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Tremorgenic Toxins from Penicillia

III. Tremortin Production by *Penicillium* Species on Various Agricultural Commodities[1]

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A low temperature (4 C) favors the accumulation of the mycotoxin tremortin when tremortin-producing molds are grown on various agricultural commodities. However, the rate of toxin formation is more rapid at a high temperature (20 C).

Several *Penicillium* spp. capable of producing a highly toxic tremorgen, tremortin A ($C_{37}H_{44}O_6NCl$), have been isolated from moldy commercial feedstuffs by Wilson et al. (5) and Ciegler (1). Subsequently, a second tremorgenic compound, tremortin B ($C_{37}H_{45}O_5N$), was isolated from the same fungi by Hou et al. (Can. J. Microbiol., *in press*). Tremortin production appears to be confined to several species in the subsection Fasciculata, section Asymmetrica of the genus *Penicillium* (2). Several of these species are common contaminants of grains. Various storage methods, other than drying to low moisture contents, have been studied so that fungal contamination of grain with possible subsequent mycotoxin formation can be avoided. Refrigerated storage of high-moisture grain has been considered both in this country and abroad as an attractive alternative to drying or ensiling (4).

This report is concerned with the tremortin-producing capability of four penicillia at low and moderate temperatures (4 and 20 C) on various agricultural commodities. The four cultures studied were *P. cyclopium* NRRL 3477, *P. palitans* NRRL 3468, *P. crustosum* NRRL 5186, and *P. puberulum* NRRL 1889; all had been isolated from moldy commercial feedstuffs.

Forty grams of each commodity was placed in 300-ml Erlenmeyer flasks. To each flask was added 32 ml of distilled water, except 16 ml of water was added to rice; flasks plugged with cotton were autoclaved for 15 min at 121 C. Each flask was inoculated with 1 ml of a spore suspension prepared by adding 50 ml of sterile distilled water to 1- to 2-week-old slant cultures. Flasks were incubated statically at 4 and 20 C. All samples were prepared in duplicate, and each flask was assayed in duplicate.

Flasks were harvested at different times, and the grains from each flask were extracted with 120 ml of chloroform-methanol (2:1, v/v) in a Waring Blendor. After the macerated material was filtered through filter paper, 5 ml of the filtrate (containing about 10 to 100 μg of tremortin) was evaporated to dryness for each assay. The tremortins were extracted from the residual solids with three 2-ml volumes of diethyl ether. By this procedure, tremortins were separated from some phospholipids and gumlike substances. The extracts were combined, evaporated to a small volume, and applied to a glass plate coated with Silica Gel G-HR (Merck) which was developed with chloroform-acetone (93:7, v/v). Tremortin A and B standards were applied to the same plate and after development were sprayed with 1% $FeCl_3$ in butanol followed by gentle heating. Plate areas containing tremortins from the extracted grains and corresponding to R_F values of the authentic tremortins (A, R_F of 0.42; B, R_F of 0.56) were scraped off and extracted with acetone. Quantitative analyses for the extracted tremortins were made by a colorimetric method previously described (3).

The four molds grew well at both temperatures on all of the commodities studied. Data for tremortin production after various incubation periods at 4 and 20 C are given in Table 1. Tremortins were produced on all commodities studied except soybeans, peanuts, and cottonseed. Peak production was usually attained by 25 days at 20 C and after 120 days at 4 C. In addition, the amount of tremortin produced was strain- and commodity-dependent. *P. crustosum*, a common contaminant of refrigerated foodstuffs, produced considerable quantities of tremortins at 4 C, particu-

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TABLE 1. *Production of tremortins by four Penicillium species on various grains at various times and temperatures*

Grain	Temp (C)	Total tremortins (mg/kg of grain)																			
		10 days				25 days				50 days				85 days				120 days			
		A[a]	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Barley	4	0	0	0	0	0	0	0	0	40	0	24	58	111	0	93	420	395	31	156	740
	20	55	0	41	104	212	20	300	120	222	16	180	103	201		138	100				
Oats	4	0	0	0	0	0	0	0	0	32	0	0	32	480	0	98	615	525	35	102	745
	20	76	0	10	88	387	17	83	250	312	15	66	135	240		51	123				
Rice	4	0	0	0	0	54	0	24	28	264	0	80	236	295	0	117	250	237	28	100	189
	20	184	0	144	180	255	37	187	208	144	28	147	84	67	21	108	86				
Rye	4	0	0	0	0	8	0	0	0	193	0	32	109	337	0	44	335	400	29	33	330
	20	123	0	70	66	265	25	126	99	290	18	84	70	240	11	61	66				
Sorghum	4	0	0	0	0	8	0	0	0	44	0	22	36	88	0	46	62	116	31	66	125
	20	58	0	86	64	186	25	445	107	125	18	325	70	103	11	292	51				
Wheat	4	0	0	0	0	0	0	0	0	42	0	65	275	432	0	127	345	482	34	168	317
	20	79	0	73	76	409	20	108	154	308	16	61	79	278		67	67				
White corn	4	0	0	0	0	0	0	0	0	71	0	64	76	138	0	82	180	372	24	210	413
	20	94	0	88	100	321	30	397	157	235	25	240	138	161	14	147	76				
Yellow corn	4	0	0	0	0	0	0	0	0	72	0	24	20	120	0	55	114	337	24	204	362
	20	60	0	40	52	278	30	241	143	229	25	211	114	158	13	114	73				

[a] A, *P. palitans;* B, *P. puberulum;* C, *P. cyclopium;* D, *P. crustosum.*

larly on barley and oats. *P. puberulum* was a poor producer regardless of substrate or temperature. The ratio of tremortin A to B varied with the fungus and the commodity but usually approximated 6 to 10:1.

Although low temperature favored toxin accumulation, a higher rate of production occurred at 20 C. Data indicate that refrigeration is not a satisfactory method for long-term storage of high-moisture grains, for even when toxin production did not occur fungal growth was profuse.

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