

Production of Cyclopiazonic Acid by *Aspergillus tamarii* Kita

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Production of the mycotoxin cyclopiazonic acid by *Aspergillus tamarii* Kita is reported for the first time. Examination of 23 isolates of the fungus showed that 22 produced the toxin under the culture conditions utilized.

Contamination of foods and feed by mycotoxins is a significant problem that can lead to severe economic losses. Cyclopiazonic acid (CPA) is an indole-tetramic acid mycotoxin that has been isolated from several species of fungi and has been reported as a natural contaminant of corn (4) and peanuts (8). Previously, the toxin has been reported to be produced by *Penicillium cyclopium* Westling, *P. patulum*, *P. viridicatum*, *P. puberulum*, *P. crustosum*, *P. camemberti*, *Aspergillus flavus*, *A. versicolor*, and *A. oryzae* (6, 9-11, 13, 17, 19, 21, 22). The 50% lethal dose of CPA in rats was 36 mg/kg in males and 63 mg/kg in females when dosed orally. Intraperitoneal injection in males produced a 50% lethal dose of 2.3 mg/kg (20). Recent feeding studies with broiler chickens showed that purified CPA incorporated into the ration at 100 ppm produced high mortality and decreased weight gain (2).

Aspergillus tamarii Kita is classified in the *A. flavus* group of fungi and often has been isolated from substrates that yielded isolates of *A. flavus* Link, *A. parasiticus* Speare, and related fungi. *A. tamarii* is one of the organisms involved in a disease of cocoa (*Theobroma cacao*) that results in internal moldiness and gives finished products such as chocolate an unpleasant flavor and odor (18). The fungus has been associated with a dry rot disease of cashews (3) and with contamination of pistachios during the latter stages of nut development (15). Manabe and Tsurata (14) found that a high percentage of grain samples from Southeast Asia were contaminated by *A. tamarii*, and Moreno-Martinez and Christensen (16) showed that the fungus was one of the major contaminants of black and white pepper. Finally, Leon-Cazares et al. (12) showed that compounds isolated from a strain of *A. tamarii* (NRRL 429) produced an almost 100% inhibition of the mitotic process of peripheral blood lymphocytes.

The purpose of this communication is to re-

port the production of CPA by several isolates of *A. tamarii*.

During routine screening of fungi for toxigenicity, several isolates of *A. tamarii* were found to be toxigenic by the method of Kirksey and Cole (7). The isolates were provided by Robert Hill and had been isolated from peanuts grown in experimental plots at the National Peanut Research Laboratory (5). Seventeen isolates were cultured on Difco mycological broth plus 15% sucrose and 2% yeast extract (50 ml/500-ml Erlenmeyer flask) for 10 days at 25 to 28°C. Cultures were extracted by homogenization with chloroform, dried over anhydrous sodium sulfate, and analyzed by thin-layer chromatography (TLC) for the presence of CPA. These analyses were performed on precoated silica gel 60 F-254 plates (5 by 10 cm; EM Laboratories, Inc., Elmsford, N.Y.) and on identical plates that were pretreated by dipping in 2% aqueous oxalic acid and drying for 1 h at 100°C. Internal and external standards of CPA were included on each plate, and plates were developed in a solvent system containing toluene-ethyl acetate-formic acid (5:4:1, vol/vol/vol). Developed plates were sprayed first with 1% *p*-dimethylaminobenzaldehyde in ethanol and then with 50% ethanolic sulfuric acid.

Six additional isolates of *A. tamarii* (NRRL 427, 428, 429, 436, 1654, and 4960) supplied by D. T. Wicklow from the Agricultural Research Service Culture Collection, Peoria, Ill., were also tested for CPA production. One isolate, NRRL 427, was cultured on Difco mycological broth plus 15% sucrose and 2% yeast extract in 40 Fernbach flasks (200 ml of media per 2,800-ml flask) for confirmation of CPA production by TLC and spectroscopic analyses of the purified toxin. Cultures were extracted by homogenization with chloroform, and CPA was purified in the manner previously described (4).

The metabolite purified from the mass culture of *A. tamarii* NRRL 427 was concluded to be

CPA based on TLC and spectroscopic characteristics. Crystals from methanol had a melting point of 243 to 245°C and a TLC behavior identical to that of authentic CPA, i.e., an R_f of 0.68 on oxalic acid-impregnated plates and a blue color reaction after spraying. On plates not impregnated with oxalic acid, the spot tailed extensively but produced the same blue color reaction after spraying. This behavior on TLC has been used previously as a confirmatory test for the presence of CPA (4). The UV spectrum of the purified metabolite showed $\lambda_{\max}^{\text{MeOH}}$ 225, 253, 275(sh), 284, and 292(sh) nm, and low-resolution mass spectral analysis showed a molecular ion at nominal mass m/e 336. These data established that the material purified from *A. tamarii* cultures was CPA.

Of the 23 *A. tamarii* isolates examined for CPA production, 22 produced the toxin under the culture conditions utilized. Only strain NRRL 4960 was a nonproducer, but this isolate had unusual growth characteristics and appeared to be an atypical member of the species. The 22 toxigenic strains produced dense mycelial mats with abundant aerial sporulation of the typical dark greenish-brown coloring, whereas strain NRRL 4960 grew completely submerged with no aerial sporulation.

This survey shows that a significant percentage (96%) of *A. tamarii* isolates produce CPA and sheds additional light on the biochemical relationship of members of the *A. flavus* group. In other studies, Gallagher et al. (4) reported that 28 of 54 (52%) *A. flavus* isolates produced CPA, whereas it has been shown that none of 47 *A. parasiticus* isolates produced CPA (J. W. Dorner, R. J. Cole, and U. L. Diener, unpublished data). It is interesting, therefore, that nearly all isolates of *A. tamarii* produced CPA and none produced aflatoxin. About half of the *A. flavus* isolates produced CPA (4), and from various studies nearly half of the isolates produced aflatoxins, but usually only B₁ and B₂. No isolate of *A. parasiticus* was shown to produce CPA, but almost all produced aflatoxins B₁ and G₁ (J. W. Dorner, R. J. Cole, and U. L. Diener, unpublished data). Therefore, in these three species, there appears a biosynthetic relationship ranging from CPA production only (*A. tamarii*) to aflatoxin production only (*A. parasiticus*), with an intermediate stage that can produce both, neither, or only one of the two toxin types (*A. flavus*).

Since *A. tamarii* is a contaminant of various commodities, the finding that 22 of 23 strains produce CPA certainly has a potential significance with regard to the safety of those commodities. In fact, as CPA is being found as a metabolite of increasing numbers of fungal species, its potential as a contaminant of foods and

feeds becomes greater. Although CPA is not as acutely toxic as aflatoxin B₁ (50% lethal dose in 90-g male rats, 36 mg/kg per os for CPA [20] compared to 7.2 mg/kg per os in 100-g male rats for aflatoxin B₁ [1]), further evaluation of its importance as a feed contaminant is needed.

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