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A NEW SPECIES OF PENICILLIUM, P. SCABROSUM

JENS C. FRISVAD*
ROBERT A. SAMSON** and AMELIA C. STOLK**

A new species of *Penicillium*, *P. scabrosum*, was found repeatedly in soil samples from temperate regions of the world, especially northern Europe and Canada. It occurs in high frequencies in wheat and barley field soils together with *P. janczewskii* Zaleski. The species has also been found on fleshy fungi and in foods, particularly as a spoilage organism in food containing lipid and cereal-containing feedstuff. It produces many unknown strongly coloured secondary metabolites. Known mycotoxins from the species include fumagillin, viridicatin, and viridicatol.

An yet undescribed species was repeatedly isolated from soil and food samples and is characterized by strongly coloured colonies, one- to two-staged branched penicilli, very rough conidiophore stipes and rough-walled, globose conidia. Such isolates were encountered in earlier investigations and taxonomists had called them *P. canescens* Sopp, *P.* cf. *atrovenetum* G. Smith (Gams & Domsch, 1970), *P.* cf. *paxilli* Bain., or *P. aurantiogriseum* Dierckx. The isolates representing this taxon are different from all these species and are, therefore, described below as *P. scabrosum*. The specific epithet refers to the conspicuously roughened stipes of the penicilli.

MATERIALS AND METHODS

Isolates of *P. scabrosum* were obtained from food, feedstuff, and soil samples using direct and dilution plating on DG18, DRBC, PRYES or Czapek Dox agar media [see King & al. (1986) and Samson & van Reenen-Hoekstra (1988) for formulations]. They were strongly yellow on PRYES agar in both obverse and reverse, and typically yellow-, orange- or red-brown (usually in concentric differently coloured zones) on Czapek-based media.

The isolates were screened for secondary metabolites using thin-layer chromatography (TLC) (Filtenborg & al., 1983) and high-performance liquid chromatography (HPLC), using the method of Frisvad & Thrane (1987).

Penicillium scabrosum Frisvad, Samson & Stolk, *spec. nov.*—Figs. 1, 2

Stipites conidiophororum asperati, conidiophora bis vel ter verticillata, conidia asperulata, globosa, 2.4–3.2 µm diam.; phialides angustae, collulo conspicuo, fere elongato terminatae. Coloniae reversum luteum vel aurantiobrunneum. Substantiae metabolicae: fumagillinum et nonnulla viridicatina. — Typus: Herb. IMI 285533 (vivus CBS 420.89), isolatus e grano *Zeae maydis*, in Dania, dec. 1983.

* The Technical University of Denmark, Department of Biotechnology, Building 221, DK-2800 Lyngby, Denmark.
** Centraalbureau voor Schimmelcultures, P.O. Box 273, 3740 AG Baarn, The Netherlands.

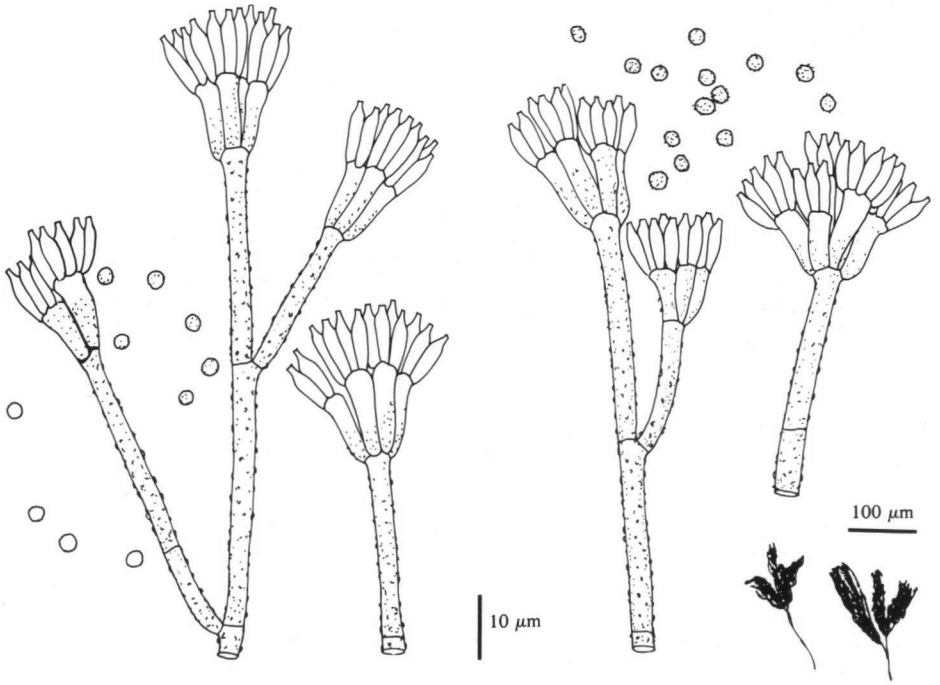


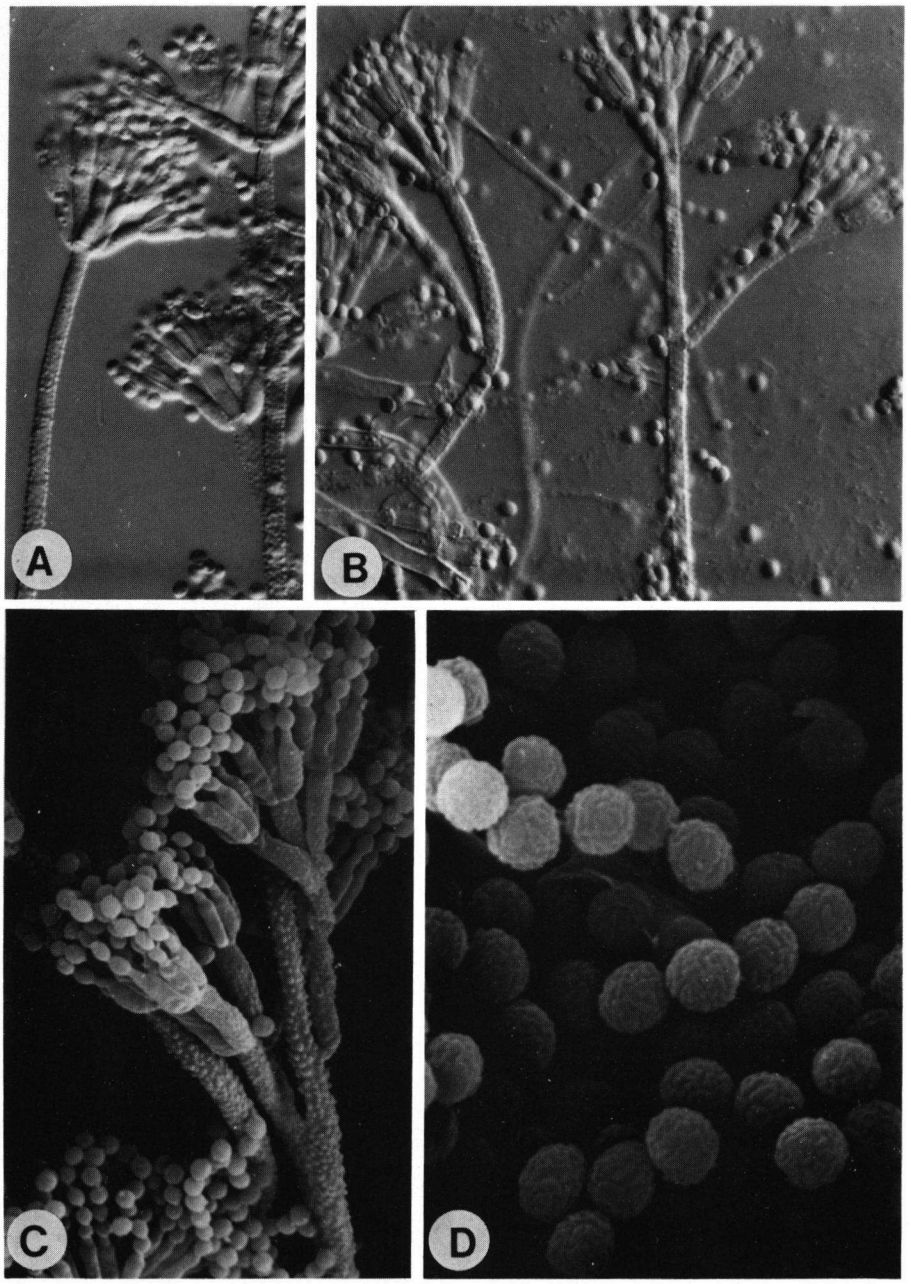
Fig. 1. Camera lucida drawing of the conidiophores and conidia of *P. scabrosum*.

Diagnosis.—Stipes conspicuously roughened, conidiophores bi- and terverticillate, conidia rough, globose (2.4–3.2 µm diam.), phialides slender, with a well-defined, abruptly narrowed collulum; colony reverse yellow to orange-brown on Czapek-based media. Metabolites: fumagillin and viridicatins.

Description.—Conidiophore stipes 200–400 × 3–4 µm, arising from subsurface and surface hyphae, consistently conspicuously roughened and often encrusted. Penicilli predominantly biverticillate, with a comparatively short and compact terminal verticil of 3–6 somewhat appressed to slightly divergent roughened metulae, 10–20 × 2.5–4.0 µm, and often with a relatively low, conspicuously roughened ramus, which occurs at an angle of about 45°, measuring 15–25 × 2.5–4.0 µm. Phialides 5–12 on each metula, slender, with a well-defined, abruptly narrowed collulum, phialides measuring 7–11 × 2.0–2.5 µm. Conidia globose to subglobose, rough-walled, often more or less echinulate, measuring 2.4–3.2 µm, adhering at first in parallel chains, forming loose columns on each metula, later becoming tangled.

Colonies on Czapek-yeast autolysate agar (CYA) 26–32 mm diam. after one week at 25°C, of strictly velutinous texture, plane and only seldom radiately wrinkled, with good

Fig. 2. *Penicillium scabrosum*, conidiophores and conidia. — A, B. Nomarski interference contrast light microscopy (× 800). — C, D. Scanning electron micrographs (× 1300 and × 4200 respectively).


A
B
C
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sporulation, mycelium white and/or yellow, conidia bluish green en masse (Methuen 24–26 D–F 3), reverse characteristically strongly coloured, bright yellow, orange or yellow-, orange- or red-brown, often in conspicuous concentric zones, exudate often present, yellow or coloured like the reverse, the yellow to yellow-brown colour often diffusing into the agar (this is often more pronounced on malt extract agar, MEA, and Czapek agar), odour insignificant. Colonies on MEA 21–31 mm diam. after one week at 25°C, velutinous to floccose, with good sporulation, conidia dark bluish green en masse, reverse yellow to orange, the colour often diffusing into the agar.

Colonies on 2% Difco Yeast extract-15% sucrose agar (YES) agar conspicuously yellow in both reverse and obverse, radially wrinkled, 32–38 mm diam. after one week at 25°C, good sporulation. On CYA at 5°C colonies 2–4 mm diam. and on CYA at 37°C no growth. Growth on creatine-sucrose agar (Frisvad, 1985) very weak, with no or poor acid production.

ISOLATES EXAMINED.—ON VARIOUS SUBSTRATES: IMI 285533 (ex type) = FRR 2950 = CBS 420.89 = IBT[1] 3736, ex corn, Denmark, Dec. 1983, J.C.F.[2]; IMI 304296, ex mouldy *Flammulina velutipes*, Harderbos, Flevoland, the Netherlands, Nov. 1985, J.C.F.; IBT JHAT, ex mouldy *Armillarea mellea*, Sandbjeg, Denmark, Oct. 1986, J.C.F.; IBT NEE, Air spora, fruit juice production plant, Hørsholm, Denmark, J.C.F.; IBT 3733, 3734, 3737, 3738, 3892, 3897, ex Hollandaise sauce, March 1984, Odense, Denmark, J.C.F.; IBT 3735, ex potato, Lyngby, Denmark, 1985, J.C.F.; IBT B 699, ex swine feed, Oslo, Norway, H. Stenwig; IBT BB2/P4, ex onion, Lyngby, Denmark, 1984, J.C.F.; IBT MLP 6984.3, ex mouldy liver paste, Holbæk, Denmark, 1982, Per Godtfredsen; IBT FRO 15, ex bean sprouts, Lyngby, Denmark, 1986, J.C.F.; IBT BEDF 4 & 8, ex stone, Bedford, Great Britain, 1986, J.C.F.; IBT ALK 35-4, indoor airspora, Denmark, 1988, J.C.F. — ON CEREALS: IBT 3528, wheat (21% moisture content), UK, K.A. Scudamore; IBT KB 7, barley containing 2.83 ppm ochratoxin A, Denmark, 1980, J.C.F.; IBT SA 55, barley (21% water), Ans, Denmark, Feb. 1979; IBT Gamma 3, barley containing 0.432 ppm ochratoxin A, Gudhjem, Den-mark, Jan. 1979, J.C.F. — ON SOIL: CBS 355.68 (= IBT 3739) (as *P.* cf. *atrovenetum*), ex wheat-field soil, Kitzeberg, Kiel, FRG, 1968, W. Gams; CBS 632.70, the Netherlands, J.H. van Emden; CBS 922.70 (= IBT 3740) (as *P.* cf. *paxilli*), the Netherlands, J.H. van Emden; CBS 520.73 (= IBT 3341), Saskatoon, Saskatchewan, Canada, R.A.A. Morrall (SSF 73); CBS 420.89, ex wheat-field soil, Flakkebjerg, Denmark, 1985, S. Elmholt; IMI 304293, ex barley field soil, Flakkebjerg, Aug. 1985, S. Elmholt; IMI 304294, Hven, Sweden, Aug. 1985, J.C.F.; IMI 304295, Teresienstadt, Czechoslovakia, Oct. 1984, J.C.F.; IMI 309316, J.C.F.; IBT HOJ 1, Jagersveld near Lelystad, the Netherlands, May 1985, J.C.F; IBT KLIM 2, Klitmøller, Denmark, Nov. 1986; IBT ISTA 2, lake side 200 km from Istanbul, Turkey, 1986, J.C.F.; IBT KNAJ 2 & 5, Knardijk, the Netherlands, Oct. 1986, J.C.F.; IBT HOUT 4 & 5, Houtribdijk, the Netherlands, Oct. 1986, J.C.F.

Penicillium scabrosum is characterized by conspicuously roughened conidiophore stipes and finely roughened globose to subglobose conidia. The penicilli are predominantly one-stage-branched, but a lower branch often occurs. The new taxon resembles *P. atrovenetum* G. Smith (1956), but this species has only finely roughened stipes and definitely echinulate conidia. Moreover *P. atrovenetum* grows more slowly on MEA (18–22 mm diam. after one week at 25°C). The two taxa have no secondary metabolites in common. Among the ca. 100 different secondary metabolites produced by *P. scabrosum*, cyclopenin, cyclopenol, and viridicatin are antibiotically active and fumagillin is antiprotozoan (Cole & Cox, 1981). In contrast, *P. atrovenetum* produces 3-nitropropionic acid and atroventin (Frisvad & Filtenborg, 1990).

[1] IBT = collection of the Institute of Biotechnology, Lyngby.
[2] J.C.F. = Jens C. Frisvad.

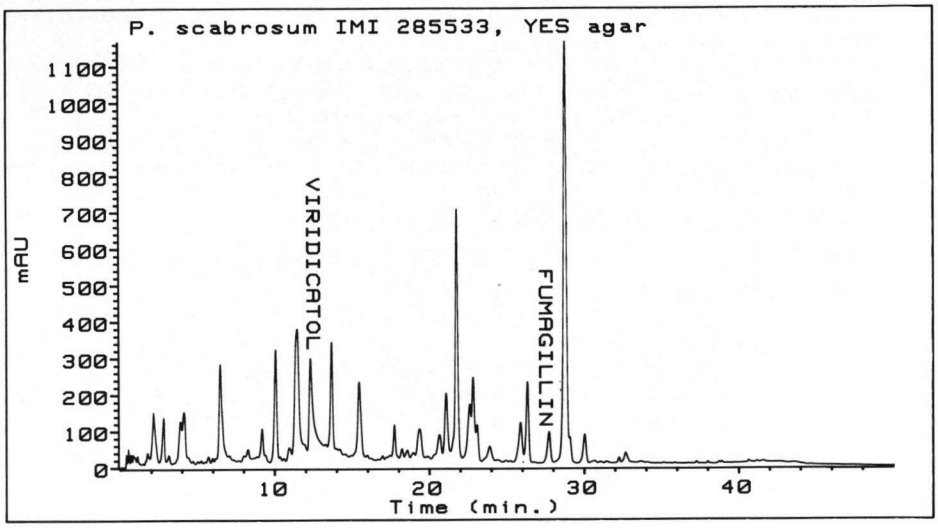

Fig 3. HPLC trace of a chloroform/methanol extract of the culture ex type of *P. scabrosum*.

A total number of 154 isolates of *P. scabrosum* were recovered from soil and food and feed samples and the most important isolates are listed under the isolates examined. We have isolated *P. scabrosum* repeatedly from cultivated soil in the Netherlands and Denmark. Isolates from wheat-field soil in Germany (e.g. CBS 355.68) which were originally assigned to *P. atrovenetum* by Gams & Domsch (1970) proved to be *P. scabrosum*. All the isolates examined have the same profile of secondary metabolites as evaluated by TLC, including several yellow- and blue-fluorescent compounds, both before and especially after treatment of the TLC plates with cold 50% sulphuric acid. Viridicatin, viridicatol, and fumagillin were among the blue-fluorescent metabolites and their identity was confirmed by HPLC with diode array detection.

Penicillium scabrosum should be placed in *Penicillium* subgenus *Penicillium* section *Divaricatum* Raper & Thom ex Pitt series *Atroveneta* Stolk & Samson (see Stolk & Samson, 1985).

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