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Simple Screening Method for Molds Producing Intracellular Mycotoxins in Pure Cultures

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A simple screening method for molds producing the intracellular mycotoxins brevianamide A, citreoviridin, cyclopiazonic acid, luteoskyrin, penitrem A, roquefortine C, sterigmatocystin, verruculogen, viomellein, and xanthomegnin was developed. After removing an agar plug from the mold culture, the mycelium on the plug is wetted with a drop of methanol-chloroform (1:2). By this treatment the intracellular mycotoxins are extracted within seconds and transferred directly to a thin-layer chromatography plate by immediately placing the plug on the plate while the mycelium is still wet. After removal of the plug, known thin-layer chromatographic procedures are carried out. The substrate (Czapek yeast autolysate agar) and growth conditions (25°C for 7 days) used by *Penicillium* taxonomists proved suitable for the production of the mycotoxins investigated when 60 known toxigenic isolates and 865 cultures isolated from foods and feedstuffs were tested with this screening method.

A simple screening method for toxigenic molds in pure cultures on agar substrates has previously been described (16). This is a very time- and resource-saving method compared with other screening methods (2, 5, 23, 34), and it has already proved valuable in investigations where numerous isolates had to be screened (4, 17). The use of the method is, however, restricted to pronounced extracellular mycotoxins such as aflatoxins, citrinin, kojic acid, mycophenolic acid, 3-nitropropionic acid, ochratoxins, patulin, penicillic acid, PR-toxin, T-2 toxin, and zearalenone, as it is based on diffusion of the mycotoxin into the substrate. This means that important mycotoxins like roquefortine C, penitrem A, sterigmatocystin, and several others, which are chiefly intracellular (see reference 29), cannot be detected with this screening method. To overcome this limitation without losing the advantages of the screening method, the method described in this paper was developed.

MATERIALS AND METHODS

Fungi. A total of 60 known toxigenic cultures (from culture collections) and 865 isolates from foods and feedstuffs were tested with the screening method. The taxonomy of the cultures from culture collections is not treated in this paper, but the remaining 865 cultures were identified according to the following taxonomic treatments: the penicillia according to the methods of Pitt (27), Ciegler et al. (7), and Frisvad (17), and the aspergilli according to the methods of Raper and Fennell (30), Samson (32), and Christensen (6). The cultures were maintained on malt extract agar (27) and Czapek yeast autolysate agar (CYA) (27) at 0.5°C.

Substrates. The cultures were three point inoculated on yeast extract-sucrose agar (34), yeast extract-glucose agar (16, 17), CYA (27, 34), potato-glucose-yeast extract agar (potato extract [Difco Laboratories, Detroit, Mich.], 4 g; glucose, 20 g; yeast extract [Difco], 5 g; agar, 20 g; water, 1 liter), and malt extract agar (E. Merck AG, Darmstadt, Germany; art. 5398) (23). The cultures were incubated at 25°C for 7 days.

Mycotoxin analysis. One or more agar plugs were cut out of a mold colony (near the center) with a flame-sterilized stainless steel tube (inner diameter, 0.4 cm). The plugs were removed by using a flame-sterilized scalpel or needle. By means of a syringe, a drop of extraction liquid was placed directly on the mycelium or conidia. While still wet, the mycelium side of the plug was gently pressed against the application line on a thin-layer chromatography (TLC) plate and then removed immediately. After drying the application spot, the procedure could be repeated with other plugs. The TLC plates (Merck precoated silica gel G, art. 5721; with and without oxalic acid impregnation) were activated for 2 h at 110°C. To produce oxalic acid-impregnated TLC plates, precoated plates were dipped in an 8% methanolic solution of oxalic acid for 2 min and air dried overnight.

Combinations of chloroform or ethyl ether with ethanol, methanol, or acetone were compared for efficiency in mycotoxin extractions. The most efficient of these solvent mixtures was chloroform-methanol (2:1 [vol/vol]), so this mixture was used in the screening method.

Representatives of cultures which did not produce the expected mycotoxins were analyzed by using a stomacher extraction technique (17) after 1 and 2 weeks of incubation.

The following standard toxins were used as external and internal standards: sterigmatocystin (Karl Roth, Karlsruhe, Federal Republic of Germany); luteoskyrin

TABLE 1. Procedures for detection of the mycotoxins included in the screening method

Mycotoxin	TLC developing systems[a] (references)	Treatments[b]
Brevianamide A	TEF (23), CA (41)	VIS (41), UV (41)
Citreoviridin	TEF (10), CM (37)	VIS (37), UV (37)
Cyclopiazonic acid	CI[c] (35), EPA (22)	EHRLICH (18), $FeCl_3$ (18)
Luteoskyrin	AHW (38), TEF (34)	UV (34), ANIS (34)
Penitrem A	HE (12), CA (9)	$AlCl_3$, $FeCl_3$ (9)
Roquefortin C	CMA (33), CAP (19)	$Ce(SO_4)_2$ (19), EHRLICH (40)
Sterigmatocystin	TEF (34), BMA (34)	$AlCl_3$ (1), ANIS (34)
Verruculogen	TEF (11), CA (11)	VIS (11), H_2SO_4 (11)
Viomellein	BMA (8), TEF (31)	VIS (8), NH_3 (8)
Xanthomegnin	BMA (8), TEF (31)	VIS (8), NH_3 (8)

[a] Abbreviations: TEF, toluene–ethyl acetate–90% formic acid (5:4:1 [vol/vol/vol]); CA, chloroform-acetone (9:1 [vol/vol] or 93:7 [vol/vol]); CM, chloroform-methanol (9:1 [vol/vol]); CI, chloroform-isobutylmethylketone (4:1 [vol/vol]); EPA, ethyl acetate–2-propanol–28% NH_3 in water (20:15:10 [vol/vol/vol]); AHW, acetone–*n*-hexane–water (4:2:1 [vol/vol/vol]); HE, hexane-ethyl acetate (6:4 [vol/vol]); CMA, chloroform–methanol–28% NH_3 in water (90:10:1 [vol/vol/vol]); CAP, chloroform-acetone-propane-2-ol (85:15:20 [vol/vol/vol]); BMA, benzene-methanol-acetic acid (24:2:1 [vol/vol/vol]).

[b] Abbreviations: VIS, viewed under normal visible light; UV, viewed under UV light at 366 nm; EHRLICH, 1% (wt/vol) *p*-dimethylbenzaldehyde in 96% ethanol was sprayed on the TLC plate, and the plate was dried under a hair dryer and placed over HCl fumes for 10 min; $FeCl_3$, 1% (wt/vol) $FeCl_3$ in butane-1-ol; ANIS, 0.5% *p*-anisaldehyde (vol/vol) in ethanol-acetic acid-concentrated H_2SO_4 (17:2:1 [vol/vol/vol]); $AlCl_3$, 20% (wt/vol) $AlCl_3$ in 96% ethanol; Ce $(SO_4)_2$, 1% Ce $(SO_4)_2$ (wt/vol) in 6 N H_2SO_4; H_2SO_4, 50% (vol/vol) concentrated H_2SO_4 in water; NH_3, exposure to NH_3 vapors for 1 min. See the original references for details on the colors of the mycotoxins after different treatments.

[c] On oxalic acid-treated plates.

(Sigma Chemical Co., St. Louis, Mo.); penitrem A and cyclopiazonic acid (from L. Leistner, Bundesanstalt für Fleischforschung, Kulmbach, Federal Republic of Germany); brevianamide, xanthomegnin, and viomellein (from A. Ciegler, Southern Regional Research Center, New Orleans, La.); verruculogen (from R. T. Gallagher, Ruakura Agricultural Research Center, Hamilton, New Zealand); citreoviridin (from A. E. de Jesus, Council for Scientific and Industrial Research, Pretoria, South Africa); and roquefortine C (from U. L. Diener, Auburn University, Auburn, Ala., and H.-J. Rehm, University of Münster, Federal Republic of Germany).

In the prescreening, all mycotoxins were detected by using toluene–ethyl acetate–90% formic acid (5:4:1) (40) and external standards. Roquefortine C and cyclopiazonic acid had very low R_f values in that developing system. The toxins were visualized in daylight and UV light at 366 and 254 nm (UV_{366} and UV_{254}), before and after treatment with a 50% solution of sulfuric acid (11). The production of any particular mycotoxin was confirmed by using external and internal standards in optimal developing systems, with toxins visualized as listed in Table 1.

The visualization of penitrem A with $AlCl_3$ has not previously been reported. It was performed by spraying the TLC plate with a 20% (wt/vol) solution of $AlCl_3$ in 96% ethanol (7) and heating for 5 min at 120°C. The toxin was bluish green in daylight and reddish brown in UV_{366}. Seventy nanograms of penitrem A could be detected on the TLC plate.

RESULTS AND DISCUSSION

The chemical detection of the individual toxins was never disturbed by interfering metabolites, in spite of the omission of sample purification before application on TLC, as described in the present method. A limited number of other metabolites were indeed observed, but they were always well separated from the mycotoxins. In a few cultures, confirmation of the toxins was questionable due to weak responses on the TLC plate. In these cases, the amount of toxin applied to the plate was increased by superimposed application of three plugs, whereas extension of extraction time did not improve the result. Several protein–lipid-separating solvent systems were tested in the extraction procedure. The chloroform-methanol (2:1) system appeared as optimal for toxin extraction as it is for lipid extraction (20). The release of the toxin from the mycelium effected by simply adding this solvent mixture may be explained by the polar solvent breaking the protein-lipid bonds in membranes by denaturating the proteins, with the less polar solvent helping to dissolve the lipids (20).

Different substrates were used in optimizing toxin production. Certain important variations within toxins and isolates were observed, but of main interest was that the substrate CYA, although not always the best, appeared useful for all isolates, except for roquefortine C production from a few *Penicillium roquefortii* isolates. To include these isolates, cultures on yeast extract-sucrose agar could be used. The incubation time needed to detect toxin production was less than the specified 7 days for many cultures, but this

TABLE 2. Detection of mycotoxin production on CYA at 25°C from a selected number of known toxigenic mold isolates

Mycotoxin	Mold isolate	Strains (references)[a]
Brevianamide A	*Penicillium brevicompactum*	IMI 40225 (3)
	P. viridicatum	NRRL 963 (41), Purdue 66-68-2 (41), Sp 931 (23)
Citreoviridin	*P. citreoviride*	CBS 920.70[b], NRRL 2046[b], NRRL 2579[b]
	P. citrinum	Sp 865 (23, 24)
	P. miczynskii	Sp 340 (23, 24)
	P. pulvillorum	CSIR 1405 (25), CSIR 1406 (25)
Cyclopiazonic acid	*Aspergillus flavus*	NRRL 3251 (18)
	P. camembertii	CBS 299.48 (22, 36), ATCC 42009 (22, 36), Sp 1133 (36)
	P. crustosum	Sp 607 (24)
	P. patulum	CSIR 1082 (21), CSIR 1399 (24)
	P. puberulum	Sp 524 (24)
	P. cyclopium	Sp 603, Sp 605, Sp 608, Sp 613 (24)
	P. viridicatum	Sp 119 (24)
Luteoskyrin	*P. islandicum*	CBS 587.68[b], NRRL 1036 (14)
Penitrem A	*P. commune*	AUA 827 (40)
	P. crustosum	NRRL 968, NRRL 1983, NRRL 5186 (9), Sp 458, Sp 1191 (24)
	P. cyclopium	NRRL 3476[c], NRRL 3477 (9), NRRL 6093 (39)
	P. granulatum	NRRL 2036 (9)
	P. martensii	NRRL 2034 (9)
	P. olivinoviride	NRRL 958 (9)
	P. palitans	NRRL 3468[c] (9)
Roquefortine C	*P. commune*	AUA 827 (40)
	P. crustosum	G. Engel 6842 (13)
	P. cyclopium	NRRL 6093 (39)
	P. roquefortii	NRRL 849 (33), Sp 860, Sp 1066, Sp 1077, Sp 1079 (23, 24)
Sterigmatocystin	*Aspergillus versicolor*	CBS 600.65[b], Frank H9, H22, 519, 543 (26)
Verruculogen	*P. estinogenum*	76S9FC9[d] (28)
	P. piscarium	NRRL A-14996 (=Sp 306) (24)
	P. simplicissimum	Sp 863 (23)
	P. verruculosum	NRRL 5881 (=ATCC 24640)[d] (11, 15, 28)
Viomellein	*P. viridicatum*	NRRL 963, NRRL A-15402, NRRL A-15505, NRRL A-19118 (8), Purdue 66-68-2 (31)
Xanthomegnin	*P. viridicatum*	NRRL 963, NRRL A-15402, NRRL A-15505, NRRL A-19118 (8), Purdue 66-68-2 (31), Sp 931 (23)

[a] The cultures are listed as received or listed in culture collection catalogues.
[b] Toxin production by these isolates has been stated in personal communications to us. Furthermore, these species are generally accepted as producers of the toxins mentioned (29). See also the culture collection catalogues from the Commonwealth Mycological Institute (CMI, 1982), the American Type Culture Collection (ATCC, 1982), and the Centraalbureau voor Schimmelcultures (CBS, 1978).
[c] Equals *P. crustosum* (28).
[d] Equals *P. simplicissimum* (28).

period was necessary to detect all tested toxigenic cultures.

The detection limits of the method cannot be specified quantitatively from this investigation. But, as indicated in Table 2, it proved sufficient compared with alternative methods (2, 5, 23, 34) for the detection of toxin production from all 60 tested known toxigenic mold isolates.

As a further illustration of the sensitivity of this method, the results of the screening of some of our own isolates from foods and feedstuffs are listed in Table 3.

TABLE 3. Detection of mycotoxin production on CYA at 25°C from isolates of known toxigenic mold species from foods and feedstuffs

Mycotoxin	Species	No. of isolates investigated	No. producing the toxin
Brevianamide A	*Penicillium brevicompactum*	28	28
	P. viridicatum I	320	7
Citreoviridin	*P. miczynskii*	8	8
Cyclopiazonic acid	*Aspergillus flavus*	5	5
	P. camembertii	24	24
	P. griseofulvum	12	12
	P. puberulum	15	15
Luteoskyrin	*P. islandicum*	7	6
Penitrem A	*P. crustosum*	66	66
Roquefortine C	*P. crustosum*	66	66
	P. roquefortii	39	39[a]
Sterigmatocystin	*Aspergillus versicolor*	38	38
Verruculogen	*P. simplicissimum*	3	3
Viomellein	*P. viridicatum* I	320	320
Xanthomegnin	*P. viridicatum* I	320	320
	P. aurantiogriseum	300	188

[a] Toxin from a few *P. roquefortii* isolates was only detected after several superimposed applications on the TLC plate or in cultures on yeast extract-sucrose agar.

The sensitivity of the method appears to be sufficient as far as the majority of the species are concerned, since all tested isolates of 11 species produced the expected intracellular toxins. However, within *Penicillium aurantiogriseum* (xanthomegnin) and *Penicillium viridicatum* I (brevianamide A) the toxins could not be detected in the cultures of a considerable number of the isolates tested. The demonstration of isolates not producing the expected toxins was checked with an alternative screening method (stomacher extraction technique [17, 23]), but no disagreement was observed. In other words, we have not been able to demonstrate any false-negative results with the screening method.

The described method is meant for the screening of intracellular mycotoxins, but it has often proved useful for the screening of typical extracellular mycotoxins as well. However, the combination of this method and the screening method for extracellular mycotoxins (16) is necessary to achieve sufficient sensitivity in the general screening of molds in pure culture for their ability to produce known mycotoxins. The test can be performed in connection with mold identification procedures, since identical incubation conditions and substrates can be used for these purposes. This offers a very fast and simple way to confirm the identity of a mold isolate with important mycotoxicological characteristics.

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LITERATURE CITED

1. **Athnasios, A. K., and G. O. Kuhn.** 1977. Improved thin layer chromatographic method for the isolation and estimation of sterigmatocystin in grains. J. Assoc. Off. Anal. Chem. **60**:104–106.
2. **Barr, J. G., and G. A. Dawney.** 1975. A multiple inoculation technique for the screening of fungal isolates for the evaluation of growth and mycotoxin production on agar substrates. J. Sci. Food Agric. **26**:1561–1566.
3. **Bird, B. A., and I. M. Campbell.** 1982. Disposition of mycophenolic acid, brevianamide A, asperphenamate, and ergosterol in solid cultures of *Penicillium brevicompactum*. Appl. Environ. Microbiol. **43**:345–348.
4. **Blaser, P., and W. Schmidt-Lorenz.** 1981. *Aspergillus flavus* Kontamination von Nüssen, Mandeln und Mais mit bekannten Aflatoxin-Gehalten. Lebensm. Wiss. Technol. **14**:252–259.
5. **Bullerman, L. B.** 1974. A screening medium and method to detect several mycotoxins in mold cultures. J. Milk Food Technol. **37**:1–3.
6. **Christensen, M.** 1981. A synoptic key and evaluation of species in the *Aspergillus flavus* group. Mycologia **73**:1056–1084.
7. **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.
8. **Ciegler, A., L. S. Lee, and J. J. Dunn.** 1981. Production of naphthoquinone mycotoxins and taxonomy of *Penicillium viridicatum*. Appl. Environ. Microbiol. **42**:446–449.
9. **Ciegler, A., and J. I. Pitt.** 1970. Survey of the genus *Penicillium* for tremorgenic toxin production. Mycopathol. Mycol. Appl. **42**:119–124.
10. **Cole, R. J., J. W. Dorner, R. H. Cox, R. A. Hill, H. G. Cluter, and J. M. Wells.** 1981. Isolation of citreoviridin from *Penicillium charlesii* cultures and molded pecan fragments. Appl. Environ. Microbiol. **42**:677–681.
11. **Cole, R. J., J. W. Kirksey, J. H. Moore, B. R. Blakenship, U. L. Diener, and N. D. Davis.** 1972. Tremorgenic toxin from *Penicillium verruculosum*. Appl. Microbiol. **24**:248–256.
12. **de Jesus, A. E., P. S. Steyn, F. R. van Heerden, R. Vleggaar, P. L. Wessels, and W. E. Hall.** 1981. Structure and biosynthesis of the penitrems A-F, six novel tremor-

genic mycotoxins from *Penicillium crustosum*. J. Chem. Soc. Chem. Commun. **6**:289–291.
13. **Engel, G., and M. Teuber.** 1978. Simple aid in the identification of *Penicillium roquefortii* Thom. Growth in acetic acid. Eur. J. Appl. Microbiol. Biotechnol. **6**:107–111.
14. **Enomoto, M., and I. Ueno.** 1974. *Penicillium islandicum* (toxic yellowed rice)-luteoskyrin-islanditoxin-cyclochlorotine, p. 303–326. *In* I. H. F. Purchase (ed.), Mycotoxins. Elsevier/North-Holland, Amsterdam.
15. **Fayos, J., D. Lokensgard, J. Clardy, R. J. Cole, and J. W. Kirksey.** 1974. Structure of verruculogen, a tremor producing peroxide from *Penicillium verruculosum*. J. Am. Chem. Soc. **96**:6785–6787.
16. **Filtenborg, O., and J. C. Frisvad.** 1980. A simple screening-method for toxigenic moulds in pure cultures. Lebensm. Wiss. Technol. **13**:128–130.
17. **Frisvad, J. C.** 1981. Physiological criteria and mycotoxin production as aids in identification of common asymmetric penicillia. Appl. Environ. Microbiol. **41**:568–579.
18. **Gallagher, R. T., J. L. Richard, H. M. Stahr, and R. J. Cole.** 1978. Cyclopiazonic acid production by aflatoxigenic and non-aflatoxigenic strains of *Aspergillus flavus*. Mycopathologia **66**:31–36.
19. **Gorst-Allman, C. P., and P. S. Steyn.** 1979. Screening methods for the detection of thirteen common mycotoxins. J. Chromatogr. **175**:325–331.
20. **Hanahan, D. J.** 1960. Lipid chemistry, p. 12–13. John Wiley & Sons, Inc., New York.
21. **Holzapfel, C. W.** 1968. The isolation and structure of cyclopiazonic acid, a toxic product of *Penicillium cyclopium* Westling. Tetrahedron **24**:2101–2119.
22. **Le Bars, J.** 1979. Cyclopiazonic acid production by *Penicillium camembertii* Thom and natural occurence of this mycotoxin in cheese. Appl. Environ. Microbiol. **38**:1052–1055.
23. **Leistner, L., and C. Eckardt.** 1979. Vorkommen toxinogener Penicillien bei Fleisch Erzeugnisse. Fleischwirtschaft **59**:1892–1896.
24. **Leistner, L., and J. I. Pitt.** 1977. Miscellaneous *Penicillium* toxins, p. 639–653. *In* J. V. Rodricks, C. W. Hesseltine, and M. A., Mehlmann (ed.), Mycotoxins in human and animal health. Pathotox Publishers, Park Forest South, Ill.
25. **Nagel, D. W., P. S. Steyn, and B. de Scott.** 1972. Production of citreoviridin by *Penicillium pulvillorum*. Phytochemistry **11**:627–630.
26. **Orth, R.** 1976. Wachstum und Toxinbildung von Patulin- und Sterigmatocystin-Bildende Schimmelpilzen unter kontrolierter Atmosphäre. Z. Lebensm. Unters. Forsch. **160**:359–366.
27. **Pitt, J. I.** 1979. The genus *Penicillium* and its teleomorphic states *Eupenicillium* and *Talaromyces*. Academic Press, Inc., London.
28. **Pitt, J. I.** 1979. *Penicillium crustosum* and *Penicillium simplicissimum*: the correct names for two common species producing tremorgenic mycotoxins. Mycologia **71**:1166–1177.
29. **Purchase, I. H. F. (ed.).** Mycotoxins. Elsevier/North-Holland, Amsterdam.
30. **Raper, K. B., and D. I. Fennell.** 1965. The genus *Aspergillus*. The Williams & Wilkins Co., Baltimore.
31. **Robbers, J. E., S. Hong, J. Tuite, and W. W. Carlton.** 1978. Production of xanthomegnin and viomellein by species of *Aspergillus* correlated with mycotoxicosis produced in mice. Appl. Environ. Microbiol. **36**:819–823.
32. **Samson, R. A.** 1979. A compilation of the aspergilli described since 1965. Stud. Mycol. **18**:1–40.
33. **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.
34. **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.
35. **Steyn, P. S.** 1969. The separation and detection of several mycotoxins by thin-layer chromatography. J. Chromatogr. **45**:473–475.
36. **Still, P. E., C. Eckardt, and L. Leistner.** 1978. Bildung von Cyclopiazonsäure durch *Penicillium camemberti* Isolate von Käse. Fleischwirtschaft **58**:876–877.
37. **Ueno, Y.** 1974. Citreoviridin from *Penicillium citreo-viride* Biourge, p. 283–302. *In* I. H. F. Purchase (ed.), Mycotoxins. Elsevier/North-Holland, Amsterdam.
38. **Ueno, Y., and I. Ishikawa.** 1969. Production of luteoskyrin, a hepatotoxic pigment, by *Penicillium islandicum* Sopp. Appl. Microbiol. **18**:406–409.
39. **Vesonder, R. F., L. Tjarks, W. Rohwedder, and D. O. Kieswetter.** 1980. Indole metabolites from *Penicillium cyclopium* NRRL 6093. Experientia **36**:1308.
40. **Wagener, R. E., N. D. Davis, and U. L. Diener.** 1980. Penitrem A and roquefortine production by *Penicillium commune*. Appl. Environ. Microbiol. **39**:882–887.
41. **Wilson, B. J., D. T. C. Yang, and T. M. Harris.** 1973. Production, isolation, and preliminary toxicity studies of brevinamide A from *Penicillium viridicatum*. Appl. Environ. Microbiol. **26**:633–635.