APPLIED MICROBIOLOGY, Dec. 1974, p. 1094-1096
Vol. 28, No. 6

Toxigenic Aspergilli and Penicillia Isolated from Aged, Cured Meats

M. T. WU,[1] J. C. AYRES, AND P. E. KOEHLER
Department of Food Science, University of Georgia, Athens, Georgia 30602

Received for publication 30 July 1974

Eighty-nine cultures of *Aspergillus* and 54 cultures of *Penicillium* isolated from aged, cured meats were tested for toxicity to chicken embryos. Two of 22 isolates of *A. ruber*, 5 of 28 *A. repens*, 2 of 12 *A. sydowi*, 1 of 12 *A. restrictus*, 2 of 7 *A. amstelodami*, 1 of 2 *A. chevalieri*, and an *A. fumigatus* isolate exhibited toxicity. Similarly, 2 of 15 isolates of *P. expansum*, 1 of 3 *P. notatum*, 1 of 2 *P. brevi-compactum*, and 1 of 8 *Penicillium* spp. were found to be the most toxic. Among these fungi, the chloroform extract from the growth of an *A. sydowi* isolate showed the greatest toxicity. There was no direct or indirect evidence that aged, cured meats contain toxic metabolites.

More than 800 mold cultures were isolated and identified from country cured hams and fermented sausages (3, 9). Aflatoxin-, ochratoxin-, sterigmatocystin-, and citrinin-producing strains were isolated and identified from country cured hams (1, 2, 8, 9, 12). Although these workers identified aspergilli and penicillia, their interest was largely confined to strains that produced specific mycotoxins such as aflatoxins, ochratoxins, sterigmatocystins, and citrinin. No examination was made for the gross toxicity production of other mycotoxins. Many of the same mold species commonly found on aged, cured meats were also isolated from cereal and legume products (6). In Scott's study (6), 46 of 228 mold strains examined caused death of ducklings within 14 days. These strains included 27 isolates of *Aspergillus* and 12 isolates of *Penicillium* that caused acute toxicosis in ducklings.

Working with stored lots of corn, Richard et al. (5) isolated and identified 42 strains of *Aspergillus* and 19 of *Penicillium* that caused the death of mice and ducklings. They also found that water or ether extracts of all moldy corn samples exhibited some degree of toxicity. Later, Semeniuk et al. (7) showed that 166 strains of *Aspergillus*, including 73 species and 9 varieties, were toxigenic.

The present investigation was undertaken to determine the toxicity of various aspergilli and penicillia to chicken embryos.

Eighty-nine cultures of *Aspergillus* spp. and 54 cultures of *Penicillium* spp. previously (1 to 4 years ago) isolated in this laboratory from aged, cured meats were screened for toxicity. Cultures were maintained by periodical transferring to new media. They still retained original characteristics. These cultures were incubated on slants of Czapek-Dox agar for 2 weeks at 27 C and then stored at 4 C. Then 10^5 spores from each *Aspergillus* culture were transferred to 50 ml of yeast extract (2%) sucrose (20%) medium in 250-ml Erlenmeyer flasks and incubated at 27 C for 3 weeks; 10^5 spores of each culture of *Penicillium* were added to 50 ml of potato dextrose broth (Difco) in 250-ml Erlenmeyer flasks and incubated at 27 C for 4 weeks. Six flasks were used for each culture to provide sufficient material for extraction.

After incubation, the contents of the flasks were sterilized by autoclaving at 121 C for 15 min, and 10 ml of culture filtrate was removed from each. The remaining material from the six flasks was transferred to a 1-quart (ca. 0.95 liter) Mason jar and extracted with chloroform with the aid of a high-speed mixer. The extraction procedure was repeated three times, using 100 ml of chloroform for each extraction. The chloroform extracts were combined, concentrated in a flash evaporator, and diluted to 5 ml with chloroform.

Chicken egg air sac inoculations (10) were used to assay the toxicity of culture filtrates and chloroform extracts. Groups of 10 fertile White Leghorn eggs were inoculated with 0.02 ml of the test materials before incubation. The groups of eggs that failed to hatch (0% hatchability) were assayed further by using duplicate tests of 20 eggs each. Various dilutions of the test material were made with either water or chloroform to determine the level of the tox-

[1] Present address: Nutrition and Food Science Department, Utah State University, Logan, Utah 84322.

icity. Control groups of eggs were injected with chloroform, sterilized uninoculated yeast extract sucrose medium, or potato dextrose broth.

All *A. ruber* isolates were toxic to chicken embryos, especially those cultures extracted with chloroform; more than 50% hatchability was observed for only 2 of the 22 isolates. Sterilized, uninoculated yeast extract sucrose medium, potato dextrose broth, and chloroform were not toxic to chicken embryos. There was 0% hatchability in broth culture filtrate and the chloroform extract (0.02 ml) from 2 of the 22 isolates of *A. ruber* (Table 1). Tenfold dilutions (0.002 ml) of the concentrated chloroform extracts of strains M120 and M161 exhibited 0% hatchability. Further dilution decreased their toxicity to the same degree (i.e., 60% hatchability).

Among 28 isolates of *A. repens*, five chloroform extracts (.02 ml) exhibited 0% hatchability. Half of the isolates showed 10 to 50% toxicity while nine showed 0 to 40% toxicity. One-tenth dilutions (0.002 ml) of the chloroform extracts showed little toxicity. None of the culture filtrates produced 0% hatchability of chicken embryos.

After inoculation of fertile eggs with culture filtrates of chloroform extracts of isolates of *A. sydowi*, 11 of 12 culture filtrates, but only 4 of 12 chloroform extracts, demonstrated greater than 50% hatchability. Chloroform extracts of two isolates of *A. sydowi* (M12 and XVII/17) produced 0% hatchability. Isolate M12 was more toxic than XVII/17. There was 0% hatchability of chicken embryos with a 1,000-fold dilution (0.00002 ml) of chloroform extract from M12. Insofar as the amount of chloroform extract required to cause 0% hatchability is concerned, *A. sydowi* is the most toxic fungal strain found in this study. Zero percent hatchability resulted when chloroform extracts of two isolates of *A. amstelodami*, M300 and M306, were used; the other five isolates demonstrated little toxicity. In an earlier study, Rabie et al. (4) reported a strain of *A. amstelodami* that was highly toxic to rabbits, poultry, and ducklings.

Chloroform extracts of only 1 of 12 isolates of *A. restrictus*, *A. restrictus* XIII/23, exhibited 0% hatchability. A 10-fold dilution (0.002 ml) of this isolate still exhibited 0% hatchability. Only 4 of the 12 *A. restrictus* isolates had more than 50% hatchability.

Both the culture filtrates and chloroform extracts of a single *A. fumigatus* strain, M48,

TABLE 1. *Toxicity of culture filtrates and chloroform extracts of aspergilli and penicillia to chicken embryos as determined by egg hatchability*

Species	Strain	Hatchability[a] (%)							
		Culture filtrate		Chloroform extract					
		Undiluted (0.02 ml)[b]	1:10 diluted (0.002 ml)	Concentrate (0.02 ml)[b]	1:10 (0.002 ml)	1:20 (0.001 ml)	1:100 (0.0002 ml)	1:1,000 (0.00002 ml)	1:2,000 (0.00001 ml)
A. ruber	M120	0	75	0	0	60			
	M161	0		0	0	60			
A. repens	M121	60		0	90				
	M160	30		0	60				
	VIII/35	100		0	50				
	VIII/39	50		0	95				
	VIII/42	100		0	80				
A. sydowi	M12	100		0	0	0	0	0	75
	XVII/17	0	40	0	0	15			
A. amstelodami	M300	100		0	85				
	M306	90		0	70				
A. restrictus	XIII/23	100		0	0	35			
A. fumigatus	M48	0	30	0	0	45			
A. chevalieri	M236	0	65	0	90				
P. expansum	M170	90		0	0	65			
	M208	100		0	0	80			
P. notatum	M17	60		0	55				
P. brevi-compactum	M280	90		0	25				
Penicillium species	M177	50		0	0	80			

[a] 20 eggs per trial; percent hatchability of control eggs, eggs inoculated with sterilized yeast extract sucrose medium, potato dextrose broth, and chloroform were 100, 90, 100, and 100, respectively.

[b] 0.02 ml of concentrate or of diluted material was inoculated for all tests. The milliliter amounts shown represent equivalents of the original concentrate.

and one of two *A. chevalieri*, i.e., M236, exhibited toxicity to chicken embryos. According to Wilkinson and Spilsbury (11), *A. chevalieri* produced gliotoxin, an antibiotic of considerable toxicity. Culture filtrates and chloroform extracts from single isolates of *A. mangini* (M105), *A. aurantiobruneus* (IX/2), and *A. pseudoglaucus* (VIII/44) and from two unidentified *Aspergillus* spp. isolates (M23 and M136) demonstrated little toxicity.

None of the *Penicillium* cuture filtrates tested exhibited 0% hatachability. However, chloroform extracts of *P. expansum* M170 and M208, *P. notatum* M17, *P. brevi-compactum*, M280, and *Penicillium* spp. M177 failed to hatch. Half or more of the chicken embryos inoculated with chloroform extracts of 9 of 15 isolates *P. expansum*, 2 of 3 *P. notatum*, 1 of 2 *P. brevi-compactum*, 1 of *P. cyclopium*, 7 of 7 *P. viridicatum*, 2 of 3 *P. lanoso-coeruleum*, 2 of 4 *P. simplicissimum*, 2 of 3 *P. micznyskii*, 1 of 1 *P. parvum*, 2 of 2 *P. urticae*, and 1 of 2 *P. roqueforti* hatched. One must conclude that insofar as these particular representatives of aspergilli and penicillia are concerned, the aspergilli demonstrate greater toxicity to chicken embryos.

Although it is apparent that most of the fungi tested have some degree of toxicity, no direct evidence is presented that cured meats contain toxic metabolites produced by these fungi. Further research on the identification of the unknown toxic metabolites is essential before one could conclude that these aspergilli and penicillia produce harmful metabolites that might constitute a potential hazard to public health.

This investigation was supported by grant FD-00155-07 from the Office of Research Grants, Food and Drug Administration.

LITERATURE CITED

1. Escher, F. E., P. E. Koehler, and J. C. Ayres. 1973. Production of ochratoxin A and B on country cured ham. Appl. Microbiol. **26**:27-30.
2. Halls, N. A., and J. C. Ayres. 1973. Potential production of sterigmatocystin on country cured ham. Appl. Microbiol. **26**:636-637.
3. Leistner, L., and J. C. Ayres. 1968. Molds and meats. Fleischwirtschaft **48**:62-65.
4. Rabie, C. J., W. A. Deklerk, and M. Terblanche. 1964. Toxicity of *Aspergillus amstelodami* to poultry and rabbits. S. Afr. J. Agr. Sci. **7**:341-346.
5. Richard, J. L., L. H. Tiffany, and A. C. Pier. 1969. Toxigenic fungi associated with stored corn. Mycopathol. Mycol. Appl. **38**:313-326.
6. Scott, D. B. 1965. Toxigenic fungi isolated from cereal and legume products. Mycopathol. Mycol. Appl. **25**:213-222.
7. Semeniuk, G., G. S. Harshfield, C. W. Carlson, C. W. Hesseltine, and W. F. Kwolek. 1971. Mycotoxins in *Aspergillus*. Mycopathol. Mycol. Appl. **43**:137-152.
8. Strzelecki, E., H. S. Lillard, and J. C. Ayres. 1969. Country cured ham as a possible source of aflatoxin. Appl. Microbiol. **18**:928-939.
9. Sutic, M., J. C. Ayres, and P. E. Koehler. 1972. Identification and aflatoxin production of molds isolated from country cured hams. Appl. Microbiol. **23**:656-658.
10. Verrett, M. J., J. P. Marliac, and J. McLaughlin, Jr. 1964. Use of the chick embryo in the assay of aflatoxin toxicity. J. Ass. Off. Agr. Chem. **47**:1003-1006.
11. Wilkinson, S., and J. F. Spilsbury. 1965. Gliotoxin from *Aspergillus chevalieri*. Nature (London) **206**:619.
12. Wu, M. T., J. C. Ayres, and P. E. Koehler. 1974. Production of citrinin by *Penicillium viridicatum* on country cured ham. Appl. Microbiol. **27**:427-428.