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# Mycotoxin-Producing Strains of *Penicillium viridicatum*: Classification into Subgroups

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Fifty-two isolates of *Penicillium viridicatum* Westling were divided into three groups based on ability to produce ochratoxin and/or citrinin, color, growth rate, type of growth, odor, and isolation source. Members of group I resemble one of the representative strains of *P. viridicatum* described in the literature; those belonging to group II differ from group I strains in several characteristics; group III is a heterogeneous series of highly variable isolates. Although three subgroupings can be recognized, retention of all isolates in the species *P. viridicatum* is deemed most appropriate at this time. Spore macerates of all isolates were examined for virus-like particles but none were detected.

Various isolates identified as *Penicillium viridicatum* Westling are capable of producing one or more mycotoxins including ochratoxin (5, 15), citrinin and oxalic acid (8), penicillic acid (7), a hepatorenal toxin (3), and a substance causing photosensitization in mice (4). However, other isolates appear to produce no demonstrable mycotoxins.

A limited number of reports indicate that fungal secondary metabolite synthesis, including mycotoxins, may be affected by the presence of virus-like particles (VLP) (1, 2, 11). VLP have been observed throughout the cytoplasm in thin sections of hyphae and spores of *P. stoloniferum* and *P. brevi-compactum* (9).

The purpose of our experiments was to determine if there was any correlation between cultural characteristics, mycotoxin synthesis, and possible occurrence of VLP in 52 isolates of *P. viridicatum*.

This investigation was presented at the 73rd annual meeting of the American Society for Microbiology, Miami Beach, Florida, May 6 to 11, 1973.

## MATERIALS AND METHODS

**Cultures.** Cultures were obtained from the Agricultural Research Service Culture Collection of the Northern Laboratory and from the Centraalbureau voor Schimmelcultures, Baarn, Netherlands. During the course of the investigation, cultures were maintained on Difco yeast malt (YM) agar slants.

For taxonomic studies, cultures were three-point inoculated on Czapek solution agar (Cz) and Blakeslee's malt agar (BMA) in 100-mm petri dishes: Cz: sucrose, 30 g; $NaNO_3$, 2 g; $K_2HPO_4$, 1 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; KCl, 0.5 g; $FeSO_4 \cdot 7H_2O$, 0.01 g; agar, 15 g; water, 1 liter; BMA: malt extract, 20 g; peptone, 1 g; dextrose, 20 g; agar, 20 g; water, 1 liter. Cultures were incubated 10 to 14 days at 25 C.

To obtain quantities of conidia for VLP analyses, the fungi were inoculated from slants onto white bread cubes (1 $cm^3$) sterilized for 10 min at 121 C in 300-ml Erlenmeyer flasks. The bread had no preservatives added and was purchased at a local bakery. After 10 days of incubation at 28 C, a single heavily molded cube was added to 225 to 250 g of sterile cubed bread in 2.8-liter Fernbach flasks; flasks were again incubated for 10 days. Conidia were recovered by the addition of 500 ml of 1:10,000 sterile aqueous Triton X-100, gentle agitation to dislodge conidia, and filtration through cheesecloth. Spore suspensions were centrifuged and washed twice with sterile water to remove starch. Approximately 18 to 22 g (wet weight) of spore paste (5–6 g dry weight) were recovered per flask.

**VLP detection.** Sufficient spore paste to give 1 g dry weight was suspended in 45 ml of 0.1 M phosphate buffer, pH 7.2. Suspensions were placed in 75-ml glass Bronwill flasks containing 45 g of 0.5-mm glass beads and homogenized for 2 min at 4,000 rpm with a Bronwill mechanical cell homogenizer (Braun Model MSK) bathed in a $CO_2$ stream; stream flow was adjusted to prevent freezing of the flask contents. Spore suspensions and homogenizing flasks were kept cold in an ice bath prior to processing. Homogenized spores were examined under the microscope to determine extent of spore rupture and then were centrifuged at 8,000 rpm ($7.7 \times 10^3$ *g*) to remove spore debris; the clear supernatant fluid was recentrifuged at 32,000 rpm ($10.5 \times 10^4$ *g*) for 2.5 h. The supernatant fluid was discarded and the pellet was suspended in 1.5 ml of phosphate buffer. This suspension was filtered through a membrane filter (0.45 $\mu$m pore size; Millipore Corp.); a drop was placed on a Formvar-

coated copper grid and allowed to dry. A drop of 1% uranyl acetate was placed on the grid and blotted off after 10 s. The grid was rinsed three times with distilled water, air-dried, and examined under an RCA EMU-3 electron microscope for the presence of VLP.

**Mycotoxin analyses.** A 500-ml amount of YES broth (2% Difco yeast extract plus 4% sucrose) in Fernbach flasks was inoculated with one cube of molded bread per flask and incubated at 28 C as still cultures for 8 to 10 days. The broths were decanted, adjusted to pH 2.5 with HCl, and extracted twice with an equivalent volume of $CHCl_3$. The solvent, after being dried with anhydrous $Na_2SO_4$, was removed by flash evaporation, and the residual solids were dissolved in 2 ml of $CHCl_3$. Portions (10 $\mu$liters) were spotted on Brinkmann Silica Gel N-HR thin-layer chromatographic (TLC) plates, along with penicillic acid, citrinin, and ochratoxin reference samples, and the plates were developed in chloroform-ethyl acetate-formic acid (60:40:1). Plates were examined under ultraviolet light (UV) for ochratoxin and citrinin which fluoresce green and bright yellow, respectively, and then were exposed to ammonia to produce a blue fluorescent penicillic acid derivative (6). The yellow fluorescence of citrinin tends to mask the green fluorescence of ochratoxin since both, when present, are at about the same $R_f$. However, citrinin ceases to fluoresce after ammonia exposure, revealing an intense blue-fluorescing ochratoxin-ammonia derivative.

Penicillic acid was confirmed by reactions with both ammonia and with diphenylboric acid ethanolamine to give blue-fluorescing derivatives; ochratoxin A by reaction with ammonia to give a blue-fluorescing derivative and by methyl ester formation (12); citrinin by reaction with $FeCl_3$ to give an orange derivative. The $R_f$ values were also compared by TLC in other solvent systems (7).

**GLC of volatiles.** Cultures were grown in bottles for 10 days on BMA, barley, and corn; the bottles were stoppered, and the headspace was sampled by means of a 2-ml pressure-type syringe; 1 ml of gas was injected onto a gas-liquid chromatography (GLC) column. Separation of volatile substances was carried out with a Bendix model 2500 gas chromatograph equipped with a flame ionization detector. Columns (1.83 by 2 mm, inner diameter) packed with Poropak type P, 80/100 mesh (Waters Associates), were temperature programmed from 50 to 120 C at 5 C/min. Nitrogen was used as the carrier gas at a flow rate of 50 ml/min. Injection port and detector were 210 and 250 C, respectively.

Coupled GLC-mass spectrometry (MS) (Dupont Model CEC) was used to obtain mass spectra of the major substance produced by *P. viridicatum* NRRL 5572. This compound was identified by comparing its GLC retention time and mass spectrum to those of an authentic standard.

## RESULTS AND DISCUSSION

**Analyses for VLP.** None of the 51 strains of *P. viridicatum* examined revealed the presence of VLP. It is possible, however, that VLP could be found if even larger masses of conidia or mycelia were examined. However, it appears unlikely that such low concentrations of VLPs would, should they occur, affect secondary metabolite synthesis by *P. viridicatum*.

**Culture characteristics and mycotoxin synthesis.** All isolates used in this survey had been identified as *P. viridicatum* by one or more investigators. Identification was based on Raper and Thom's *Manual of the penicillia* (13) in which species diagnoses are based primarily on cultural and morphological characteristics displayed on Cz. Differences on BMA are not emphasized as diagnostic. Survey isolates growing on this latter medium could be arranged into several groups on the basis of color, texture, growth rate of colonies, and odor. This separation was supported by a comparison of the original source of the isolates and their ability to produce ochratoxin and citrinin.

**Group I.** The 22 strains in group I (Table 1) were isolated primarily from plant sources and produced no detectable ochratoxin or citrinin. Intraperitoneal injection of the $CHCl_3$ extracts of the culture broths (YES medium) into mice resulted in no noticeable toxicity. One culture (NRRL 5570) produced penicillic acid and appears to be intermediate between groups I and II; the colony pattern and odor on BMA are as group I, on Cz as group II.

In morphological and cultural characters on Cz and BMA (Fig. 1), these isolates duplicate NRRL 963, cited by Raper and Thom (13) as the primary culture used in their description of *P. viridicatum* and shown, on Cz, in color plate VIII of the Manual. On BMA, colonies are plain, rapidly and heavily sporing, thinning, and granular or slightly fasciculate at the margins, attaining a diameter of 6 to 6.5 cm in 2 weeks at 25 C. Conidia are bright yellow-green when young, quickly becoming forest green and remaining so in age; conidial chains are in columns, shattering easily; reverse is yellow-gold. Odor is penetrating, woodsy, earthy, *Streptomyces*-like, stifling. Penicilli are large and asymmetrically branched and indistinguishable from many other species in the Fasciculata.

Isolates conforming with this description are among the most common penicillia isolated from moldy grains. They represent the yellow-green end of the *P. viridicatum-martensii* continuum discussed under taxonomic implications.

**Group II.** The 17 cultures in this group (Table 2) also originated from plant sources and most produced both ochratoxin and citrinin;

TABLE 1. *Characteristics of P. viridicatum, group I*

| Culture no. | Source | | Toxin synthesis | | Colony characters on BMA[a] | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Geographical | Substrate | Ochratoxin | Citrinin | Diameter | Surface texture | Color | Reverse | Odor |
| NRRL 963 | Washington, D.C. | Air | ND[b] | ND | S[c] | SF[d] | FG[e] | O[f] | W[g] |
| NRRL 3586 | Michigan | Wheat flour | ND | ND | S | SF | FG | O | W |
| NRRL 3600 | Pennsylvania | Wheat | ND | ND | S | SF | FG | O | W |
| NRRL 5569 | Kansas | Corn | ND | ND | S | SF | FG | O | W |
| NRRL 5570 | Unknown | Oregano | ND | ND | S | SF | GFG[h] | GY[i] | W |
| A-14307 | Michigan | Wheat flour | ND | ND | S | SF | FG | O | W |
| A-15059 | Wisconsin | Corn | ND | ND | S | SF | FG | O | W |
| A-15105 | Montana | Wheat flour | ND | ND | S | SF | FG | O | W |
| A-15402 | Texas | Wheat flour | ND | ND | S | SF | FG | O | W |
| A-17919 | Indiana | Corn | ND | ND | S | SF | FG | O | W |
| A-18341 | Kansas | Corn | ND | ND | S | SF | FG | O | W |
| A-18343 | Kansas | Corn | ND | ND | S | SF | FG | O | W |
| A-18563 | Michigan | Wheat | ND | ND | S | SF | FG | O | W |
| A-18565 | Michigan | Wheat | ND | ND | S | SF | FG | O | W |
| A-18567 | Michigan | Wheat | ND | ND | S | SF | FG | O | W |
| A-18609 | Michigan | Wheat | ND | ND | S | SF | FG | O | W |
| A-18611 | Michigan | Wheat | ND | ND | S | SF | FG | O | W |
| A-18616 | Michigan | Wheat | ND | ND | S | SF | FG | O | W |
| A-18876 | Michigan | Wheat | ND | ND | S | SF | FG | O | W |
| A-19118 | Michigan | Wheat | ND | ND | S | SF | FG | O | W |
| A-19121 | Michigan | Wheat | ND | ND | S | SF | FG | O | W |

[a] BMA, Blakeslee's malt agar.
[b] ND, not detected.
[c] Broadly spreading, rapid.
[d] Slightly fasciculate marginally.
[e] Forest green.
[f] Orange.
[g] Woodsy, earthy, penetrating.
[h] Grayed forest green.
[i] Golden yellow, diffusible.

two produced only citrinin and four produced only ochratoxin.

On Cz, colonies are 2.0 to 2.5 cm in diameter in 12 days at 25 C, indistinctly zonate, consisting of a tough, somewhat raised and radially wrinkled white to light cream mycelium; scant to abundant clear or faintly yellow exudate is produced in small to conspicuous droplets on the mycelium; poorly sporulating from the center outward; reverse colorless through various yellow shades to brown; continuing to grow and reaching diameters of 4.5 to 5.0 cm at 1 month, in some strains remaining almost velvety but in the majority becoming strongly fasciculate (Fig. 2). Fasciculation, not evident in young cultures, appears to develop as a result of growth and regrowth over exudate droplets. Sporulation is heaviest in the fascicles which are formed in concentric zones, leaving nonsporulating white basal mycelium exposed in alternate zones. Young conidia in both velvety and fasciculate

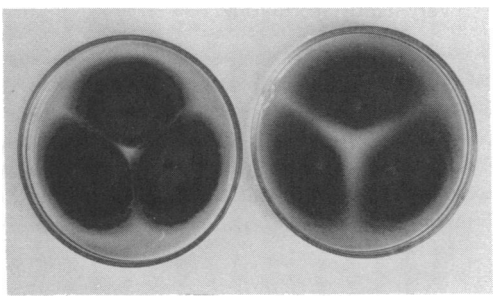

FIG. 1. *Strain NRRL 963 representative of group I P. viridicatum on Cz (left) and BMA (right), 12 days, 24 C.*

strains are definitely blue-green but quickly change to yellow-green and retain this color in age. Such a change was originally described for *P. verrucosum* Dierckx which was reduced to synonymy with *P. viridicatum* by Raper and Thom (13) and was cited as a characteristic of

TABLE 2. *Characteristics of P. viridicatum, group II*

| Culture no. | Source | | Toxin synthesis | | Colony characters on BMA | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Geographical | Substrate | Ochra-toxin | Citrinin | Diameter | Surface texture | Color | Reverse | Odor |
| NRRL 3710[a] | Canada | Wheat | + | + | M[b] | V[c] | VG[d] | PRB[e] | P(?)[f] |
| NRRL 3712 | Canada | Wheat | + | − | R[g] | F[h] | GG[i] | UC[j] | P[k] |
| NRRL 5571 | Michigan | Wheat | + | + | R | F | GG | PRB | P(?) |
| NRRL 5572 | Canada | Wheat | + | − | R | F | GG | RB[l] | P |
| NRRL 5583 | Canada | Beans | − | + | R | F | GG | PRB | P(?) |
| A-17732 | Canada | Wheat | + | + | R | F | GG | UC | P(?) |
| A-18686 | Michigan | Wheat | + | + | R | F | GG | PRB | P(?) |
| A-18689 | Michigan | Wheat | + | + | R | F | GG | PRB | P(?) |
| A-20216 | Canada | Beans | + | + | R | F | GG | RB | P(?) |
| A-20217 | Canada | Peanuts | + | − | R | SF[m] | GG | RB | P |
| A-20218 | Canada | Peanuts | + | + | R | F | GG | PRB | P(?) |
| A-20219 | Canada | Beans | − | + | M | SF | LGG[n] | UC | FW[o] |
| A-20221[a] | Canada | Beans | + | + | R | F | GG | PRB | P(?) |
| A-20222 | Canada | Beans | + | + | R | F | GG | RB | P |
| A-20223 | Canada | Beans | + | + | M | Fl[p] | VLS[q] | PRB | P(?) |
| A-20224 | Canada | Animal feed | + | + | R | F | GG | RB | P(?) |
| A-20225[a] | Canada | Peanuts | + | − | M | SF | GG | UC | P(?) |

[a] Some color change on Czapek's solution agar or throwing sectors showing color change of group III.
[b] Moderate growth, 3.0 to 3.5 cm in diameter at 2 weeks.
[c] Velvety.
[d] Yellow-green.
[e] Pale reddish brown.
[f] 2-Pentanone?
[g] Restricted growth, 2.0 to 2.5 cm in diameter at 2 weeks.
[h] Fasciculate.
[i] Slightly grayed green.
[j] Uncolored or light flesh.
[k] 2-Pentanone.
[l] Red-brown.
[m] Slightly fasciculate.
[n] Light gray-green.
[o] Faint woodsy.
[p] Floccose.
[q] Very lightly sporulating.

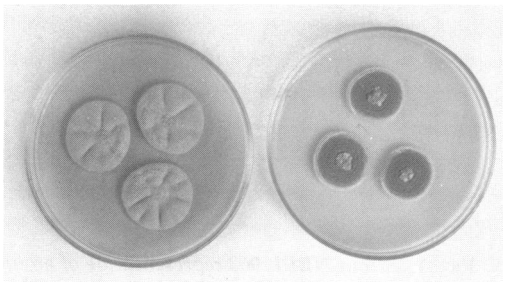

FIG. 2. *Strain NRRL 5572 representative of group II P. viridicatum on Cz (left) and BMA (right), 12 days, 24 C.*

*P. palitans* Westling strains used by Scott et al. (14).

Colonies on BMA are restricted, 2.0 to 2.5 cm in 12 days at 25 C, grayed yellow-green, granular to definitely fasciculate marginally but, as on Cz, continuing to grow and reaching a diameter of 3.5 to 4.0 cm at 1 month, becoming zonate and more conspicuously fasciculate during later stages of growth. Strains that remain almoust velvety on Cz are more gray and less fasciculate on BMA. Conidial chains in columns tend to form crusts; reverse ranges from uncolored to pale yellow to reddish-orange in age showing some red at colony centers. Penicillus morphology is similar to that of strains in group I (Fig. 3). Most produce a solvent odor, strong in some, barely detectable in others. The volatile material from NRRL 5572 has been identified by GLC and MS as 2-pentanone.

None of the cultures cited by Raper and Thom as representative of *P. viridicatum* can be included in this group of isolates. They have been encountered most often on toxic grain from Canada and Michigan. Incubation of isolation plates at 15 C, rather than 25 C, encourages their development.

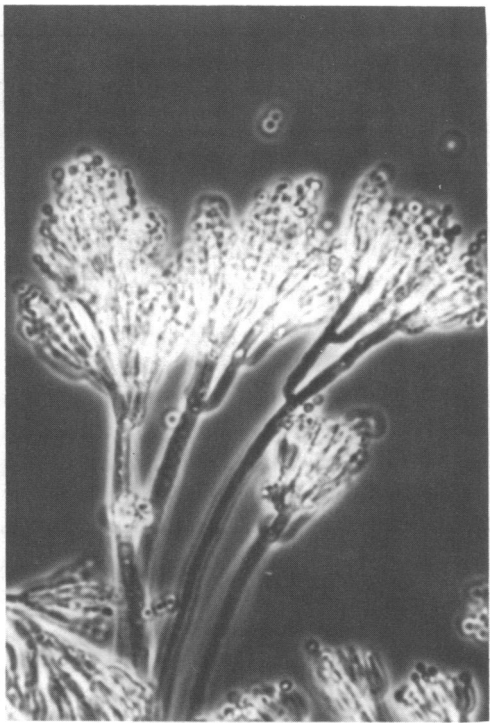

FIG. 3. *Penicilli of strain NRRL 5583, representative of group II P. viridicatum, showing morphology of strains included in both groups I and II, ×875.*

**Group III.** Group III (Table 3) is a heterogeneous assemblage of 13 strains isolated from meat or air in a meat packing plant. The majority were isolated quite recently from European mold-fermented sausages and have a history of variability in cultural pattern and color as well as ability to produce ochratoxin. At one time, all have produced the toxin; four strains have lost this trait. None produced either citrinin or penicillic acid.

Colonies on Cz (Fig. 4) are close-textured, velvety, 3 to 3.5 cm in diameter in 12 days at 25 C, plane to radially wrinkled, sporulating abundantly in fairly bright yellow-green shades but quickly becoming various gray-brown shades; reverse uncolored to drab pink to orange-brown; exudate scant, occurring as very small droplets within the basal mycelium; odor faint, rather pleasant.

Colonies on BMA (Fig. 4) are plain, close-textured and velvety or showing an overgrowth of aerial mycelium to give a slightly floccose surface; 3.0 to 3.5 cm in diameter in 12 days, reverse uncolored to light cream or dull pale yellow; odor faint, rather pleasant.

Penicilli of these isolates most frequently consist of a terminal verticil of three or four somewhat divaricate metulae 10 to 15 μm long, each bearing a verticil of fairly numerous parallel phialides averaging about 10 μm in length with distinct coidium-bearing tips 1 to 2 μm long (Fig. 5). In more complex penicilli, branches are long and only somewhat appressed and bear either a similar verticil of metulae and phialides or phialides only. Conidia are mostly globose to subglobose, smooth or nearly so, 3 to 4 μm in longer axis, in occasional strains more definitely elliptical but otherwise as above.

In growth habit on Cz, in the color change to gray-brown and in the details of the penicilli and conidia, these strains satisfy Raper and Thom's description of either *P. olivino-viride* Biourge or *P. puberulum* Bainier. Conidial color on Cz is yellow-green as described for *P. olivino-viride* rather than the blue-green of *P. puberulum*. However, they have only a faint and not unpleasant odor on both Cz and BMA as compared with the strong odors described for both species, and they are unlike the type and representative strains cited for either species. One culture (NRRL 1160) cited by Raper and Thom as *P. viridicatum*, but not included in this study, most nearly approximates these isolates.

Despite certain cultural and morphological differences, the following two cultures have been included in group III because of their similar origin, their lack of distinctive odors, and their toxin production pattern.

Strain NRRL 3711, yellow-green and in age showing only slight reduction in intensity of color, has a history of having developed velvety sectors that showed the color change to brown. As it exists in our collection today, the culture appears more funiculose than fasciculate on Cz. Penicilli and conidia are as described for the sausage isolates.

Strain NRRL 1161, cited as *P. viridicatum* by Raper and Thom, is velvety and definitely blue-green when young on Cz but becomes somewhat fasciculate and assumes a yellowish tinge and remains green with age (1 month). In these characters, it resembles cultures of group II to some degree. Colonies on BMA also resemble those of group II, but are somewhat more widely spreading, considerably less fasciculate, and fail to produce the characteristic odor. Penicilli and conidia of this isolate also resemble those of group II.

The considerable differences that separate the isolates of group III from those of groups I and II may mirror a selection effect of the substrate from which they were isolated, i.e., low carbohydrate, high protein, and lipids (primarily saturated). This hypothesis will be subjected to future experimentation.

**Odor.** The solvent-like odor produced by

TABLE 3. *Characteristics of P. viridicatum, group III*

| Culture no. | Source | | Toxin synthesis | | Colony characters on BMA | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Geographical | Substrate | Ochra-toxin | Citrinin | Diameter | Surface texture | Color | Reverse | Odor |
| NRRL 1161 | Canada | Air in meat-packing plant | + | – | M[a] | SF[b] | G-BG[c] | VC[d] | FP[e] |
| NRRL 3711 | Canada | Ham | + | – | M | Fl[f] | G-YG[g] | UC | FP |
| NRRL 5573 | Italy | Sausage | – | – | M | V[h] | G-YG | UC | FP |
| NRRL 5574 | Italy | Sausage | + | – | M | V | YG | UC | FP |
| NRRL 5582 | Italy | Sausage | + | – | M | V | YG[i] | UC | FP |
| A-19166 | Italy | Sausage | – | – | M | SF | G-YG | VC | FP |
| A-19169[j] | Italy | Sausage | – | – | M | V-Fl | G-YG | UC | FP |
| A-19171 | Italy | Sausage | + | – | M | V | YG | UC | FP |
| A-19173[j] | Italy | Sausage | + | – | M | V | YG | UC | FP |
| A-19174 | Italy | Sausage | + | – | M | SF | G-YG | UC | FP |
| A-19175[j] | Italy | Sausage | – | – | M | V | YG | UC | FP |
| A-19176[j] | Italy | Sausage | + | – | M | SF | YG | UC | FP |
| A-19179b | Yugoslavia | Sausage | + | – | M | Fl | NS[k] | UC | B[l] |

[a] Moderate, 4.0 to 4.5 cm.
[b] Slightly fasciculate.
[c] Grayed blue-green.
[d] Uncolored or in palest flesh tone.
[e] Faint, pleasant.
[f] Floccose.
[g] Grayed yellow-green.
[h] Velvety.
[i] Yellow-green.
[j] Unstable, throwing sectors of differing color or texture or both on BMA.
[k] Nonsporulating.
[l] Butyric?

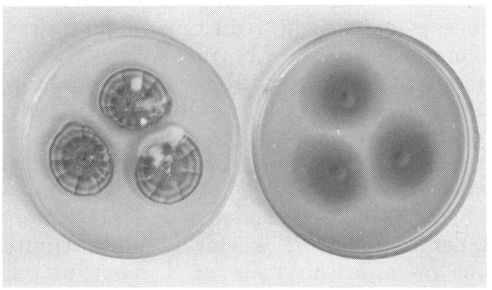

FIG. 4. *Strain NRRL 5574 representative of the majority of strains in group III on Cz (left) and BMA (right), 12 days, 24 C.*

NRRL 5572 (group II) or BMA was identified as 2-pentanone by GLC and MS (Fig. 6 and 7). Although the odor was not apparent when the culture was grown on barley or corn, it could be detected by GLC. The odors of other strains in group II were determined organoleptically on BMA. The strong woodsy or earthy odor of group I gave no peaks on GLC, indicating a very low concentration of what is probably a mixture of volatile substances (10).

**Taxonomic implications.** Exact delineation of many species in the genus *Penicillium* is extremely difficult. This is nowhere more apparent than in the Fasciculata section of the Asymmetrica.

The Lanata, Funiculosa, and Fasciculata sections of the Asymmetrica are separated on the basis of colony texture, as their names suggest. However, since determination of texture is necessarily subjective, sectional placement can be difficult. Raper and Thom (13) were acutely aware of the intergradations existing not only between species but also between these sections and repeatedly advised users of their Manual to consider species in more than one section.

Those species in the section Lanata (*lanoso-viride*, *lanoso-coreruleum*, *biforme*, *commune*, and *lanoso-griseum*) and the Funiculosa (*psittacinum*, *terrestre*, and *solitum*) that are distinguished by subtle differences in conidial color are those which show greatest resemblance to and are probably most often confused with the similarly separated *P. viridicatum*, *P. cyclopium*, and *P. expansum* series of the section Fasciculata.

In most of the species concerned, morphological differences are essentially nonexistent. Penicilli are asymmetric, large, usually with one or more appressed branches in addition to the

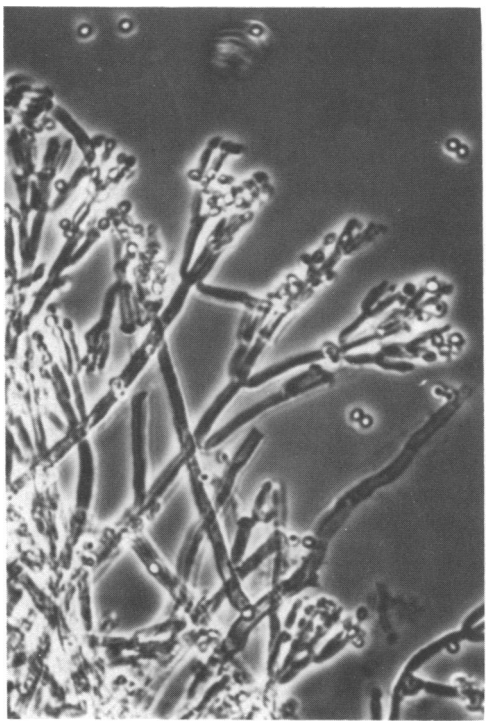

FIG. 5. *Penicilli of strain NRRL 5582, also representative of group III, ×875.*

main axis, each terminated successively by veticils of metulae and phialides. Conidiophores are comparatively long and coarse and may be either smooth or rough. Conidia range from globose to elliptical, frequently in the same species, and are smooth or delicately roughened.

Most species have been described as producing strong odors. Terms such as "actinomyces-like," "suggesting mushrooms," "moldy," "penetrating," "earthy," or occasionally "sourish" or "aromatic" have been used to distinguish between these odors.

Representatives of many of the species assigned to these sections show a marked tendency to vary under continued laboratory culture. This variability, frequently reflected in the loss of a strain's ability to produce a specific secondary metabolite, has plagued many investigators. Whether such variation results from an inherent genetic instability, selection due to change of substrate, the mutational effect of toxic secondary metabolites, or other factors, is not known.

Strains of *Penicillium* isolated from moldy foods and feedstuffs encountered during mycotoxin investigations in recent years have blurred even further the lines between species. Among new isolates from corn and wheat, there appears to be a color continuum from the characteristic yellow-green of *P. viridicatum* to the distinctly blue-green of *P. martensii* of the *P. cyclopium* series. A similar, but not entirely consistent, progression is observed in the amount and color of diffusible pigment produced by these isolates on Cz slants: from yellow through orange and maroon to purplish. Colony patterns are almost identical. Differences in morphology are nebulous and a matter of degree rather than sharply defined. The two cultures used to illustrate *P. viridicatum* (NRRL 963) and *P. martensii* (NRRL. 2029) in color plate VIII of the Manual of the Penicillia appear to represent the distal ends of the continuum.

Examination of the cultures cited by Raper and Thom as representative of *P. viridicatum* reveals a considerable range of intraspecies variation. Their broad concept of the species is clearly apparent in their description. This con-

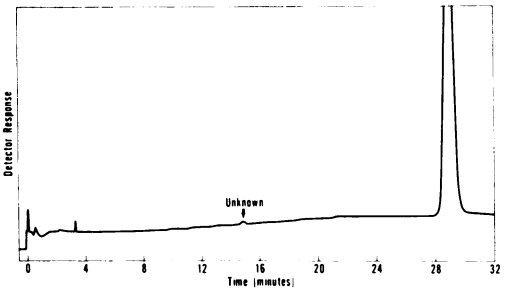


FIG. 6. *Gas chromatogram of the volatile substance produced by P. viridicatum NRRL 5572 grown on BMA.*

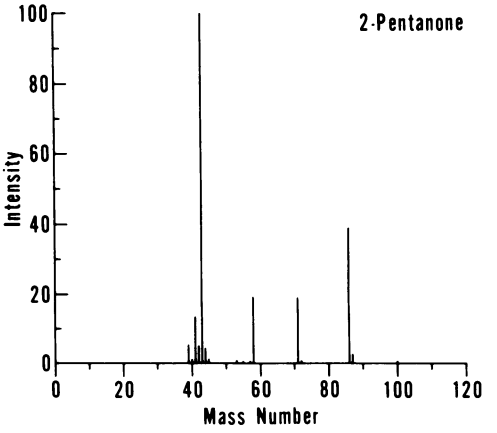


FIG. 7. *Mass spectrogram of the volatile substance produced by P. viridicatum NRRL 5572 grown on BMA. The mass chromatogram of pure 2-pentanone is superimposible.*

cept is not contested and the fact that the composite of characters they used to delineate the species was selected after years of experience in the continued observation and cultivation of many strains is fully recognized. We cannot say, with certainty, that all of our isolates would have been identified as *P. viridicatum* by Raper and Thom. We believe that those separated into group I would definitely have been included. Those of group III may represent either *P. olivino-viride* or *P. puberulum*, although they fail to conform with type or representative strains of these species or with the restrictedly growing strains of *P. viridicatum* recognized by Raper and Thom. No counterpart for the isolates in group II has been found among type and representative strains of either *P. viridicatum* or those other species in the sections Lanata, Funiculosa, and Fasciculata that Raper and Thom believed to show interrelationship with *P. viridicatum*.

The restricted growth on both Cz and BMA, the distinctive odor, and the toxin production pattern of this group of isolates perhaps would justify their being described as a new subspecies or variety. This is not being done, however, since they lack distinctive morphological characteristics; production of similar metabolic products does not provide an adequate basis for recognition of a new taxon. In addition, GLC detection of 2-pentanone in one of these isolates does not constitute indisputable proof that the odor of the other isolates, organoleptically identified as similar by several individuals, is due to this same compound.

Until additional evidence is available, we prefer to apply the "group" system devised by Raper and Thom for *P. citrinum*, leaving all of the isolates in *P. viridicatum* but acknowledging the existence of recognizable subgroupings within the species.

The strains of *P. viridicatum* in this survey can be separated into three groups according to the following key: (1) Conidial color remaining yellow-green in age. (a) Colonies on BMA spreading, granular or slightly fasciculate at margins in age . . . group I. (b) Colonies on BMA restricted, usually conspicuously fasciculate at margins in age . . . group II. (1) Conidial color changing to gray-brown shades in age . . . group III.

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