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Production of Citrinin by *Penicillium viridicatum* on Country-Cured Ham

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Seven strains of *Penicillium viridicatum* isolated from country-cured ham produced citrinin in potato dextrose broth and on country-cured ham. None of the strains produced detectable amounts of citrinin at 10 C. The optimal temperature range for citrinin production was 25 to 30 C.

Citrinin, an antibiotic and nephrotoxin, is produced by many species of penicillia and aspergilli (2, 6, 9, 10). It was first recognized as a powerful antibiotic (7, 14), but later was found to damage the kidneys of test animals, retard growth, and eventually cause death (1, 3, 4). Scott et al. detected citrinin in moldy wheat, oat, rye, and barley in Canada (13). Therefore, its presence on food and feed could constitute a hazard to humans and animals.

Country-cured hams are very popular in the southeastern United States (5). The surface of these hams is often covered by the growth of different species of molds including *Penicillium viridicatum* (8). In recent years, citrinin production by *P. viridicatum* has been substantiated (3, 4, 12, 13). This investigation was undertaken to determine if such strains of *P. viridicatum* produce citrinin on country-cured ham. The effect of various temperatures on citrinin production by these strains was also determined.

Seven strains of *P. viridicatum*, M-110, M-115, M-133, M-165, M-206, M-218, and M-240, previously isolated from country-cured hams by Leistner and Ayres (8) and received as soil cultures, were screened for production of citrinin on potato dextrose broth and on ham slices. Inocula containing 10^6 spores of each strain of *P. viridicatum* were added to 100 ml of potato dextrose broth (Difco) in 500-ml Erlenmeyer flasks and were incubated at 10, 15, 25, and 30 C for 7 to 21 days. At the end of 7, 14, and 21 days of incubation, the contents were transferred to 0.946-liter Mason jars and extracted with chloroform. Boneless slices of country-cured ham (0.5 to 1.0 cm thick and weighing 30 g) were cut from fully cured and aged hams, surface-sterilized by dipping in 1% NaOCl for 1 min, rinsed with sterilized, distilled water, and blotted dry on sterile cheese cloth. For inoculation, these slices were swabbed with 1.0 ml of a spore suspension containing 10^6 spores of *P. viridicatum*. Three inoculated slices were hung in a sterilized 0.946-liter Mason jar with a lid to which a metal hook had been soldered; the jar was then incubated at 10, 15, 25, and 30 C for various time periods. After incubation, the ham slices were cut into small pieces for extraction of citrinin.

For quantitative determination of citrinin, all cultures were extracted with chloroform by homogenizing in a high-speed blender. The crude extract was filtered through Whatman no. 1 filter paper, and the chloroform layer was separated in a separatory funnel. The extraction was repeated three times with 50 ml of chloroform per extraction. The chloroform extracts were combined and concentrated in a flash evaporator; concentrated extracts were diluted to 5 ml with chloroform. Citrinin was separated from extracts by thin-layer chromatography on Adsorbosil-1 (Applied Science Laboratories, State College, Pa.) by using toluene-ethyl acetate-formic acid 6:3:1 (vol/vol/vol) as developing solvent (11). A Photovolt fluorodensitometer (Photovolt Corp., N.Y.) was used to compare the intensity of fluorescence of the samples with that of standards.

As shown in Table 1, all seven strains of *P. viridicatum* produced citrinin on potato dextrose broth, although in different amounts. At 10 C, growth of the mold was very poor, and no toxin could be detected. At 15 C, *P. viridicatum* gave poor growth, and citrinin was not detected until after 21 days of incubation. The optimal temperature for toxin production by all strains was between 25 and 30 C. At 30 C incubation, toxin production began earlier than that at 25 C, but after 21 days of incubation, there was

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TABLE 1. *Production of citrinin by P. viridicatum on potato dextrose broth at various temperatures*

Strain no.	Citrinin (μg/100 ml of broth)				
	15 C	25 C		30 C	
	21 days	14 days	21 days	14 days	21 days
M-110	63[a]	55	238	205	227
M-115	76	79	211	184	221
M-133	185	204	441	435	464
M-165	163	140	337	278	292
M-206	88	65	257	245	286
M-218	96	56	184	196	211
M-240	76	69	213	205	228

[a] Mean of four replications.

little difference in toxin production by the same strain at the two temperatures. Strain M-133 produced the largest amount of citrinin among the seven strains tested.

Production of citrinin by *P. viridicatum* on sliced ham is shown in Table 2. As on potato dextrose broth, all seven strains produced citrinin on ham slices. There was no growth of mold on ham slices at 10 C, and growth was poor within the first week at 15 C. A small amount of citrinin (84 to 296 μg) was detected after 21 days of incubation. At 25 C, toxin production increased sharply after 14 days of incubation. There was little difference in the amount of toxin at 25 to 30 C after 21 days of incubation.

Most country-cured hams are aged from four to eight months at 21 to 32 C (5); often molds grow during this period. Because our study shows that all seven strains of *P. viridicatum* are capable of producing the mycotoxin in appreciable amounts on sterilized slices, it is possible that these molds might produce this toxin during aging and could constitute a potential hazard to human health. Also, there is evidence that some mold-infected grains contain up to 80 parts per million of citrinin (13), and this mycotoxin has drawn attention in Canada. Our study further shows that at temperatures below 15 C, very little or no toxin was produced. Whenever possible, ham should be kept at a low temperature during storage.

TABLE 2. *Production of citrinin by P. viridicatum on country-cured ham at various temperatures*

Strain no.	Citrinin (μg/slice)				
	15 C	25 C		30 C	
	21 days	14 days	21 days	14 days	21 days
M-110	135[a]	109	225	216	277
M-115	173	97	280	242	261
M-133	289	244	658	672	663
M-165	296	312	547	569	578
M-206	144	110	398	355	376
M-218	155	173	418	427	459
M-240	84	104	327	358	369

[a] Mean of three slices.

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