

**PENICILLIUM DONKII SP. NOV. AND SOME OBSERVATIONS ON  
SCLEROTIAL STRAINS OF PENICILLIUM FUNICULOSUM**

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(With one Text-figure)

A description and drawings of a new species of *Penicillium*, *P. donkii*, are presented. *Penicillium purpurogenum* Stoll var. *rubri-sclerotium* Thom is considered a synonym of *P. funiculosum* Thom. Some observations are recorded, especially in connection with the cultural appearance of sclerotial strains of *P. funiculosum*.

From arable soil in Alaska a number of cultures were isolated by L. K. Oliver and sent to the 'Centraalbureau' for identification. Among these one strain of *Penicillium* turned out to be sufficiently different from all known members of the genus to warrant its description as a new species. Since this isolate is characterized by dark brown, soft, sclerotium-like structures, it is compared with two other species of *Penicillium* which are also known to produce brown or black pseudoparenchymatous sclerotia: *P. novae-zeelandiae* and *P. funiculosum*.

***Penicillium donkii* Stolk, sp. nov.—Text-fig. 1**

Coloniae in agaro Czapekii 25 °C, fere celeriter crescunt, modice floccosae, medio elato, albae vel bubalinae, conidiis griseo-viridibus abundantibus; nonnumquam pauca corpora alba setosa sclerotialia adsunt. Reversum primo roseolum, deinde brunneum. Coloniae 20 °C lentius crescunt, numerosa corpora alba setose formant, penicilli paucis intermixta.

Coloniae in agaro maltoso 25 °C celeriter expansae, stratum laxe textum modice sporulans formant; reversum brunneum transparet; corpora setose nulla vel rara. 20 °C corpora setosa abundant, penicillis intermixta.

Hyphae vegetativae primo hyalinae, demum luteolae, 2-6  $\mu\text{m}$  diametro.

Conidiophora plerumque e hyphis aeriis, marginem versus nonnumquam e hyphis submersis oriuntur, simplicia, septata, hyalina, 20-300  $\times$  2-3  $\mu\text{m}$ , parietibus levibus vel fere levibus, sursum dilatata, 4-5.5  $\mu\text{m}$  diametro. Penicilli monoverticillati. Phialides 10-12 verticillatae, e basi cylindrica et tubo conidiifero subito constricto, circa 1  $\mu\text{m}$  longo, constant, 7.5-10  $\times$  2.2-2.5  $\mu\text{m}$ . Conidia subglobosa vel paene ellipsoidea, diluta viridia, 2.0-2.7  $\times$  1.5-2.3  $\mu\text{m}$ , levia vel fere levia, columnas ad 200  $\mu\text{m}$  longas formant.

Corpora sclerotialia brunnea, plerumque discreta, globosa, 60-120  $\mu\text{m}$  diametro, mollia, pseudoparenchymatosa, multis hyphis septatis, hyalinis, fere rectis, deorsum ramosis, radiantibus ad 300  $\mu\text{m}$  longis, 1.5-2  $\mu\text{m}$  crassis, circumdata, quae corpus brunneum omnino complectuntur. Status perfectus ignotus.

Typus CBS 188.72, isolatus a L. K. Oliver e solo agresti in Alaska.

Colonies on Czapek agar growing fairly rapidly, attaining a diameter of 4-4.5 cm within two weeks at 25 °C, azonate, consisting of a thin, loose-textured, more or

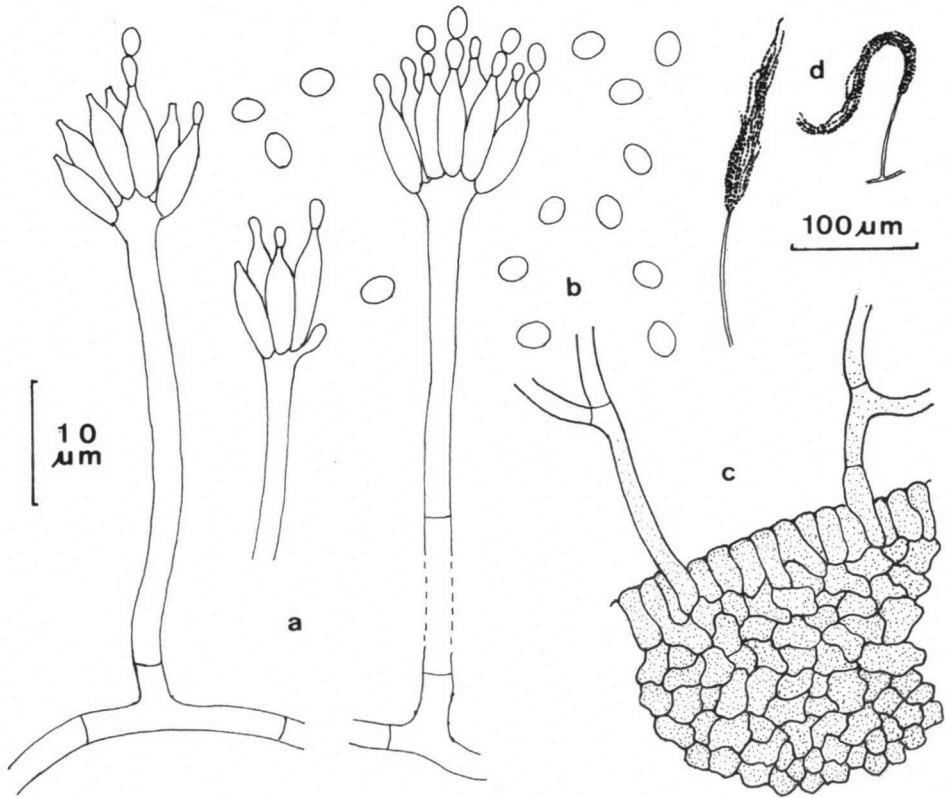


Fig. 1. *Penicillium donkii*, CBS 188.72. — a. Penicilli. — b. Conidia. — c. Section through a sclerotium-like body. — d. Habit sketches of penicilli showing conidial columns.

less floccose, basal felt; central areas raised, close-textured, nearly sterile, white to Vinaceous-Buff (Ridgway, Pl. 40; Rayner, 17''d), bearing abundant conidial structures in marginal and sub-marginal areas, occasionally producing a few, white, setose, sclerotium-like structures; surrounded by a narrow, brownish zone, consisting mainly of submerged hyphae. Conidial areas greyish green, ranging from Celandine Green to Storm Gray, becoming Grayish Olive in age (Ridgway, Pls. 47, 52, 46; Rayner, 33''b, 35''m, 21''m). Exudate lacking. Reverse at first pinkish, near Light Pinkish Cinnamon, later showing brown shades ranging from Snuff Brown to Bister, becoming Clove Brown in age (Ridgway, Pls. 29, 40; Rayner, 15''d, 15''k, 15''m, 17''m). Colonies at 20 °C growing more slowly, developing numerous white, setose, sclerotium-like bodies, usually produced near the agar surface, but sometimes also occurring within the aerial mycelium, intermixed with a few penicilli; colour white to greyish.

Colonies on malt agar attaining a diameter of 6.5 cm within two weeks at 25 °C, zonate, consisting of a loose-textured felt, light-sporing throughout; dark brown, near *Chaetura* Drab, becoming *Chaetura* Black in age (Ridgway, Pl. 46; Rayner

17''''k, 17''''m), owing to the brown colour of the reverse showing through; setose bodies lacking or scanty. Reverse dark brown like the surface. Colonies at 20 °C consisting mainly of a layer of white, setose, sclerotium-like bodies, embedded in and overgrown by a loose aerial network, bearing abundant conidial structures, surrounded by a broad, brownish marginal zone consisting mainly of submerged hyphae. Reverse like at 25 °C.

Vegetative hyphae at first hyaline, later becoming yellowish brown, smooth-walled, 2–6 µm in diameter.

Conidiophores arising usually from the basal felt and from overgrowing aerial mycelium, in marginal areas sometimes developing from submerged hyphae, unbranched, septate, hyaline, variable in length, ranging from 20–300 µm in length by 2–3 µm in diameter, with walls smooth or nearly so, rarely covered with a few encrustations, apices enlarged, about 4–5.5 µm in width. Penicilli monoverticillate. Phialides in crowded whorls up to 10 or 12 in a verticil, consisting of a cylindrical base tapering abruptly to a short conidium-bearing tube (about 1 µm long), measuring 7.5–10 × 2.2–2.5 µm. Conidia subglobose to ellipsoidal, pale greenish, 2–2.7 × 1.5–2.2 µm, smooth or nearly so, forming well-defined, sometimes slightly twisted columns up to 200 µm in length.

Sclerotium-like bodies usually discrete, globose, 60–120 µm in diameter, hyaline when young, but soon becoming brown, soft, pseudoparenchymatous, consisting of comparatively small, irregular cells, about 2–3 µm in diameter, with the surface cells radially arranged; bearing numerous, septate, hyaline (at the base slightly brownish), radiating, fairly straight hyphae, up to 300 µm in length by 1.5–2 µm in diameter, a few times branched at the basal parts, completely obscuring the brown inner bodies and giving the structures a conspicuous white appearance even in old cultures. Perfect state not observed.

The species is mesophilic, optimum temperature 20–25 °C, maximum temperature somewhat above 35 °C. Development of setose bodies is more pronounced at 20 °C than at 25 °C.

TYPE CULTURE: CBS 188.72, isolated by L. K. Oliver from arable soil in Alaska.

The species is named after the late Dutch mycologist Dr. M. A. Donk.

*Penicillium donkii* cannot be placed satisfactorily in any of the series of the genus proposed by Raper & Thom (1949). The structures of the penicilli, the conidial columns, and the shape of the phialides are reminiscent of the *P. thomii* series. However, the soft, brown, sclerotium-like structures of the present species are quite different from the true sclerotia characteristic of the species of the *P. thomii* series. The sclerotia of the latter are typically very hard and gritty, consisting of large, almost colourless, polygonal cells with very thick walls. The dark brown, sclerotium-like bodies of *P. donkii* are suggestive of the reddish brown sclerotia produced by occasional strains of *P. funiculosum* Thom and the black sclerotia characterizing *P. novae-zeelandiae* Beyma. The sclerotia of the latter two species are much larger. They are pseudoparenchymatous and consist of large, thick-walled, brown cells, which are quite different from those of *P. donkii*. Moreover, they are not surrounded by long, conspicuous, radiating hyphae. In addition, *P. donkii* differs from the two species mentioned in producing monoverticillate penicilli.

Although classified by Raper & Thom in the *Biverticillata-Symmetrica*, *P. novae-zeelandiae* does not produce the lanceolate phialides characteristic of this section. The

penicilli of *P. novae-zeelandiae* are in much better agreement with those of the *P. raistrickii* series, but the sclerotia of *P. novae-zeelandiae* are quite different from those occurring in this series on account of their structure and black colour.

#### PENICILLIUM FUNICULOSUM Thom

*Penicillium funiculosum* Thom in U.S. Dept. of Agr., Bur. Anim. Ind. 118: 69. 1910.

*Penicillium purpurogenum* Stoll var. *rubri-sclerotium* Thom in Mycologia 7: 142. 1915.

*Penicillium purpurogenum* Stoll var. *rubri-sclerotium* was introduced by Thom (1915) for strains which have the deep red reverse in common with the parent species, but differ from *P. purpurogenum* in developing dark red to dark brown sclerotia on the surface of the agar. The type strain of this variety (CBS 270.35 = NRRL 1064) has lost its capacity to produce sclerotia and no longer develops its characteristic red reverse. The strain now produces fairly deep, funiculose, broadly spreading colonies. Examination of CBS 270.35 proved the conidial structures and the conidia to be quite different from those of *P. purpurogenum*. The conidiophores and especially the penicilli show greenish brown colours, thus agreeing completely with the corresponding structures of CBS 329.48 (= NRRL 1032a) regarded by Raper & Thom (1949) as representative of *P. funiculosum*. In addition, the conidial structures of CBS 365.48 (= NRRL 1066), identified by Raper & Thom (1949) as *P. purpurogenum* var. *rubri-sclerotium*, are also identical with those of *P. funiculosum*. The rate of growth and the red colour of the reverse of these sclerotial strains agree very well with Thom's original description of *P. funiculosum* (1910, p. 69) and with CBS 329.68.

As there is no character other than the production of sclerotia to separate *P. purpurogenum* var. *rubri-sclerotium* from *P. funiculosum*, a character that becomes useless as soon as the sclerotial strains have lost their sclerotia, the maintenance of this variety seems to have little sense.

Colonies of new isolates of sclerotial strains of *P. funiculosum* (CBS 883.72 and CBS 884.72) grow rapidly on most media but especially on malt agar. They are velvety or nearly so, at least near the margin, while trailing hyphae or strands of hyphae are observed only in the central areas. On Czapek the reverse is deep red. While marginal areas in some strains may be dark brown owing to the production of sclerotia, the latter occur only scantily in others. Sclerotium production is most abundant on malt agar at 25 °C. On this medium sclerotia are abundantly produced throughout the cultures, typically occurring in concentric zones. The sizes of the conidiogenous structures agree with those of the non-sclerotial strains. Conidiophores and penicilli are definitely green to brown coloured. Sclerotia are reddish brown to dark brown, globose to ellipsoid, often confluent, about 200–300  $\mu\text{m}$  in diameter, pseudoparenchymatous, commonly overgrown by a very thin, inconspicuous web of hyphae, often bearing a few conidial structures.

Raper & Thom (1949: 619) described an isolate of *P. funiculosum* (NRRL 1132) which produced reddish brown to dark brown sclerotium-like bodies resembling

those of the strains considered by them to represent *P. purpurogenum* var. *rubri-sclerotium*. According to these authors the sclerotia of NRRL 1132 are primarily produced within the substrate, whereas those of *P. purpurogenum* var. *rubri-sclerotium* were stated to develop on the agar surface. However, in the fresh isolates described above the sclerotia develop within the agar as well as on the agar surface.

Sclerotial strains have a strong tendency to degenerate. After a few transfers the colonies become more funiculose while after some years of subsequent laboratory cultivation the number of sclerotia decreases. This ultimately leads to the loss of the capacity to produce sclerotia. However, on Czapek agar some strains produce 'wet' colonies which consist mainly of submerged hyphae.

According to Raper & Thom's conception (1949), *P. funiculosum* is an extremely variable species, especially as regards the appearance of the colony. Broadly spreading as well as restrictedly growing strains belong to it. The colour of the colonies varies depending upon the amount and colour of the aerial mycelium which is usually yellow, red, but occasionally colourless. Moreover, the reverse may show such different colours as pink, red, yellowish or brownish. The older penicilli of the strains are often characterized by a greenish colour which may be lacking in young structures, but very striking in sclerotium-producing strains. The conidia of these strains are all very much alike.

A more detailed examination of a larger number of strains of *P. funiculosum* is necessary to decide on the desirability of a subdivision into varieties, based on cultural appearance and rate of growth.

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