellates during the earliest symptoms of the disease and the correlated increase and spread of the flagellates in the phloem as the disease progresses suggest that the organisms may be pathogenic to the palms.*

Although the existence of the plant-infecting trypanosomatid flagellate *Phytomonas* (*1*) has been known for nearly 70 years, relatively little is known about the flagellate's relationship to its hosts. The flagellate has been found chiefly in laticiferous plants (*2*), and in those plants it is apparently confined to the latex-bearing cells—the laticifers (*3*). Most investigators suggest that the flagellate is nonpathogenic to its laticiferous plant hosts (*2*, *4*). However, a few reports from Europe and elsewhere do suggest that the flagellate may be pathogenic to some of its latex-bearing hosts (*5*). The lygaeid hemipteran *Oncopeltus* is known to be