Toxigenic Fungi in Food

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Forty-five fungal isolates from moldy supermarket foods were tested for toxicity to brine shrimp, and twenty-two of these isolates were subsequently tested for toxicity to chicken embryos. Highly toxigenic fungi were Cladosporium sphaerospermum from a bakery product, Fusarium oxysporum from carrots, F. solani from cabbage, Aspergillus niger and Penicillium corylophilum from bread, P. cyclopium and P. herquei from corn meal, P. lanosum from onions, P. steckii from chocolate syrup, Penicillium sp. from jelly, and Rhizopus nigricans isolates from sweet potato, applesauce, and strawberries. Approximately one-third of the fungal cultures were moderately to highly toxigenic to brine shrimp and chicken embryos, while several additional cultures were slightly toxigenic.

Mycotoxicoses have been well documented in animals, but there is an increasing need for research in the area of human health (7). Some of the implications of mycotoxins to human health have been recently reviewed (1, 4). The extent of mycotoxin hazard to man can be better assessed, if the identity of the fungi involved is known. This report presents results of a portion of a screening program in which fungi were routinely isolated from a variety of human foods, identified, and tested for toxicity to brine shrimp in primary bioassays and chicken embryos in secondary bioassays.

Forty-five fungi were isolated from visibly moldy foods purchased or obtained with the cooperation of personnel in a local supermarket and obtained from home refrigerators during 1973–74. Isolation was by direct plating on YE agar (2% dextrose, 0.7% yeast extract [Difco], 0.5% KH$_2$PO$_4$, and 2% agar), from which the fungi were monocultured at room temperature (25 to 30°C) on either Czapek-Dox or potato-dextrose agar for identification as described by Morgan-Jones and Booth (Toxin-Producing Fungi, in preparation). The fungi were then grown in 1-liter Erlenmeyer flasks on nutrient-amended shredded wheat (3, 6), which had been autoclaved 15 min at 121°C twice in 24 h. The cultures were incubated 14 to 21 days at 25°C.

Moldy substrates were extracted with chloroform-ethanol (80:20), filtered, and evaporated under an airstream at room temperature. Extracts for the brine shrimp bioassay were taken up in 95% ethanol, while extracts for chicken embryo bioassays were suspended in corn oil by methods previously reported (3). Controls consisting of extracts of un inoculated nutrient-amended shredded wheat were included in all bioassays.

Results of the brine shrimp primary bioassay revealed that nearly 44% of all isolates were toxic to brine shrimp (Table 1). Of seven Aspergillus isolates only A. niger from onion and bread was toxic. Three of six Fusarium isolates, two of four Mucor cultures, six of nine Rhizopus isolates, and seven of 17 Penicillium isolates were toxic. Nontoxic isolates and their sources were: Aspergillus flavus — diet bread, A. chevalieri — cake, A. repens — coconut cake, A. fumigatus — sweet roll, A. niger — strawberry, Cladosporium sphaerospermum — honeybun, Fusarium oxysporum — onion, F. solani — cabbage, Fusarum sp. — okra, Geotrichum candidum — squash, Mucor globosum — eggplant, M. hiemalis — sweet potato, Penicillium frequentans — tomato, P. lanosum — cucumber, cheese, green pepper, okra, squash, and potato, P. notatum — peach, P. waksmani — tomato, Penicillium sp. — water chestnut, Rhizopus arrhizus — peach, and R. nigricans — sweet potato and squash. Nineteen isolates toxigenic and three isolates nontoxigenic to brine shrimp were subsequently bioassayed with chicken embryos (Table 1). Fourteen of these 22 isolates (64%) were highly toxic to chicken embryos, causing 60 to 100% mortality. These were: A. niger (#769), C. sphaerospermum (#533), F. oxysporum (#597), F. solani (#625), P. corylophilum (#765), P. cyclopium (#535), P. herquei (#630), P. lanosum (#650), P. nigricans (#748), P. steckii (#551), Penicillium sp. (#707), R. nigricans (#620), R. nigricans

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### Table 1. Toxicity of fungal extracts to brine shrimp and chicken embryos

| AUA culture number | Fungus                  | Food source       | Brine shrimp mortality* | Chicken embryos deaths/ total eggs |
|--------------------|-------------------------|-------------------|-------------------------|-----------------------------------|
| Check              | Uninoculated medium     | onion             | 0                       | 1/20                              |
| 647                | Aspergillus niger       | bread             | 2                       | 10/20                             |
| 769                | A. niger                | honey bun         | 0                       | 14/20                             |
| 533                | Cladosporium sphaeropspernum | orange         | 2                       | 5/10                              |
| 553                | Fusarium lateritium     | carrot            | 1                       | 10/10                             |
| 597                | F. oxysporum            | cabbage           | 0                       | 10/10                             |
| 625                | F. solani               | squash            | 2                       | 5/10                              |
| 665                | Fusarium sp.            | squash            | 1                       | 10/20                             |
| 664                | Mucor fragilis          | bread             | 2                       | 10/10                             |
| 765                | Penicillium corylophilum| corn meal         | 2                       | 10/10                             |
| 555                | P. cyclopium            | corn meal         | 1                       | 10/10                             |
| 630                | P. herquei              | onion             | 2                       | 6/10                              |
| 650                | P. lanosum              | peach             | 2                       | 10/10                             |
| 748                | P. nigricans            | chocolate syrup  | 2                       | 13/20                             |
| 551                | P. steckii              | diet jelly        | 2                       | 8/10                              |
| 707                | Penicillium sp.         | tomato            | 1                       | 5/10                              |
| 644                | Rhizopus nigricans      | onion             | 2                       | 4/10                              |
| 648                | R. nigricans            | peach             | 2                       | 3/10                              |
| 598                | R. nigricans            | sweet potato      | 0                       | 10/10                             |
| 620                | R. nigricans            | applesauce        | 2                       | 7/10                              |
| 675                | R. nigricans            | corn meal         | 2                       | 4/10                              |
| 606                | R. nigricans            | strawberry        | 2                       | 10/10                             |
| 618                | R. nigricans            |                   |                         |                                   |

*2 = 60 to 100% mortality; 1 = 20 to 59%; 0 = 0 to 19%.

(#675), and *R. nigricans* (#618). These 14 highly toxigenic isolates represent 31% of all the fungi isolated from foods. Five other isolates, *A. niger* (#647), *F. lateritium* (#553), *Fusarium sp.* (#665), *M. fragilis* (#664), and *R. nigricans* (#644) exhibited low to moderate toxicity. Thus, approximately 42% of the fungi initially isolated from foods were at least moderately toxic to brine shrimp and/or chicken embryos.

Initially screening was with brine shrimp, which are regarded as sensitive test organisms for mycotoxins (5, 9). However, Curtis et al. (2) have shown that some fungi elaborate naturally occurring fatty acids that are toxic to brine shrimp. Thus, toxicity towards brine shrimp should be confirmed with at least one additional test organism. In this research, fungi that were toxic to brine shrimp were subsequently tested with chicken embryos (11 except in the case of *M. globosus* (#595), which died in culture between bioassays. However, certain isolates not toxic to brine shrimp were also tested with chicken embryos, namely, *C. sphaerospernum* and *F. solani*. *R. nigricans* (#620) was included in the second bioassay because previous tests had indicated that it was toxigenic.

Extracts from approximately one-third of the fungi used in this study were moderately to highly toxic to brine shrimp and chicken embryos. Saito et al. (10) found that 22.5% of 247 fungal isolates from foods in Japan were moderately to highly toxic to HeLa cells and 30% to mice. Approximately 36% of 531 fungal strains from foods in the diet of rural Bantu in Eastern Transvaal and Swaziland (8) were moderately or very highly toxic to ducklings. Our isolates were from conspicuously molded foods from a local supermarket, and from the refrigerators and kitchens of consumers. One wonders what happens to such foodstuff in localities where extreme hunger exists and concern is on quantity rather than quality. A potential hazard could be compounded by the fact that the undernourished are generally more susceptible to toxicants than are individuals with more adequate diets. This investigation did not determine whether the foodstuffs actually contained known mycotoxins or were toxic per se. It was only determined that 42% of the moldy foodstuffs investigated were invaded by strains of fungi capable of elaborating toxic substances. Additional work appears warranted to determine the toxic compounds and whether the toxigenic isolates elaborate mycotoxins on the foods from which they were isolated.

Several of the toxigenic isolates are not generally recognized mycotoxin producers. These are *C. sphaerospernum*, *F. lateritium*, *P. corylophilum*,
philum, P. herquei, P. lanosum, P. steckii, and R. nigricans. Fungi from meat were not included in this investigation, since moldy meat was not observed in the supermarket. Wu et al. (12) in their investigation of fungi on meat found that toxigenic species of Aspergillus were more prevalent than toxigenic species of Penicillium. Penicillium was the most frequently isolated group of fungi in our study, with 41% of 17 isolates being toxigenic to brine shrimp and chicken embryos. Thus, our data are similar to that of Saito et al. (10) in that the most prevalent toxigenic fungi in foods were Penicillium species. The apparent predominance of Penicillium species may reflect the influence of refrigeration; it has been our observation that Penicillium is very competitive at low temperature storage of high moisture foods.

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