APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Mar. 1981, p. 568-579
0099-2240/81/030568-12$02.00/0
Vol. 41, No. 3

Physiological Criteria and Mycotoxin Production as Aids in Identification of Common Asymmetric Penicillia

JENS C. FRISVAD

Food Technology Laboratory, The Technical University of Denmark, DK-2800 Lyngby, Denmark

The taxonomy of the asymmetric (predominantly terverticillate) penicillia is based on morphological differences that leave identification difficult. The application of physiological criteria facilitated the identification of the common asymmetric penicillia investigated. Changes in the placement of some strains of these penicillia made the connection to mycotoxin-producing ability clearer. The classical criterion of conidium color was deemphasized and replaced by the following criteria: (i) growth on nitrite-sucrose agar and (ii) growth and acid (and subsequent base) production on creatine-sucrose agar (containing bromocresol purple). Other criteria used or developed were: (iii) growth on sorbic acid plus benzoic acid agar (50 + 50 ppm, pH 3.8), (iv) growth on an agar containing 1,000 ppm propionic acid (pH 3.8), (v) growth on an agar containing 0.5% acetic acid, (vi) growth at 37°C, (vii) growth rate on an agar containing 0.1% pentachloronitrobenzene, (viii) production of extracellular tricaproinase, and (ix) fasciculation on a medium containing 10 ppm botran (2,6-dichloro-4-nitroanilin). The pattern of extracellular metabolites after thin-layer chromatography was used as a chemotaxonomic criterion. The species investigated, the number of isolates investigated, and the toxins which some of these isolates produce were: *Penicillium roqueforti* (18) (patulin), *P. citrinum* (11) (citrinin), *P. patulum* (9) (patulin and griseofulvin), *P. expansum* (patulin and citrinin), *P. hirsutum* (13), *P. brevicompactum* (19), and *P. chrysogenum* (12). Widespread species of the *P. cyclopium*, *P. viridicatum*, and *P. expansum* series of Raper and Thom (*A Manual of the Penicillia*, 1949) were subdivided into four new groups: "P. crustosum pA" (29) (penitrem A), "P. melanochlorum" (29), "P. cyclopium p" (119) (penicillic acid and infrequently penitrem A), and "P. viridicatum o-c" (43) (ochratoxin A and citrinin). "P. viridicatum o-c" was separated from "P. cyclopium p" due to its ability to grow on nitrite as sole nitrogen source. The species and groups investigated were related to the new taxonomic classification of the genus *Penicillium* according to Pitt.

The difficulties in identifying the asymmetric penicillia because of the absence of distinct characteristics have been pointed out repeatedly (5, 6, 28, 33, 35). The colony texture and color used in *Penicillium* taxonomy are often artificial and subjective criteria (20). Raper and Thom (33) were aware of the difficulties and indicated that the subgrouping of the non-divaricate asymmetric penicillia in velutina, lanata, funiculosa, and fasciculata might separate species that are biochemically and physiologically alike. Furthermore, most of the common asymmetric penicillia are difficult to group on the basis of micromorphological criteria alone. Pitt (30) mentions transition forms between *Penicillium brevicompactum* and *P. puberulum*, species considered clearly separable by Raper and Thom (33). Samson et al. (35) consider their broad species *P. verrucosum* as nearly inseparable micromorphologically from *P. roqueforti*.

Physiological criteria in *Penicillium* taxonomy have not been used extensively because molds in general have a sufficiently varied morphology to allow identification to the species level (21). Some investigators have, however, used physiological criteria in the subdivision of the penicillia, such as the ability to grow on a medium containing 0.5% acetic acid, separating *P. roqueforti* from other penicillia (8), or the ability to grow on nitrite (1, 8) or creatine (8) as sole nitrogen source. Pitt (28, 29, 30) uses colony diameter at different temperatures and water activities to separate sections, series, and species in *Penicillium*.

This investigation aimed to establish physiological criteria for the identification of common asymmetric penicillia. The mycotoxin-producing ability of the species treated was emphasized. The species investigated were the common asymmetric penicillia in foods and feeds: *P. cy-*

clopium, *P. viridicatum*, *P. expansum*, *P. citrinum*, *P. brevicompactum*, *P. patulum*, and *P. chrysogenum* (19, 20), and species that occur frequently in Danish products such as *P. roqueforti*, *P. crustosum*, *P. verrucosum* var. *melanochlorum*, and *P. hirsutum*.

MATERIALS AND METHODS

Source and maintenance of cultures. The strains were obtained from culture collections (Table 1) or isolated from Danish and Italian products (Table 2). The cultures were maintained on potato dextrose agar (PDA) (Difco) at 0.5°C.

All of the isolates were identified by the method of Raper and Thom (33) with the following exceptions. *P. hirsutum* was identified by the method of Pitt (30). The members of the *P. chrysogenum* series (33) were identified by the method of Samson et al. (34). *P. viridicatum* was subdivided in the three subgroups mentioned by Ciegler et al. (5). *P. palitans* as described by Raper and Thom (33) was divided into *P. verrucosum* var. *melanochlorum* (called "P. melanochlorum") and isolates which could be placed together with *P. cyclopium*, *P. crustosum*, or *P. viridicatum* (deemphazising the dark colors of such isolates). The name *P. patulum* was used in place of *P. urticae* (30, 33). As the new manual of the penicillia by Pitt (30) was published recently, Table 3 shows the relations between the species defined by Pitt (30) and the species/groups analyzed in this work.

Media and growth conditions. The strains were identified on Czapek agar (33) and Blakeslee malt agar (33). These cultures were incubated at 25°C for 14 days. Furthermore the strains were identified by the method of Pitt (30) using Czapek yeast autolysate agar (CYA), malt extract agar (MEA), and 25% glycerol nitrate agar. These cultures were incubated at 25°C for 7 days. Furthermore, the strains were incubated on glucose yeast extract (GY) agar (9, 41) (glucose, 20 g; yeast extract [Difco], 5 g; agar [Difco], 20 g; water, 1 liter) at 37°C for 7 days.

Toxin production media used were: yeast extract sucrose (YES) agar (15, 38) (yeast extract, 20 g; sucrose, 150 g; agar, 20 g; water, 1 liter) and GY agar (9).

During preliminary tests on a few typical strains from the different species, it was noted that of many widely used nitrogen sources (ammonium chloride, ammonium nitrate, creatine, nitrite, cystein, casein, and other N sources), creatine and nitrite were very useful. The results concerning different carbon sources were not promising. The ability of the penicillia to produce different extracellular enzymes (proteases, lipases, and amylases) was tested, and of the lipases, tricaproinase showed the most consistent results. The following media were developed as selective and indicative media.

(i) Media with different N sources. Nitrite-sucrose (NO2) agar consisted of the following: $NaNO_2$, 3 g; sucrose, 30 g; $K_2HPO_4 \cdot 3H_2O$, 1.3 g; mineral solution, 10 ml; agar, 20 g; water, 1 liter.

Creatine-sucrose (CREA) agar consisted of: creatine, $1H_2O$ (Merck, p.a.), 3 g; sucrose, 30 g; $K_2HPO_4 \cdot 3H_2O$, 1.3 g; mineral solution, 10 ml; bromocresol purple, 0.05 g; agar, 20 g; water, 1 liter. After autoclaving, the pH of the medium was adjusted to 8.0 with sterile 1 N NaOH solution.

The mineral solution was made as follows: KCl, 5 g; $MgSO_4 \cdot 7H_2O$, 5 g; and $FeSO_4 \cdot 7H_2O$, 0.1 g, were dissolved in 100 ml of water.

(ii) Lipase medium. Tricaproin agar consisted of Czapek basal medium (Difco), 35 g; $CaCl_2 \cdot 2H_2O$, 0.4 g; agar, 20 g; water, 1 liter. After autoclaving, 1 g of sterile tricaproin (glycerine tricaproate) (Fluka, techn.) per liter of basal medium was added to the molten agar (50°C). The mixture was homogenized in an atomixer (MSE) (12,000 rpm, 1 min).

(iii) Media with different fungicides. Media with different fungicides included PDA (Difco) with 0.1% pentachloronitrobenzene (Fluka, pract.) (PCNB). The pH was adjusted to 6.5 after mixing the ingredients. After autoclaving, the pH of the medium was readjusted to 6.5. The medium was agitated very thoroughly before and after autoclaving and before pouring the medium in the petri dishes.

Another medium with a fungicide was PDA (Difco) containing 10 ppm (10 mg/liter) botran (2,6-dichloro-4-nitro aniline) (the Technical University of Denmark, the botran was recrystallized twice in acetone), which was called PDAB. A 10-mg amount of botran dissolved in 10 ml of acetone was added to 1 liter of molten PDA medium (50°C).

GY agar with 50 ppm sorbic acid and 50 ppm benzoic acid (pH 3.8) (GYBS) was a third medium in this group. Sodium benzoate, 0.09 g, and potassium sorbate, 0.067 g, were added to 1 liter of GY agar. After autoclaving, the pH of the medium was lowered to 3.8 with sterile 1 N HCl.

GY agar with 1,000 ppm propionic acid (pH 3.8) (GYP) was another medium in this group. A 1.375-g amount of sodium propionate was added to 1 liter of GY agar. After autoclaving, the pH of the medium was lowered to 3.8 with sterile 1 N HCl.

GY agar with 5,000 ppm acetic acid (GYA) was the last medium in this group. After autoclaving, glacial acetic acid was added to a final concentration of 0.5% (vol/vol). pH was adjusted to 3.8.

These media were three-point inoculated using spore suspensions of the molds (10^6 to 10^7 conidia per ml of suspension medium). Suspension medium consisted of: NaCl, 8.5 g; Tween 80 (Merck, p.a.), 1 g; water, 1 liter. The cultures were incubated at 20°C and observed from the day 4 of incubation.

Inoculation of apples. Spore suspensions (as described) of all the isolates of *P. verrucosum* var. *melanochlorum*, *P. expansum*, and *P. crustosum*, and other penitrem A producers were inoculated into surface-sterilized apples (Grästen and Spartan varieties) using a preparation needle. The apples were surface sterilized by dipping in a 1% solution of NaOCl (1 min) and washed two times in sterile water. The diameter of the rot was recorded after 7 and 14 days of incubation (incubation temperature, 20°C).

Mycotoxin analysis. Mycotoxin analyses were performed the day 4, 5, 7, 14, and 21 of incubation using the "agar plug method" (9). Agar plugs were cut out at the border of a colony grown on GY and YES agar with a flamed stainless-steel tube (inner diameter,

TABLE 1. *Molds from culture collections used in this investigation, (subdivided by their physiological characteristics)*

New species/group	Received as	No.	Source[a]	Mycotoxins produced[b]
P. roqueforti	*P. roqueforti*	NRRL 849	A	PR-toxin[c] + roquefortine[c]
	P. roqueforti	111	A	PR-toxin[c]
	P. roqueforti	143	A	PR-toxin[c]
	P. roqueforti	145	A	
	P. roqueforti	198	A	PR-toxin[c]
	P. roqueforti	Sp. 572	B	Patulin
	P. roqueforti	Sp. 581	B	Patulin
P. brevicompactum	*P. brevicompactum*	IMI 92044	C	Mycophenolic acid[c]
	P. brevicompactum	IMI 94149	C	Mycophenolic acid[c]
	P. brevicompactum	IMI 40225	D	Mycophenolic acid[c]
	P. brevicompactum	IMI 143520ii	D	
	P. brevicompactum	IMI 92219	D	
	P. puberulum	IMI 17456	D	Mycophenolic acid[c]
"P. viridicatum o-c"	*P. viridicatum* (II)	NRRL 5571	E	Ochratoxin A + citrinin
	P. viridicatum (II)	NRRL 5583	E	Citrinin
	P. viridicatum (II)	NRRL 3712	E	Ochratoxin A
	P. viridicatum (III)	NRRL A-19179[b]	E	Ochratoxin A
	P. viridicatum (III)	NRRL 5573	E	
	P. verrucosum var. *verrucosum*	Sp. 134	B	Ochratoxin A
	P. verrucosum var. *verrucosum*	Sp. 991	B	Ochratoxin A
	P. cyclopium	NRRL 6065	E	Ochratoxin A
P. citrinum	*P. citrinum*	NRRL 1843	E	Citrinin
	P. citrinum	Sp. 458	B	Citrinin
	P. citrinum (V)[d]	398f	A	Citrinin
P. expansum	*P. expansum*	CBS 325.48	F	
	P. expansum	CP 554	G	Patulin
	P. expansum	Sp. 248	B	Patulin
	P. expansum	Sp. 454	B	Patulin + citrinin
	P. expansum	597	A	Patulin + citrinin
	P. expansum	599	A	Patulin
	P. expansum	603	A	Patulin + citrinin
"P. crustosum pA"	*P. crustosum*	NRRL 968	E	Penitrem A
	P. crustosum	NRRL 1983	E	Penitrem A
	P. crustosum	NRRL 5186	E	Penitrem A
	P. palitans	NRRL 3468	E	Penitrem A
	P. cyclopium	NRRL 3476	E	Penitrem A
	P. cyclopium	NRRL 3477	E	Penitrem A
	P. verrucosum var. *cyclopium*	Sp. 458	B	Penitrem A
	P. verrucosum var. *cyclopium*	Sp. 1191	B	Penitrem A
	P. terreste	IMI 89384	D	
	P. pseudocasei	IMI 68235	D	
"P. melanochlorum"	*P. viridicatum*	IMI 154731	D	
	P. cyclopium	IMI 89374	C	
"P. cyclopium p"	*P. lanosocoeruleum*	NRRL 888	E	
	P. aurantiocandidum	NRRL 884	E	
	P. aurantiovirens	IMI 34846ii	D	Penicillic acid
	P. aurantiovirens	IMI 92199	D	Penicillic acid
	P. cyclopium	NRRL 1888	E	Penicillic acid
	P. verrucosum var. *cyclopium*	Sp. 789	B	Penicillic acid
	P. verrucosum var. *cyclopium*	Sp. 948	B	Penicillic acid
	P. verrucosum var. *cyclopium*	Sp. 875	B	Penicillic acid + S-toxin[c]

0.4 cm). The plugs were placed directly on the precoated thin-layer chromatography plates (Merck, Silica Gel G, Art. 5721, activated at 100°C for 20 min). The plugs were removed after 10 s (about 7.5 μl was transferred to the plate), and the application spots were allowed to dry.

TABLE 1 *Continued*

New species/group	Received as	No.	Source[a]	Mycotoxins produced[b]
	P. martensii	NRRL 3612	E	Penicillic acid
	P. martensii	NRRL 3612		Penicillic acid (white mutant)
	P. verrucosum var. *cyclopium*	CBS 744.74	F	Penicillic acid
	P. viridicatum (I)	NRRL 5570	E	Penicillic acid
	P. viridicatum (I)	NRRL 5569	E	
	P. viridicatum (I)	NRRL A-14307	E	
	P. viridicatum (I)	IMI 49096	C	Penicillic acid
	P. puberulum	NRRL 3564	E	Penicillic acid
	P. palitans	NRRL 2033	E	
	P. olivinoviride	NRRL 2028	E	
	P. olivinoviride	NRRL 961	E	
	P. olivinoviride	NRRL 958	E	Penitrem A
	P. cyclopium	NRRL 1899	E	Penitrem A (−)[e] + penicillic acid
P. hirsutum	*P. corymbiferum*	NRRL 2032	E	
P. patulum	*P. patulum*	NRRL 993	E	
	P. patulum	ATCC 9260	C	Patulin + griseofulvin
	P. patulum	NRRL 2159-A	E	Patulin + griseofulvin
	P. griseofulvum	CBS 125.27	F	Patulin + griseofulvin
	P. patulum	CSIR 1399	B	Patulin + griseofulvin + cyclopiazonic acid[c]
	P. patulum	Sp. 242	B	Patulin + griseofulvin
	P. patulum	511	A	Patulin + griseofulvin
	P. patulum	512	A	Patulin + griseofulvin
	P. patulum	514	A	Patulin + griseofulvin
P. chrysogenum	*P. chrysogenum*	NRRL 1951	E	Penicillin[c]
	P. chrysogenum	IMI 178521	D	
Transition form between *P. citrinum* and *P. chrysogenum*	*P. citrinum* (III)	LKF ZL 17	C	Citrinin (trace amounts)

[a] Sources of the microorganisms: A, The Bundesforschungsanstalt für Lebensmittelfrischhaltung, Karlsruhe, Federal Republic of Germany; B, The Bundesanstalt für Fleischforschung, Kulmbach, Federal Republic of Germany; C, Løvens Kemiske Fabrik, Ballerup, Denmark; D, The Commonwealth Mycological Institute, Kew, England; E, The Agricultural Research Service Culture Collection, Peoria, Ill.; F, The Centraalbüreau voor Schimmelcultures, Baarn, the Netherlands; G, The Royal Veterinary and Agricultural University, Copenhagen, Denmark.

[b] As stated by the suppliers and/or confirmed by the agar plug method and extraction analysis.

[c] Not tested.

[d] This mold was obtained as a sector in *P. cyclopium* var. *album* 398, and it is similar to the description of *P. citrinum* NRRL 783 A given in reference 33, p. 349.

[e] Could not be detected after 3 weeks of incubation (YES agar) by the extraction analysis.

Penitrem A analysis (this toxin is intracellular [32]) and analysis on molds negative in the agar plug method (9) were performed using a modification of the method of Lötzsch et al. (16). After 21 days of incubation, the agar-grown cultures (YES agar) and the agar were divided into three pieces (using a sterile scalpel). One piece was used for the agar plug method, and the remaining two pieces were placed in a sterile polyethylene bag (protected by two additional bags). After addition of 50 ml of chloroform, the contents of the bags were treated in a stomacher (Colworth 400) for 2 min. About 30 ml of the chloroform phase was dried over anhydrous Na_2SO_4 followed by filtration. After concentration of the solvent (in vacuo, 55°C, flash evaporation) to about 2 ml, portions (10 μl) were spotted on precoated Silica Gel G plates (Merck).

Chloroform solutions of the following toxin standards were spotted on the thin-layer chromatography plates: patulin and ochratoxin A (Karl Roth, Karlsruhe, Federal Republic of Germany), citrinin (Supelco, Bellefonte, Pa.), penicillic acid and griseofulvin (Aldrich Chemical Co., Milwaukee, Wis.), and penitrem A (from L. Leistner, Kulmbach, Federal Republic of Germany). The plates were developed under saturated conditions in chloroform-ethylacetate-90% formic acid (6:3:1, vol/vol) (25), chloroform-acetic acid-diethylether (17:1:3, vol/vol, vol) (7), and benzene-methanol-acetic acid (24:2:1, vol/vol/vol) (38). Furthermore, a modification of the method of Marti et al. (18; L. Leistner, personal communication) for the detection of citrinin was used. Unactivated Silica Gel G plates were dipped in an 8% methanolic solution of oxalic acid for 2 min and air dried overnight. After application of the samples, the plates were developed in chloroform-methylisobutyl-ketone (4:1, vol/vol) (40). The toxins were observed in light of different wavelengths (5, 7, 36, 38). Each toxin was visualized in at least two ways. Citrinin was detected by its fluorescence without chemical treatment and after $FeCl_3$ treatment (5). Ochratoxin A was detected by its fluorescence before and after NH_3 treatment (5). Griseofulvin was detected by its fluorescence before and after

TABLE 2. *Origin and mycotoxin-producing ability of strains isolated from foods and feeds*

Species/group	Source	No. of strains	No. of strains producing the toxins mentioned
P. roqueforti	Air, f.p.p.[a]	7	1 (patulin)
	Rye bread	3	0
	Raspberry	1	0
P. brevicompactum	Air, f.p.p.	5	0
	Barley	6	0
	Tomato	2	0
"P. viridicatum o-c"[b]	Barley	34	33 (citrinin), 12 (ochratoxin A)
	Egg powder	1	1 (citrinin)
P. citrinum	Air, f.p.p.	2	2 (citrinin)
	Barley	6	6 (citrinin)
P. expansum	Tomato	1	1 (citrinin)
	Parsley[c]	1	1 (citrinin), 1 (patulin)
	Barley	3	3 (citrinin), 2 (patulin)
	Cherry	1	1 (citrinin), 1 (patulin)
	Air, kitchen	2	2 (citrinin), 2 (patulin)
	Raspberry	2	1 (citrinin), 2 (patulin)
	Apple	3	3 (citrinin), 3 (patulin)
	Apple, blue rot	4	4 (citrinin), 4 (patulin)
	Air, f.p.p.	3	3 (citrinin), 3 (patulin)
"P. crustosum pA"	Coffee spot	1	1 (penitrem A)
	Wheat bread	3	3 (penitrem A)
	Rye	1	1 (penitrem A)
	Raspberry	1	1 (penitrem A)
	Air, f.p.p.	13	13 (penitrem A)
"P. melanochlorum"	Air, f.p.p.	15	0
	Pear	1	0
	Barley	2	0
	Tallow[d]	8	0
	Unknown	1	0
"P. cyclopium p"[e]	Oregano[c]	1	1 (penicillic acid)
	Basil[c]	1	1 (penicillic acid)
	Sage[c]	1	1 (penicillic acid)
	Air, f.p.p.	8	4 (penicillic acid)
	Fodder mix	1	1 (penicillic acid)
	Grain mix	1	1 (penicillic acid)
	Wheat	3	2 (penicillic acid)
	Barley	80	36 (penicillic acid)
P. hirsutum	Cinnamon[c]	1	0
	Coriander	1	0
	Air, kitchen	1	0
	Air, f.p.p.	1	0
	Barley	7	0
	Apple	1	0
P. chrysogenum	Air, f.p.p.	6	0
	Grain mix	2	0
	Egg powder	1	0
	Barley	1	0

[a] f.p.p., Fruit products plants.

[b] All isolates of "P. viridicatum o-c" (= *P. viridicatum* II, type III was not found) grew restrictedly and were green at time of isolation. However, after storage of some of the cultures at 0.5°C for 14 days, 7 of 20 cultures from the same barley sample were intensively blue.

[c] Isolated from Italian products.

[d] Naturally molded garnatálg (fermented minced sheep tallow) from the Faroe Islands.

[e] According to Raper and Thom (33), the 96 fresh isolates of "P. cyclopium p" were: *P. cyclopium* (44 isolates), *P. martensii* (26 isolates), *P. viridicatum* I (6 isolates), and *P. puberulum* (20 isolates).

TABLE 3. *Relations between the species/groups investigated and the new Penicillium taxonomy of Pitt (30)*

Name according to Pitt (30)	Name in this work
Subgenus *Furcatum*, series *Citrina*	
P. citrinum Thom	*P. citrinum*[a]
Subgenus *Penicillium*; section *Penicillium*, series *Expansa*	
P. expansum Link ex Gray	*P. expansum*
P. chrysogenum Thom	*P. chrysogenum*[b]
Series *Viridicata*	
P. viridicatum Westling	"P. cyclopium p" pro parte and "P. viridicatum o-c" pro parte
P. crustosum Thom	"P. crustosum pA" and "P. melanochlorum"
P. roquefortii Thom	*P. roqueforti*
P. hirsutum Dierckx	*P. hirsutum*
P. aurantiogriseum Dierckx	"P. cyclopium p" pro parte
Series *Urticicola*	
P. griseofulvum Dierckx	*P. patulum*
P. brevicompactum Dierckx	*P. brevicompactum*
P. verrucosum Dierckx	"P. viridicatum o-c" pro parte

[a] Isolates of *P. steckii* have not been included in the investigation.

[b] Isolates of *P. griseoroseum* included in *P. chrysogenum* by Samson et al. (34) is a separate species in the work of Pitt (30). Such isolates have not been included in this investigation.

p-anisaldehyde treatment (7). Penicillic acid was detected by its fluorescence after NH_3 treatment and after *p*-anisaldehyde treatment (5). Patulin was detected by its fluorescence after *p*-anisaldehyde treatment (38) and after 3-methylbenzothiazol-2-on-hydrazon-hydrochloride treatment (36). Penitrem A was detected after $FeCl_3$ treatment (6) and after *p*-anisaldehyde treatment. After the latter treatment, the penitrem A spots became dark blue in daylight (after heat treatment, 10 min, 130°C).

RESULTS

The strains investigated are listed in Tables 1 and 2. These strains were compared mutually in their physiological, macromorphological, micromorphological, and "chemotaxonomic" characteristics. The strains could be subdivided in the 11 species/groups ("taxa") listed in Tables 1 and 2 primarily by their physiological characteristics, but also by their morphological characteristics and by the mycotoxins they produce. Most strains were typical within the species or group they represent, but some of the intergrading strains listed by Pitt (30) were also included. The strains listed in Table 2 are isolates from foods and feeds. Strains similar and dissimilar in colony texture, conidium color, and colony growth rate from each species/group were investigated. The connection between the species/groups in Tables 1, 2, and 4 and the taxonomy of *Penicillium* according to Pitt (30) is shown in Table 3.

A summary of the results of the physiological tests is shown in Table 4. The tests used or developed in this investigation show clear-cut results and allowed a good subdivision in positive and negative responses. The following accepted and well defined species showed consistent patterns of responses in the physiological tests: *P. roqueforti*, *P. brevicompactum*, *P. citrinum*, *P. expansum*, *P. patulum*, *P. chrysogenum*, and *P. hirsutum*. Few strains of the latter species could grow on the GYBS and GYP medium, however.

On the basis of the results it seems relevant to propose four provisional names for groups of strains which share several physiological and chemotaxonomic properties: "P. viridicatum o-c" (o-c, ochratoxin-citrinin), "P. cyclopium p" (p, penicillic acid), "P. crustosum pA" (near or equal to *P. crustosum* sensu Raper and Thom [33]), and "P. melanochlorum" (equal to *P. verrucosum* var. *melanochlorum* sensu Samson et al. [35]). Definitive characteristics for members in the four groups, based upon examination of 29 to 119 strains in each group, are shown in Tables 5 and 6. These four groups also showed consistent patterns of responses in the physiological tests. "P. cyclopium p", however, was a very variable group of strains as was *P. cyclopium* sensu Raper and Thom (33) and corresponding species as defined by Pitt (30) or Samson et al. (35). Table 7 gives an idea of this variability. Preservative resistant isolates of "P. cyclopium p" appeared to be more active biochemically than preservative sensitive isolates. Of the lipase producers, 72% were penicillic acid producers, and of the nonproducers of tricaproinase only 33% produced penicillic acid.

The 11 species/groups listed in Table 3 (column 2) were separable if two micromorphological criteria (ornamentation of stipe and short or long phialides) and five physiological tests were used: growth on NO2 agar, growth and acid production on CREA agar, growth at 37°C, and growth rate on PCNB agar (Table 4).

All isolates of *P. expansum* and "P. crustosum pA" produced the characteristic apple rot (extensive respective restricted) described by Raper and Thom (33). One strain of "P. melanochlorum," however, produced a restricted rot in apples.

Few strains had special physiological responses. *P. citrinum* LKF ZL 17 (*P. citrinum* III sensu Raper and Thom [33]) had a response like strains of *P. chrysogenum*. *P. olivinoviride* NRRL 958 and *P. cyclopium* NRRL 1899 had a + reaction on PCNB agar. The latter isolate produced basic metabolites on CREA agar after 10 days of incubation. The type culture of *P. expansum* received from the Centraalbureau

TABLE 4. *Results of the physiological, morphological, and mycotoxicological tests for the common asymmetric penicillia listed in Tables 1 and 2*[a]

Species/group	Physiological characteristics											Morphology		Toxins produced
	37°C[b]	NO2[c]	CREA[d]	ACID[e]	BASE[f]	PCNB[g]	PDAB[h]	LIP[i]	GYBS[j]	GYP[j]	GYA[j]	Stipe	Phialides	
P. roqueforti (18)[k]	−	++	++	−	NR	w	−	−	++	++	++	Rough	Long	Patulin
P. brevicompactum (18)	−	+	−	−/w	−	w	−	++	+	−	−	Smooth	Long	
"P. viridicatum o-c" (43)	−	++	−/w	−/w	−	w	+/−	+/−	−	−	−	Rough	Long	Ochratoxin A + citrinin
P. citrinum (11)	+	++	−	−/w	−	w	−	−	−	−	−	Smooth	Long	Citrinin
P. expansum (27)	−	−	++	++	++	w	++	−	++	++	−	Smooth	Long	Citrinin + patulin
"P. crustosum pA" (29)	−	−	++	++	++	++	++	++	++	++	−	Rough	Long	Penitrem A
"P. melanochlorum" (29)	−	−	++	++	++	w	−	++	+	+	−	Rough	Long	
"P. cyclopium p" (119)	−	−	−/w	++	−	w	+/−	−/+	−/+	−/+	−	Rough	Long	Penicillic acid + (penitrem A)
P. hirsutum (13)	−	−	−/w	++	−	+	++	−	−/+	−/+	−	Rough	Long	
P. patulum (9)	−	−	−	−/w	−	w	+	+/−	−	−	−	Smooth	Short	Patulin + griseofulvin
P. chrysogenum (12)	w	−	−	−/w	−	+	−	−	−	−	−	Smooth	Long	

[a] The results were recorded after 5 days of growth. The results on the PCNB agar and GY agar (37°C) were recorded after 7 days of incubation.

[b] Growth on GY agar at 37°C. Good growth, +; colony diameter ½ to 2 mm in 5 to 7 days, w.

[c] Growth on NO2 agar. Growth nearly as on CYA agar, ++; growth, but weaker than on CYA agar, +; growth as on water agar, −.

[d] Growth on CREA agar. Growth nearly as on CYA agar, ++; growth ranging from thin growth (as on water agar) to fairly good growth (always a thin colony margin), −/w; growth as on water agar, −.

[e] Production of acid on CREA agar. Abundant acid production (the agar turns from violet to yellow in 5 to 7 days), ++; no color changes or change to red or yellow just underneath the colony in 5 days, −/w; no color changes in the medium, −.

[f] Production of basic metabolites after the production of acid. The color of the agar turns to violet again after 8 to 21 days (beginning underneath the colony), ++; the reaction of the agar does not change in time, −; the agar is always violet, NR.

[g] Rate of growth on PCNB medium. Colony diameter greater than 2 cm in 7 days, ++; colony diameter between 1 and 2 cm in 7 days, +; colony diameter less than 1 cm in 7 days, w.

[h] Fasciculation on PDAB medium. Coremiform growth, ++; fasciculate or granular growth, +; velutinous, funiculose, or lanose growth, −.

[i] Production of extracellular tricaproinase. A significant clearing zone around the colony (1 to 4 mm beyond the colony in 5 days), ++; a clearing zone just around the colony, +; no clearing zone, −.

[j] Growth on GY agar containing, respectively: 50 ppm sorbic acid + 50 ppm benzoic acid (pH 3.8) = GYBS; 1,000 ppm propionic acid (pH 3.8) = GYP; and 5,000 ppm acetic acid = GYA. Growth unaffected by the preservatives, ++; growth weaker than on GY agar, +; no growth within 5 to 7 days, −.

[k] Total number of isolates investigated in each species/group.

TABLE 5. *Differences between "P. viridicatum o-c" and "P. cyclopium p"*

Characteristics	"P. viridicatum o-c"	"P. cyclopium p"
Names according to Raper and Thom (33) and Ciegler et al. (5)	*P. viridicatum* II *P. viridicatum* III Some slow-growing *P. cyclopium*	*P. viridicatum* I *P. olivinoviride* *P. palitans* pro parte *P. cyclopium* (fast growing) *P. martensii* *P. puberulum* pro parte *P. aurantiovirens* *P. aurantiocandidum* *P. lanosocoeruleum*
Names according to Pitt (30)	*P. verrucosum* *P. viridicatum* pro parte	*P. aurantiogriseum* *P. viridicatum* pro parte
Conidium color	Green, occasionally bluegreen	Bluegreen to green (continuum)
Extracellular color produced	No	Yes/no
NO_2^- as sole N source	Good growth	No growth
Creatine as sole N source	No or weak growth	No, weak, or fairly good growth
Acid production on creatine sucrose agar	No, or only under the reverse	Abundant
Smell on CYA agar	Weak, like spruce	Woody, earthy, moldy
Reverse on YES agar	Red brown (meat strains have yellow reverse)	Yellow to cream colored
Growth rate	Slow	Rather fast
Preservative resistance	No	Yes/no
Production of penicillic acid	No	Yes/no
Production of citrinin and/or ochratoxin A	Yes/no	No

TABLE 6. *Differences between "P. crustosum pA" and "P. melanochlorum"*

Characteristics	"P. crustosum pA"	"P. melanochlorum"
Names according to Raper and Thom (33)	*P. crustosum*	*P. palitans* pro parte
Names according to Samson et al. (35)	*P. verrucosum var. cyclopium* pro parte	*P. verrucosum var. melanochlorum*
Names according to Pitt (30)	*P. crustosum*	*P. crustosum*
Conidium color	Green to greygreen, bluegreen in peripheral zones	Dark green (specially on GY agar)
Sterile overgrowth	No	Yes (few strains no)
Production of adherent masses of conidia on malt agar	Yes	No
Fasciculation in the presence of botran	Yes	No
Limited rot produced in apples	Yes	No (one strain yes)
Growth rate in the presence of PCNB	Very high	Low
Production of penitrems	Yes (two strains no)	No
Production of three metabolites (red on thin-layer chromatography plates after anis spray, long-wave ultraviolet light)	No	Yes (few strains no)
Preservative resistance	Very good	Rather good

voor Schimmelcultures (CBS 325.48) seemed rather atypical. It was lanose in texture, sporulates late, and does not produce patulin or citrinin. This isolate had a ++ reaction on the tricaproin agar unlike all other isolates of *P. expansum*. *P. terreste* IMI 89384 and *P. pseudocasei* IMI 68235 had physiological responses like "P. crustosum pA", but they did not develop fascicles on PDAB and they did not produce penitrem A. Furthermore, it was noticed that few isolates of *P. expansum*, "P. crustosum pA" and "P. melanochlorum" developed late on the media containing preservatives (GYP and GYBS).

TABLE 7. *Penicillic acid and tricaproinase production by isolates assignable to the "P. cyclopium p"[a] group and further categorized by tolerance of preservatives*

Preservative resistance[b]	Total no. of isolates	Penicillic acid producers	Tricaproinase producers
GYP- and GYBS-	65	21 (32%)	17 (26%)
GYP+ and GYBS-	18	11 (61%)	10 (56%)
GYP- and GYBS+	10	7 (70%)	5 (50%)
GYP+ and GYBS+	22	21 (95%)	9 (41%)
Total	117	58 (50%)	41 (35%)

[a] The producers of penitrem A are not included.

[b] Symbols: +, the isolate grows on the medium within 14 days of incubation; -, the isolate does not grow within 14 days on the media.

DISCUSSION

Physiological separation of penicillia. The physiological criteria used separate the common asymmetric penicillia well (Table 4). The criteria are most useful when the asymmetric penicillia with rough stipes are to be separated. Ciegler and Pitt (6) discuss stable criteria to separate these penicillia which are very similar micromorphologically (5, 6, 28, 33, 35). The criteria shown in Table 4 are easy to record and seem to help in two ways. They facilitate the separation of species which are difficult to separate on the basis of growth on already used diagnostic agars alone and, most importantly, they link the production of specific mycotoxins to fewer "species." They also help in the identification of transitional species.

The results in Table 4 support the new *Penicillium* taxonomy of Pitt (30) in most cases. There are, however, important deviations in several cases. Results from the present study support recognition of *P. viridicatum* I, II, III, *P. crustosum*, and *P. verrucosum* var. *melanochlorum* as described and distinguished by Ciegler et al. (5), Raper and Thom (33), and Samson et al. (35), respectively. *P. crustosum* and *P. viridicatum* sensu Pitt (30) are broadly conceived in that each taxon embraces two or more types of strains that are morphologically and physiologically distinct. If the criterion of conidium color is deemphasized, the entire *P. martensii-P. viridicatum* I continuum as described by Ciegler et al. (5) represents one broad "species," "P. cyclopium p" (including *P. aurantiogriseum*, *P. olivinoviride*, *P. viridicatum* I, and *P. palitans* pro parte, the former sensu Pitt [30], the others sensu Raper and Thom [33], and Ciegler et al. [5]).

The differences between *P. cyclopium* and *P. viridicatum* (Raper and Thom [33]) and the corresponding species according to Samson et al. (35) and Pitt (30) are primarily differences in conidium color (en masse). The conidium color is an expression of the secondary metabolism of melanins and other products (4, 10) in the conidium wall, and the difference between green and bluegreen is continuous and not sharp as is ability to produce the secondary metabolites penicillic acid or ochratoxin A. As identified by the methods of Raper and Thom (33) or Samson et al. (35), *P. viridicatum* and *P. cyclopium* (*P. verrucosum* var. *verrucosum* and *P. verrucosum* var. *cyclopium*) both produce viridicatin (12, 17, 24), xanthomegnin, viomellein (39), ochratoxin A (3, 13, 23, 42), penicillic acid (5, 22), and other products (14, 31). Results from the present study and from Stack and Mislevic (39), Ciegler et al. (5), and Leistner and Eckardt (14) seem to indicate that "P. viridicatum o-c" (= *P. viridicatum* II + *P. viridicatum* III according to Ciegler et al. [5]) produces only ochratoxin A and citrinin and that "P. cyclopium p" produces many known toxins such as penicillic acid, S-toxin, brevianamid, viridicatumtoxin, xanthomegnin, viomellein, viridicatin, viridicatol, cyclopenin, cyclopenol, cyclopiazonic acid, and in few cases penitrem A. The difference in smell and growth pattern on malt agar between *P. viridicatum* I on the one hand and *P. viridicatum* II and III on the other as shown by Stack and Mislevic (39) and Ciegler et al. (5) is confirmed in the present study, and other differences between these groups are shown in Tables 4 and 5. One of the most distinct differences between "P. cyclopium p" and "P. viridicatum o-c" is the consistent difference in ability to grow on nitrite as sole nitrogen source. Another difference between "P. cyclopium p" and "P. viridicatum o-c" is that all cultures of the latter (except some cultures isolated from meat products, i.e., *P. viridicatum* III) had a red brown reverse on YES agar. Of 111 isolates of "P. viridicatum o-c," all had a red brown reverse on YES agar. All of these isolates represented *P. viridicatum* II.

In the revision of the taxonomy of the fasciculate penicillia (35), *P. crustosum* was included in *P. verrucosum* var. *cyclopium*. Furthermore, isolates like NRRL 3476 and NRRL 3477 have been identified as *P. cyclopium*, *P. viridicatum*, and *P. crustosum* by different authors (6). In this investigation, the two last-mentioned cultures, *P. palitans* NRRL 3468 and all isolates named *P. crustosum* (including Sp. 458 and Sp. 1191, which were received as *P. verrucosum* var. *cyclopium*) made up a very homogeneous group. This is in agreement with Pitt (30), but Pitt also included *P. verrucosum* var. *melanochlorum* in his concept of *P. crustosum*.

"P. crustosum pA" and "P. melanochlorum" have many characteristics in common, but they also differ in some characteristics (Table 6). "P. melanochlorum" seems to be a distinct fungus and should probably be given species status. This mold has also been found on fermented tallow used for human consumption on the Faroe Islands (unpublished results). Nearly all of the isolates of "P. melanochlorum" investigated produced three characteristic red metabolites (as seen on thin-layer chromatography plates after anisaldehyde treatment) irrespective of their sources. "P. crustosum pA" is a producer of penitrem A, but *P. cyclopium* NRRL 1899 and *P. olivinoviride* NRRL 958 also produce this toxin. These latter isolates seem to be intermediates between "P. cyclopium p" and "P. crustosum pA". Such strains have never been encountered in our laboratory, but according to Ciegler and Pitt (6) they occur infrequently in *P. olivinoviride* and *P. cyclopium*.

Reliability of the physiological criteria used. If physiological criteria are to be used in determinative taxonomy, they must be consistent and stable. The tests shown in Table 4 were stable as they gave the same results in many independent experiments. Furthermore, all of the isolates listed in Tables 1 and 2 showed consistent physiological reactions within each species (on NO2 agar, CREA agar, PCNB medium, GY agar at 37°C, and GYA agar).

The ability to grow at 37°C has been used as a taxonomic feature by Pitt (30). However, Pitt (30) mentions that some *P. citrinum* and *P. chrysogenum* isolates fail to grow at this temperature. It is probable that citrinin producers within these species all grow at 37°C. However, citrinin production and ability to grow at 37°C are not positively correlated in all penicillia (Table 4).

The ability of some species of *Penicillium* to grow on NO2 agar seems to be a very consistent criterion. Nitrite media were used earlier by Abe (1) and Engel and Teuber (8) with good results.

The ability of the penicillia to grow on CREA agar is also a consistent criterion (also found for *P. roqueforti* by Engel and Teuber [8]). However, some strains of "P. cyclopium p" and *P. hirsutum* first grew insignificantly on CREA agar, but after 8 to 14 days of incubation they grew rather well. Unlike *P. roqueforti*, *P. expansum*, "P. crustosum pA," and "P. melanochlorum," these isolates had thin colony margins on the medium. Acid production on CREA agar was also a consistent and stable criterion provided it was recorded after 5 to 7 days of incubation. *P. expansum*, "P. crustosum pA", and "P. melanochlorum" consistently produced some basic metabolites about 1 week after the abundant acid production.

Production of extracellular enzymes by molds may prove to be taxonomically useful. In some cases the production of exogenous tricaproinase is consistent. More isolates should be investigated before any conclusions are drawn. Investigation of lipase production in molds producing mycotoxins is important. Ba et al. (2) have thus found that toxigenic strains of *Aspergillus flavus* produce twice as much lipase as do non-toxigenic strains.

Criteria based on resistance towards fungicides and preservatives are not as significant taxonomically as is the ability to utilize nitrite or creatine or the ability to produce certain secondary metabolites. The former characteristics may be results of recent evolutionary adaptation to different environments. In *P. roqueforti*, however, the resistance towards preservatives seems to be genotypic.

After 2 weeks of incubation, isolates from all species/groups could grow on GYBS medium. Therefore, the data concerning resistance towards preservatives must be recorded after 5 to 7 days of incubation.

The ability of the penicillia to grow on GYP medium is a consistent criterion in most cases, but it should be used mostly as a confirmative criterion since it is inconsistent in the case of "P. cyclopium p" and *P. hirsutum* (Table 4). The following species did not grow or grew very faintly on GYP medium after 2 weeks of incubation: *P. brevicompactum*, *P. chrysogenum*, *P. citrinum*, *P. patulum*, "P. viridicatum o-c", and some strains of "P. cyclopium p" and *P. hirsutum*.

Ability to produce fascicles on PDAB (Table 4) seems to be a consistent criterion in most cases, but an inconsistency is recorded in "P. cyclopium p" and "P. viridicatum o-c". As noted by Pitt (30), fasciculation is a good confirmative and sometimes a diagnostic criterion. Mislevic (19, 20) recommends the use of this criterion in *Penicillium* taxonomy.

The growth rate of strains of "P. crustosum pA" was very high on PCNB medium, and this feature facilitated identification of these strains. Growth rate on this medium seems to be a consistent criterion, but of use for only a few species.

Table 4 shows that "P. cyclopium p" is a very variable group. An expression of this variability can be seen in Table 7. Penicillic acid producers are more prevalent among preservative "resistant" isolates. Isolates identified by the method of Raper and Thom (33) (*P. martensii*, *P. cyclopium*, and *P. viridicatum* [I]) were represented

in all of the "preservative groups" (GYP−/GYBS−, GYP+/GYBS−, etc.), but of the *P. puberulum* isolates examined only one was preservative "resistant." These isolates of *P. puberulum* (Table 2, footnote *e*) did not fit the description of Pitt (30). They were similar to *P. puberulum* NRRL 3564, but did not produce penicillic acid.

Chemotaxonomic characterization of the penicillia. All of the species/groups showed a characteristic pattern of metabolites on the thin-layer chromatography plates; however, some species/groups had two types of patterns (i.e., *P. roqueforti*). Most patterns were consistent from isolate to isolate, but in some cases one, two, and up to all metabolites could be lacking (using the agar plug method [9], the extractions of the cultures contained metabolites in common for all species/groups). Because of this fact, the usefulness of thin-layer chromatography patterns in the identification of penicillia is somewhat limited. In most cases, however, they are helpful as auxiliary and confirmatory characteristics. It should be mentioned that some of the media used in the new *Penicillium* handbook (30) are good toxin production media (CYA and MEA [14, and unpublished results]).

In the review by Scott (36a), 17 species of *Penicillium* are cited as producers of penicillic acid. If the group "P. cyclopium p" is accepted as one "species," the number of *Penicillium* species producing penicillic acid may be reduced to nine or less. Corresponding examples can be given for other mycotoxins. Table 4 shows that there seems to be a correlation between mycotoxin production and the species/groups determined using the physiological (and morphological) criteria. Only a few species seem to be able to produce more than two fundamentally different mycotoxins. In *P. roqueforti*, two "chemical races" seem to be present: the roquefortin/PR-toxin race (14, 27, 37) and the penicillic acid/patulin race (11, 14, 25, 26). In "P. cyclopium p", a xanthomegnin/S-toxin/penicillic acid/brevianamid race, a cyclopiazonic acid race, and a minor penitrem A race seem to be present (14, 39). Maybe other physiological criteria can separate such mycotoxin producers in the same species/group.

Conclusion. The present investigations have shown that certain physiological criteria can be important aids in the identification of common asymmetric penicillia. In the subgenus *Penicillium* of Pitt (30), the criteria of growth at 5°C and at 37°C can be excluded and instead the *Penicillium* isolates should be inoculated on NO2 agar and CREA agar. These media provide consistent and stable data. Furthermore, the *Penicillium* isolates could be examined during incubation using the simple agar plug method for detection of extracellular products. The pattern of metabolites may indicate at once which mycotoxins the isolate produces and, therefore, the species/group to which it belongs. The other media used in this investigation may be more variable, but in return they give the investigator data on some ecological characteristics of the isolate under examination.

On the basis of the physiological, mycotoxicological, and morphological data obtained in this investigation, it is reasonable to propose four groups of isolates (species). (i) "P. melanochlorum" (= *P. verrucosum* var. *melanochlorum* sensu Samson et al. [35]), producing no known toxins, seems to be a separate species. (ii) "P. crustosum pA" (probably equal to *P. crustosum* sensu Raper and Thom [33]) is a very homogeneous species, and it seems that fresh isolates of this mold always produce penitrem A. (iii) The nitrite-positive "P. viridicatum o-c" is also a very homogeneous "species" and is probably the only "taxon" in the subgenus *Penicillium* containing ochratoxin producers. This group comprises the strains of *P. viridicatum* II and III (5) included in *P. viridicatum* and *P. verrucosum* sensu Pitt (30). (iv) The acid-producing "P. cyclopium p" group embraces penicillic acid producers and producers of many other toxins. This group is variable, but does not include producers of citrinin or ochratoxins. "P. cyclopium p" consists of *P. aurantiogriseum* sensu Pitt (30) and *P. viridicatum* I (5) (including *P. olivinoviride* and *P. palitans* pro parte). These four groups could be the basis for the definition of four new species within the subgenus *Penicillium* and thus link the production of particular mycotoxins to fewer species.

ACKNOWLEDGMENTS

C. W. Hesseltine and D. Wicklow, Northern Regional Research Laboratory, Agricultural Research Service, Peoria, Ill., H. K. Frank, Bundesforschungsanstalt für Lebensmittelfrischhaltung, Karlsruhe, Federal Republic of Germany, and L. Leistner, Bundesanstalt für Fleischforschung, Kulmbach, Federal Republic of Germany, kindly supplied some of the cultures used in this study. I thank L. Leistner also for the penitrem A standard, Ole Filtenborg for fruitful discussions, and Ole Filtenborg and Peter Zeuthen for proofreading of the manuscript.

LITERATURE CITED

1. **Abe, M.** 1956. Studies on the classification of the Penicillia. J. Gen. Appl. Microbiol. **2**:1-344.
2. **Ba, D., M. Jemmali, and R. Drapron.** 1977. Activité lipolytique et production d'aflatoxines chez *Aspergillus flavus*. Ann. Microbiol. (Inst. Pasteur) **128B**:87-93.
3. **Barnes, J. M., P. K. C. Aystwick, R. L. Carter, F. V. Flynn, G. C. Peristianis, and W. N. Aldridge.** 1977. Balkan (endemic) nephropathy and a toxin-producing strain of *Penicillium verrucosum* var. *cyclopium*: an experimental model in rats. Lancet **i**:671-675.

4. **Bartsch, E., W. Lerbs, and M. Luckner.** 1979. Phenol oxidase activity and pigment synthesis in conidiospores of *Penicillium cyclopium*. Z. Allg. Mikrobiol. **19**:75–82.
5. **Ciegler, A., D. I. Fennell, G. A. Sansing, R. W. Detroy, and G. A. Bennett.** 1973. Mycotoxin producing strains of *Penicillium viridicatum*: classification into subgroups. Appl. Microbiol. **26**:271–278.
6. **Ciegler, A., and J. I. Pitt.** 1977. Survey of the genus *Penicillium* for tremorgenic toxin production. Mycopathol. Mycol. Appl. **42**:119–124.
7. **Ďuračková, Z., V. Betina, and P. Nemec.** 1976. Systematic analysis of mycotoxins by thin-layer chromatography. J. Chromatogr. **116**:141–154.
8. **Engel, G., and M. Teuber.** 1978. Simple aid for the identification of *Penicillium roqueforti* Thom. Growth in acetic acid. Eur. J. Appl. Microbiol. Biotechnol. **6**: 107–111.
9. **Filtenborg, O., and J. C. Frisvad.** 1980. A simple screening-method for toxigenic moulds in pure cultures. Lebensm. Wiss. Technol. **13**:128–130.
10. **Ha-Huy-Kè, and M. Luckner.** 1979. Structure and function of conidiospore pigments of *Penicillium cyclopium*. Z. Allg. Mikrobiol. **19**:117–122.
11. **Harwig, J., B. J. Blanchfield, and P. M. Scott.** 1978. Patulin production by *Penicillium roqueforti* Thom from grape. Can. Inst. Food Sci. Technol. J. **11**:149–151.
12. **Korzybski, T., Z. Kowszyki-Gindifer, and W. Korylowicz.** 1967. Antibiotics. Origin, nature and properties, vol. 2, p. 1257–1258. Pergamon Press, Oxford.
13. **Krogh, P.** 1978. Causal associations of mycotoxic nephropathy. Acta Pathol. Microbiol. Scand. Sect. A. Suppl. **269**:1–28.
14. **Leistner, L., and C. Eckardt.** 1979. Vorkommen toxinogener Penicillien bei Fleischerzeugnisse. Fleischwirtschaft **59**:1892–1896.
15. **Leistner, L., and J. I. Pitt.** 1977. Miscellaneous *Penicillium* toxins, p. 639–653. In J. V. Rodricks, C. W. Hesseltine and M. A. Mehlmann (eds.), Mycotoxins in human and animal health. Pathotox Publishers, Park Forest South.
16. **Lötzsch, R., F. Tauchmann and W. Meyer.** 1974. Einsatz des "Stomacher" in der Mykotoxin-Analytik. Fleischwirtschaft **54**:943–945.
17. **Luckner, M., L. Nover, and H. Böhm.** 1977. Secondary metabolism and cell differentiation. Molecular Biology Biochemistry and Biophysics *23*. Springer Verlag, Berlin, Heidelberg, New York.
18. **Marti, L. R., D. M. Wilson, and B. D. Evans.** 1978. Determination of citrinin in corn and barley. J. Assoc. Offic. Anal. Chem. **61**:1353–1358.
19. **Mislevic, P. B.** 1975. The effect of botran on fascicle production by species of *Penicillium*. Mycologia **67**: 194–198.
20. **Mislevic, P. B.** 1977. The genus *Penicillium*, p. 41–57. *In* T. D. Wyllie and L. G. Morehouse (ed.), Mycotoxic fungi Mycotoxins Mycotoxicosis, vol. 1. Mycotoxic fungi and chemistry of mycotoxins. Marcel Dekker, New York.
21. **Murray, I. G.** 1968. Some aspects of the biochemical differentiation of pathogenic fungi: a review. J. Gen. Microbiol. **52**:213–221.
22. **Northolt, M. D., H. P. van Egmond, and W. E. Paulsch.** 1979. Penicillic acid production by some fungal species in relation to water activity and temperature. J. Food Prot. **42**:476–484.
23. **Northolt, M. D., H. P. van Egmond, and W. E. Paulsch.** 1979. Ochratoxin A production by some fungal species in relation to water activity and temperature. J. Food Prot. **42**:485–490.
24. **Nover, L., and M. Luckner.** 1969. On the biosynthesis of cyclopenin and cyclopenol, benzodiazepides alkaloids from *Penicillium cyclopium* Westling. Eur. J. Biochem. **10**:268–273.
25. **Olivigni, F. J., and L. B. Bullerman.** 1977. Simultaneous production of penicillic acid and patulin by a *Penicillium* species isolated from cheddar cheese. J. Food Sci. **42**:1654–1657, 1665.
26. **Olivigni, F. J., and L. B. Bullerman.** 1978. Production of penicillic acid and patulin by an atypical *Penicillium roqueforti* isolate. Appl. Environ. Microbiol. **35**:435–438.
27. **Orth, R.** 1976. PR-toxin production by *Penicillium roqueforti* strains. Z. Lebensm. Unters. Forsch. **160**:131–136.
28. **Pitt, J.** 1973. An appraisal of identification methods for *Penicillium* species: novel criteria based on temperature and water relations. Mycologia **65**:1135–1137.
29. **Pitt, J. I.** 1974. A synoptic key to the genus *Eupenicillium* and to sclerotiogenic *Penicillium* species. Can. J. Bot. **52**:2231–2236.
30. **Pitt, J. I.** 1979. The genus *Penicillium* and its teleomorphic states *Eupenicillium* and *Talaromyces*. Academic Press, Inc., London.
31. **Pohland, A. E., and P. B. Mislevic.** 1976. Metabolites of various *Penicillium* species encountered in foods. Adv. Chem. Ser. **149**:110–143.
32. **Purchase, I. H. F.** 1974. *Penicillium cyclopium*, p. 149–162. *In* I. H. F. Purchase (ed.), Mycotoxins. Elsevier, Amsterdam.
33. **Raper, K. B., and C. Thom.** 1949. A manual of the Penicillia. Williams and Wilkins Co., Baltimore.
34. **Samson, R. A., R. Hadlok, and A. C. Stolk.** 1977. A taxonomic study of the *Penicillium chrysogenum* series. Antonie van Leeuwenhoek. J. Microbiol. Serol. **43**:261–274.
35. **Samson, R. A., A. C. Stolk, and R. Hadlok.** 1976. Revision of the subsection fasciculata of *Penicillium* and some allied species. Stud. Mycol. Baarn **11**:1–47.
36. **Scott, P. M.** 1974. Collaborative study of a chromatographic method for determination of patulin in apple juice. J. Assoc. Offic. Anal. Chem. **57**:621–625.
36a. **Scott, P. M.** 1977. Penicillium mycotoxins, p. 283–356. *In* T. P. Wyllie and L. G. Morehouse (ed.), Mycotoxic fungi, mycotoxins, mycotoxicosis, vol. 1. Mycotoxic fungi and chemistry of mycotoxins. Marcel Dekker, New York.
37. **Scott, P. M., and B. P. C. Kennedy.** 1976. Analysis of blue cheese for roquefortine and other alkaloids from *Penicillium roqueforti*. J. Agric. Food Chem. **24**:865–868.
38. **Scott, P. M., J. W. Lawrence, and W. van Walbeek.** 1970. Detection of mycotoxins by thin-layer chromatography: application to screening of fungal extracts. Appl. Microbiol. **20**:839–842.
39. **Stack, M. E., and P. B. Mislevic.** 1978. Production of xanthomegnin and viomellein by isolates of *Aspergillus ochraceus*, *Penicillium cyclopium* and *Penicillium viridicatum*. Appl. Environ. Microbiol. **36**:552–554.
40. **Steyn, P. S.** 1969. The separation and detection of several mycotoxins by thin-layer chromatography. J. Chromatogr. **45**:473–475.
41. **Thatcher, F. S., and D. S. Clark.** 1978. Microorganisms in food. *1.* Their significance and enumeration, 2nd ed. University of Toronto Press, Toronto.
42. **Walbeek, W. van, P. M. Scott, J. Harwig, and J. U. Lawrence.** 1969. *Penicillium viridicatum* Westling: a new source of ochratoxin A. Can. J. Microbiol. **51**:1281–1285.