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Patulin Production by Species of *Aspergillus* and *Penicillium* at 1.7, 7.2, and 12.8 C

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ABSTRACT

Two strains each of *Aspergillus clavatus* (NRRL 1980 and ATCC 9599), *Penicillium claviforme* (NRRL 1001 and 1002), *Penicillium expansum* (FM 1071 and NRRL 973), and *Penicillium patulum* (ATCC 24550 and FM 1172); and one strain of *Penicillium griseofulvum* (NRRL 2300) were inoculated into potato-dextrose broth and incubated at 1.7, 7.2, and 12.8 C for 110, 84, and 55 days, respectively. All cultures grew at all temperatures. Patulin production by *P. griseofulvum* and *P. claviforme*, NRRL 1001, was limited or inhibited at 1.7 C, whereas at 7.2 C only *P. griseofulvum*, NRRL 2300, failed to produce toxin. Patulin was produced at 12.8 C by all nine cultures.

In recent studies by Torrey and Marth (*14, 15*), *Aspergillus* and *Penicillium* species dominated molds isolated from home stored foods, both refrigerated and non-refrigerated. The mycotoxins produced by some of their isolates were aflatoxins, kojic acid, Ochratoxin A, penicillic acid, and patulin (*14*). When these isolates were tested for their ability to grow at refrigeration temperatures (*15*), no growth was detected at 8 C in *Aspergillus* species, but *Penicillium* isolates grew at 5 C if pregerminated at higher temperatures. The mean temperature limit of home refrigerators was noted as 3.9 to 11.9 C (*15*).

Several foods are known to have an indigenous flora of toxigenic species of *Aspergillus* and *Penicillium* (*1, 2, 3, 6, 8, 16, 17*), and the ability of several toxigenic strains to grow and produce toxin in refrigerated and controlled atmospheres is well documented (*1, 2, 3, 5, 7, 8, 10, 13*). *Penicillium expansum* is frequently encountered as an invader of fruits stored at refrigeration temperatures (*1, 7, 8, 10*). This species is also one of the better known producers of patulin—a toxic and carcinogenic metabolite of several species of *Aspergillus* and *Penicillium* (*4*). Patulin is stable in aqueous solution in the pH range of most fruit products and resists thermal destruction in the pH range of 3.5 to 5.5 (*9*). *P. expansum* can grow and produce patulin in the temperature range of 0 to 30 C (*12*), but for most patulin-producing species, temperatures limiting or restricting toxin production are not known.

This research explored the ability of nine strains of patulin-producing aspergilli and penicillia in five species to produce toxin at commonly used refrigeration temperatures. Because of the large number of natural in vivo substrates that would be suitable for this study, the scope was limited by the choice of a substrate known to maximize in vitro patulin production (*11*).

MATERIALS AND METHODS

Cultures used in this study were from the American Type Culture Collection (ATCC), Rockville, Md., the Northern Regional Research Center (NRRL), U.S. Department of Agriculture, Peoria, Ill., and the Food Microbiology Branch (FM), Division of Microbiology, Food and Drug Administration, Washington, D.C. They were: *Aspergillus clavatus* NRRL 1980 and ATCC 9599; *Penicillium claviforme* NRRL 1001 and 1002; *P. expansum* FM 1071 and NRRL 973; *Penicillium griseofulvum* NRRL 2300; and *Penicillium patulum* ATCC 24550 and FM 1172. Spores were produced from these cultures on potato-dextrose agar in 5-liter bottles and harvested in potato-dextrose broth with sterile glass beads.

Potato-dextrose broth (*12*) was dispensed (50 ml/flask) into 300-ml Erlenmeyer flasks and autoclaved for 30 min at 121 C. The spore suspension was added to each flask to produce a concentration $> 10^6$/ml. Each flask was shaken vigorously and incubated at 1.7, 7.2, or 12.8 C for 110, 84, and 55 days, respectively. Sampling intervals were 10 days at 1.7 C, 7 days at 7.2 C, and 5 days at 12.8 C.

Temperatures used in this study were provided by Model 805 incubators manufactured by Precision Scientific. Although rated 5 to 55 C, two of our incubators achieved and held 1.7 C without difficulty. Temperatures were adjusted and monitored with both mercury thermometers (permanently in place) and a YSI Model 425C Telethermometer. To minimize exposure to temperatures above those provided by the experimental atmospheres, each flask was preincubated before inoculation at the test temperature to be used.

At appropriate sampling intervals, duplicate broth cultures for each fungal species at each temperature were filtered through tared Whatman #2 filter paper. The paper and mycelial mat were dried for 20 to 24 h at 80 C and weighed to determine the mass of the fungal mat. This procedure was used as a semiquantitative measure of growth, and results expressed as milligrams per flask.

Patulin was determined, using 5-ml samples of filtered culture broth extracted three times with equal volumes of ethyl acetate. The extracts were dehydrated with anhydrous sodium sulfate and evaporated in vacuo at 45 C. The residue was dissolved in chloroform and spotted on thin layer silica gel (0.25 mm) along with patulin standards dissolved in chloroform. Plates were developed in benzene: methanol: acetic acid (90:5:5), and spots were made visible by forming the phenylhydrazine derivative (*10*). Quantitation was by visual comparison with standard

spots. The lower detection limit of the procedure was 0.1 μg/ml. Positive results are reported as micrograms per milliliter, and negative results as <0.1 μg/ml.

RESULTS AND DISCUSSION

All cultures germinated and grew at all temperatures. Maximum growth occurred at the highest incubation temperature (12.8 C) in only three cultures: *P. griseofulvum* NRRL 2300, *A. clavatus* NRRL 1980, and *P. expansum* NRRL 973. In all others, growth at 7.2 C was better than or equal to that of the other two temperatures. Growth was suppressed at 1.7 C in *P. griseofulvum* NRRL 2300, and *P. patulum* ATCC 24550. The data are summarized in Fig. 1.

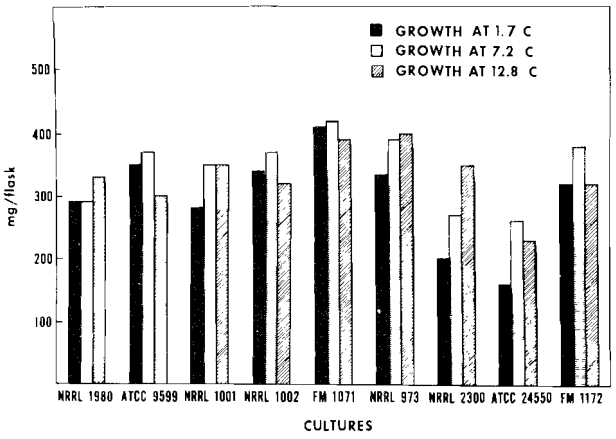

Figure 1. *Maximum culture growth in milligrams per flask.*

All cultures except *P. claviforme* NRRL 101 and *P. griseofulvum* NRRL 2300 produced >50 μg/ml of patulin at all temperatures. Both of these exceptions produced appreciable levels of toxin at 12.8 C but not at 7.2 nor 1.7 C. In contrast, *A. clavatus* ATCC 9599, *P. expansum* FM 1071, and *P. patulum* FM 1172 accumulated the highest toxin concentration at the lowest growth temperature (1.7 C). *A. clavatus* NRRL 1980, *P. claviforme* NRRL 1002, *P. expansum* NRRL 973, and *P. patulum* ATCC 24550, accumulated the highest concentration of toxin at 7.2 C. These data are presented in Fig. 2.

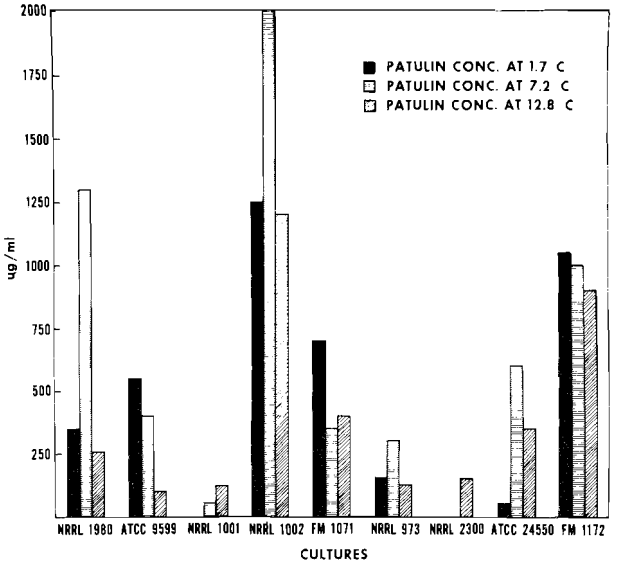

Figure 2. *Maximum patulin concentration in micrograms per milliliters culture broth.*

Table 1 gives times at which initial patulin detection, maximum toxin concentration, and maximum culture growth occurred. Maximum patulin concentration usually coincided with or followed closely maximum growth. As expected, there was considerable variation in growth and toxin production rates, both between species and between strains within a species. The most important data presented are those showing time of initial toxin detection. Patulin was detected first from 10 to 80 days at 1.7 C, 7 to 28 days at 7.2 C, and 5 to 35 days at 12.8 C. While time for maximum toxin accumulation did not coincide with initial detection, these data show that at all temperatures used, the potential for production of patulin during short (5 to 10 days) holding periods exists.

These results show the ability of all the *A. clavatus, P. expansum,* and *P. patulum* cultures and one of two *P. claviforme* cultures to produce large amounts of patulin on a suitable substrate held at the most frequently used refrigeration temperatures. The potential exists for substantial patulin production by both primary invaders such as *P. expansum* and *P. claviforme* and opportunis-

TABLE 1. *Time required for patulin detection, maximum patulin concentration, and maximum culture growth*

Culture identification	Day patulin initially detected			Day patulin maximum detected			Day maximum culture mass detected		
	1.7 C	7.2 C	12.8 C	1.7 C	7.2 C	12.8 C	1.7 C	7.2 C	12.8 C
A. clavatus									
NRRL 1980	30	14	15	100	63	35	100	63	25
ATCC 9599	80	21	10	100	77	55	100	63	40
P. claviforme									
NRRL 1001	40	28	5	100	28	20	80	49	20
NRRL 1002	10	7	5	50	42	30	50	35	30
P. expansum									
FM 1071	30	14	5	100	35	40	70	42	20
NRRL 973	50	14	5	100	70	25	110	49	35
P. griseofulvum									
NRRL 2300	ND[a]	ND	35	ND	ND	55	80	70	50
P. patulum									
ATCC 24550	20	7	5	80	42	20	70	42	20
FM 1172	30	14	5	80	28	30	80	35	20

[a]None detected.

tic decay organisms such as *A. clavatus* and *P. patulum,* when foods are stored for long periods at low temperatures. The mean temperatures of home refrigerators (*15*) would provide the opportunity for growth and substantial patulin production by seven of the nine mycotoxigenic strains tested. Whereas these in vitro studies cannot necessarily be extrapolated to in vivo conditions (*12*), they do indicate the ability of patulin-producing fungi to grow and express toxigenicity at ≤12.8 C is not unusual.

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Errata

Isolation and Identification of Lipolytic Microorganisms Found on Rough Rice from Two Growing Areas

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This paper appeared on pages 28-30, Vol. 41 (January 1978) of the *Journal of Food Protection.* Tripticase Nutrient Agar should replace Triplicate Nutrient Agar in the Microbiological examination: Bacteria sub-section of the Materials and Methods section of this paper.