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Penicillium commune, P. camembertii, the origin of white cheese moulds, and the production of cyclopiazonic acid

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The taxonomy of Penicillia producing the mycotoxin cyclopiazonic acid, including isolates classified as Penicillium aurantiogriseum *and* P. puberulum, *is reviewed on the basis of morphology, physiology, mycotoxin production and isoenzyme profiles. It is concluded that* P. puberulum, *as neotypified by Pitt in his 1979 monograph, is a synonym of* P. aurantiogriseum. *The correct name for saprophytic Penicillia producing cyclopiazonic acid is* P. commune *with* P. palitans *as a synonym. The moulds used in the manufacture of white cheeses, which are all classified in* P. camembertii, *and which also produce cyclopiazonic acid, are domesticated fungi derived from* P. commune.

Introduction

The white moulds which are used in the production of cheeses such as Camembert, Brie, Weißschimmelkäse and Neufchatel have long been regarded as domesticated fungi. Thom (1906), in describing *Penicillium camembertii*, stated 'Persistent search has failed to find a single colony [of *P. camembertii*] in America whose presence can be attributed to anything but Camembert cheese imported from Europe'. These moulds have been known by various names in cheese industries around the world: Thom (1930) and Raper and Thom (1949) accepted two species, *P. caseicola*, which produces white conidia, and *P. camembertii*, in which conidia are pale grey. Samson et al. (1977) combined the two species under the older of the two names, *P. camembertii*. Pitt (1979) agreed, considering that 'the two species are so strikingly similar that it is difficult to escape the conclusion that the strains with white conidia are mutants of the grey green: selected for, perpetuated in, and apparently confined to, cheese manufacture'.

The origin of the moulds used in the manufacture of white cheeses has aroused curiosity, for domesticated fungi are rare. Pitt (1979) speculated that FRR 2160, an isolate from spoiled New Zealand cheddar cheese, was a 'wild' *P. camembertii*. This isolate differed in a number of morphological and microscopic properties from the domesticated species, but similarities suggested a common ancestor.

The origin of *P. camembertii* became of more than academic interest when it was discovered by Still et al. (1978) that white cheese moulds may produce the mycotoxin cyclopiazonic acid. In a subsequent study of 69 *P. camembertii* isolates of both white and grey spored types,

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drawn from a wide variety of sources, Leistner and Eckardt (1979) failed to find a single isolate which was not capable of cyclopiazonic acid production. Apparently all known starter cultures of *P. camembertii* used in cheese manufacture can produce cyclopiazonic acid (Scott 1981). The discovery of a *P. camembertii* ancestor which was not mycotoxigenic could be of great potential value in producing new strains suitable for cheese manufacture.

The taxonomy of Penicillia producing cyclopiazonic acid has proved to be difficult. Leistner and Pitt (1977) reported *Penicillium cyclopium* Westling (= *P. aurantiogriseum* Dierckx) to be the main producer of this toxin, but some isolates identified as *P. puberulum* Bainier, *P. viridicatum* Westling, *P. crustosum* Thom and *P. patulum* Bainier (= *P. griseofulvum* Dierckx) produced it also. Pitt (1979) regarded all of these species as distinct, though with the name changes indicated above in brackets. Of particular interest here is his retention of *P. puberulum* as a separate species from *P. aurantiogriseum* on the basis of relatively minor differences in growth rates, colony texture and conidial colours. Toxin production was not considered.

In their study of secondary metabolite production by species in subgenus *Penicillum*, Frisvad and Filtenborg (1983) 'provisionally included' isolates of *P. puberulum* in *P. aurantiogrisum*, a species they regarded as the major producer of penicillic acid. Isolates producing cyclopiazonic acid were all assigned to a single species, *P. camembertii*, which they divided into two 'groups'. '*P. camembertii* Group I' included all of the cheese moulds placed in this species by modern taxonomists, while '*P. camembertii* Group II', centred on FRR 2160, was introduced for creatine positive isolates (Frisvad, 1981) drawn from several species accepted by Pitt (1979). Frisvad (1986) placed the neotype of *P. puberulum* in his subspecies '*P. aurantiogriseum* Group II'. In his view, the cyclopiazonic acid producers were not related to *P. puberulum*.

Williams and Pitt (1986), working from traditional taxonomic bases, enlarged the concept of *P. aurantiogriseum* so that it effectively included all *P. puberulum* isolates, but they did not resolve the fundamental confusion between isolates which produced penicillic acid, and those which produced cyclopiazonic acid.

Two studies which together resolved this question, and established the origins of *P. camembertii*, are reported in this paper. One study was carried out in the Federal Republic of Germany by two of us, while at the same time the third (R. H. C.) independently worked in Tasmania with cultures supplied from the collection at the first author's institute.

Materials and Methods

Studies in culture

About 250 isolates producing penicillic acid and/or S-toxin (Leistner 1984), or cyclopiazonic acid was studied in pure culture using the morphological and gross physiological methods of Pitt (1979). Cultures were grown on the standard plating regime of Czapek yeast extract agar (CYA) at 5, 25 and 37°C, and on malt extract agar (MEA) and 25% glycerol nitrate agar at 25°C. Cultures were examined macroscopically and microscopically from CYA and MEA at 25°C after 7 days incubation, and colony diameters were measured from all five standard conditions. Colour names in capitals and other colour nomenclature used below are from the 'Methuen Handbook of Colour' (Kornerup and Wanscher 1978). Nearly all of the cultures examined were from the collection at the Federal Centre for Meat Research, Kulmbach.

Mycotoxin assays

The cultures indicated above had previously been examined for mycotoxin production at

Kulmbach, by thin layer chromatography and other methods outlined by Leistner and Pitt (1977) and Leistner and Eckardt (1979).

Enzyme profiles

Production of pectic enzymes, amylases and ribonucleases were studied by growing cultures in the presence of suitable substrates, citrus pectin or wheat grains, followed by electrophoretic separation at low temperature and visualization by staining. Isoenzymes were then photographed and compared. The methods are given in detail by Cruickshank and Pitt (Mycologia, submitted). Cultures examined included type, authentic and other cultures classified in relevant species by the first author, including some regarded as synonyms in Pitt (1979). All cultures came from the FRR collection at CSIRO Division of Food Research, North Ryde, N.S.W., but included some originally obtained from the collection at Kulmbach.

Results

Studies at Kulmbach

At Kulmbach, detailed taxonomic studies on isolates producing either penicillic acid and/or S-toxin showed that the great majority could confidently be placed in *Penicillium aurantiogriseum*. Conidial colours of colonies grown on CYA and MEA at 25°C for 7 days were consistently greyish blue to blue green (24-25D-E3-4). Under the high power microscope, stipes from colonies grown on both media were smooth to finely roughened. This feature was not recognized by Pitt (1979) but was emphasized by Williams and Pitt (1986). Conidial colours of isolates producing cyclopiazonic acid, however, were more greenish in colour: on CYA, colours ranged from grey blue (24D-E3-4) to green (27E3), while on MEA, colours could almost always be considered to be true greens (26-27D-E3-4). Stipes of cyclopiazonic acid producers were usually distinctly, though not prominently, roughened when examined from colonies on both CYA and MEA. It became clear that two species of very similar appearance were being observed, separated readily by clear cut differences in mycotoxin production and, with care, on morphological criteria as well (Table 1).

Examination of a culture ex neotype of *P. puberulum* present in the collection at Kulmbach, Sp 916 (= FRR 2040), indicated that this should be placed in *P. aurantiogriseum* on these revised morphological grounds, as it had been on the

Table 1. Features distinguishing *Penicillium* isolates producing cyclopiazonic acid from those producing penicillic acid.

Mycotoxin	Penicillic acid	Cyclopiazonic acid
Conidial colour, CYA	Bluish green 24-25D–E3-4[a]	Bluish green to green 24-27D–E3-4
Conidial colour, MEA	Bluish green 24-25D–E3-4	Green 26-27D–E3-4
Colony diameters, mm[b]		
absolute range, CYA	20–44	24–40
absolute range, MEA	16–41	18–35
80% of isolates, CYA	30–37	30–37
80% of isolates, MEA	24–37	23–30
Stipe roughening, CYA	Smooth to finely rough (rarely rough)	Finely rough to rough (rarely smooth)
Conidia, length	Not exceeding 4 μm	Often up to 4·5 μm

[a] Colour codes from Kornerup and Wanscher (1978).

[b] Data from 167 isolates producing penicillic acid, and 94 producing cyclopiazonic acid. Cultures examined included many old and deteriorating isolates, with colony diameters outside the ranges to be expected from fresh isolates.

basis of secondary metabolism by Frisvad and Filtenborg (1983). Examination of the FRR strain of the *P. puberulum* neotype (FRR 2040) confirmed this. It was now clear that the name *P. puberulum*, neotypified by Pitt (1979) using an isolate (NRRL 1889) regarded as representative of the species by Raper and Thom (1949), is a synonym of *P. aurantiogriseum*. An equally important corollary is that the name *P. puberulum* is unavailable for the producers of cyclopiazonic acid.

The collection at Kulmbach also contained a culture ex type of *p. palitans* Westling 1911, Sp 915 (= CBS 107.11, IMI 40215, FRR 2033), which has been established to be a cyclopiazonic acid producer. Based principally on the examination of several isolates regarded by Raper and Thom (1949) as authentic for this species, Pitt (1979) had placed *P. palitans* in synonymy with *P. viridicatum*; however, in the opinion of R. A. Samson (pers. comm.) the type of *P. palitans* could not be classified in the latter species. Morphological examination of Sp 915 showed Samson's opinion to be correct: conidia on both CYA and MEA were dull green (27E4 and 26½E4 respectively), with definitely rough stipes. The early original publication date for *P. palitans*, and its morphology, together with the production of cyclopiazonic acid, indicated that it was an appropriate name for the cyclopiazonic acid producers. The possibility that an earlier valid and typified name existed could not be dismissed, but deterioration of old types in culture made accurate assessment of earlier species names difficult.

At the same time as the studies outlined above were in progress, studies on the enzyme patterns of these and other species in subgenus *Penicillium* were being undertaken at the University of Tasmania. These studies will be reported in detail elsewhere (Cruickshank and Pitt, Mycologia, submitted). With regard to the species of interest here, these studies independently produced the same conclusions as those outlined above: that isolates producing cyclopiazonic acid gave zymograms distinct from those isolates of *P. aurantiogriseum* which produced penicillic acid; that the neotype of *P. puberulum* produced zymograms characteristic of *P. aurantiogriseum*; and that the type of *P. palitans* produced zymograms characteristic of the isolates forming cyclopiazonic acid (Fig. 1). Furthermore, the cultures of *P. camembertii* examined all produced zymograms identical with, or very similar to, those of the other cyclopiazonic acid producers. Further study of type isolates of some species placed by Pitt (1979) in synonymy with *P. aurantiogriseum* and *p. puberulum* showed that the type of *P. commune* Thom 1910 produced zymograms characteristic of the cyclopiazonic acid producers also (Fig. 1). This isolate (FRR 890) was examined subsequently in the North Ryde laboratory: its morphology was also characteristic of the cyclopiazonic acid producers. Moreover, it produced a low level of cyclopiazonic acid, despite having been maintained in culture for most of the past 80 years. It can be confidently concluded that the earliest identifiable valid names for *Penicillium* isolates producing cyclopiazonic acid are *P. camembertii* and *P. commune.*

Discussion

It has been shown above that the earliest recognizable species producing cyclopiazonic acid are *P. camembertii* and *P. commune*. It is considered both logical and expedient to reserve the name *P. camembertii* for the domesticated moulds with which cheeses such as Camembert and Brie are produced, and to revive the

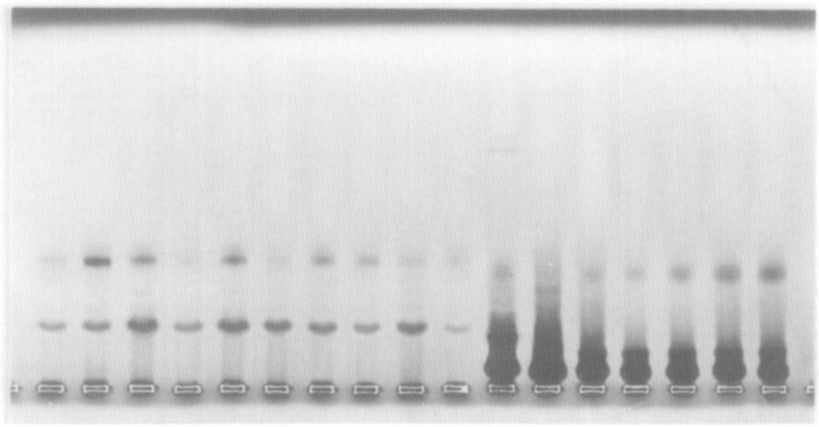

Fig. 1. Pectic (polygalacturonase) zymograms of *Penicillium* isolates. Lanes 1–10, cyclopiazonic acid producers; lanes 11–17, penicillic acid producers. 1, *P. palitans*, FRR 2033, type isolate; 2, *P. commune*, NRRL 890a, type isolate; 3–7, various isolates grouped in *P. commune* by zymogram; 8, *P. camembertii*, FRR 2160, considered to be a 'wild type' by Pitt (1979), now placed in *P. commune*; 9, *P. camembertii*, recently isolated from Camembert cheese; 10, *P. camembertii*, FRR 877, type isolate; 11, *P. aurantiogriseum*, FRR 971, neotype isolate; 12, *P. puberulum*, FRR 2040, neotype isolate; 13–17, isolates grouped in *P. aurantiogriseum* by zymogram.

name *P. commune* for isolates which are not specifically cheese moulds but which occur as ubiquitous saprophytes. The common origin of these two species is evident, both from their production of a single mycotoxin, rarely produced by other *Penicillium* species and, perhaps more convincingly, by unique and virtually identical patterns of zymograms (Fig. 1). If it is accepted that *P. camembertii* is a domesticated species, then *P. commune* must logically be its ancestral wild type. It is interesting and relevant that the type isolate of *P. commune* was isolated from cheese, and that FRR 2160, regarded by Pitt (1979) as a wild *P. camembertii*, was also isolated as a cheese spoilage fungus. Taking into account the extreme age and floccose habit of FRR 890, the type of *P. commune*, the morphological and physiological resemblance of this isolate to FRR 2160 is quite striking.

Previously, the taxonomy of the species referred to here as *P. commune* has been confused. Thom (1910, 1930) regarded *P. commune* as a ubiquitous species. Raper and Thom (1949), however, classified it in their subsection *Lanata*, to which floccose (and therefore deteriorating) species were consigned. It is probable that, in their classification, producers of cyclopiazonic acid were assigned to *P. cyclopium, P. puberulum, P. palitans* (perhaps only the type), and also other floccose species as well as *P. commune*. Samson et al. (1977) maintained *P. commune* as a rare species characterized by its floccose habit and ellipsoidal conidia. In their taxonomy, most freshly isolated cyclopiazonic acid producers would be identified, with a variety of other mycotoxigenic isolates, as *P. verrucosum* var. *cyclopium*. Pitt (1979) assigned the cyclopiazonic acid producers to either *P. aurantiogriseum* or *P. puberulum*, or in one case, FRR 2160, to *P. camembertii*, while Frisvad and Filtenborg (1983) logically created '*P. camembertii* Group II' for them. Williams and Pitt (1986) as-

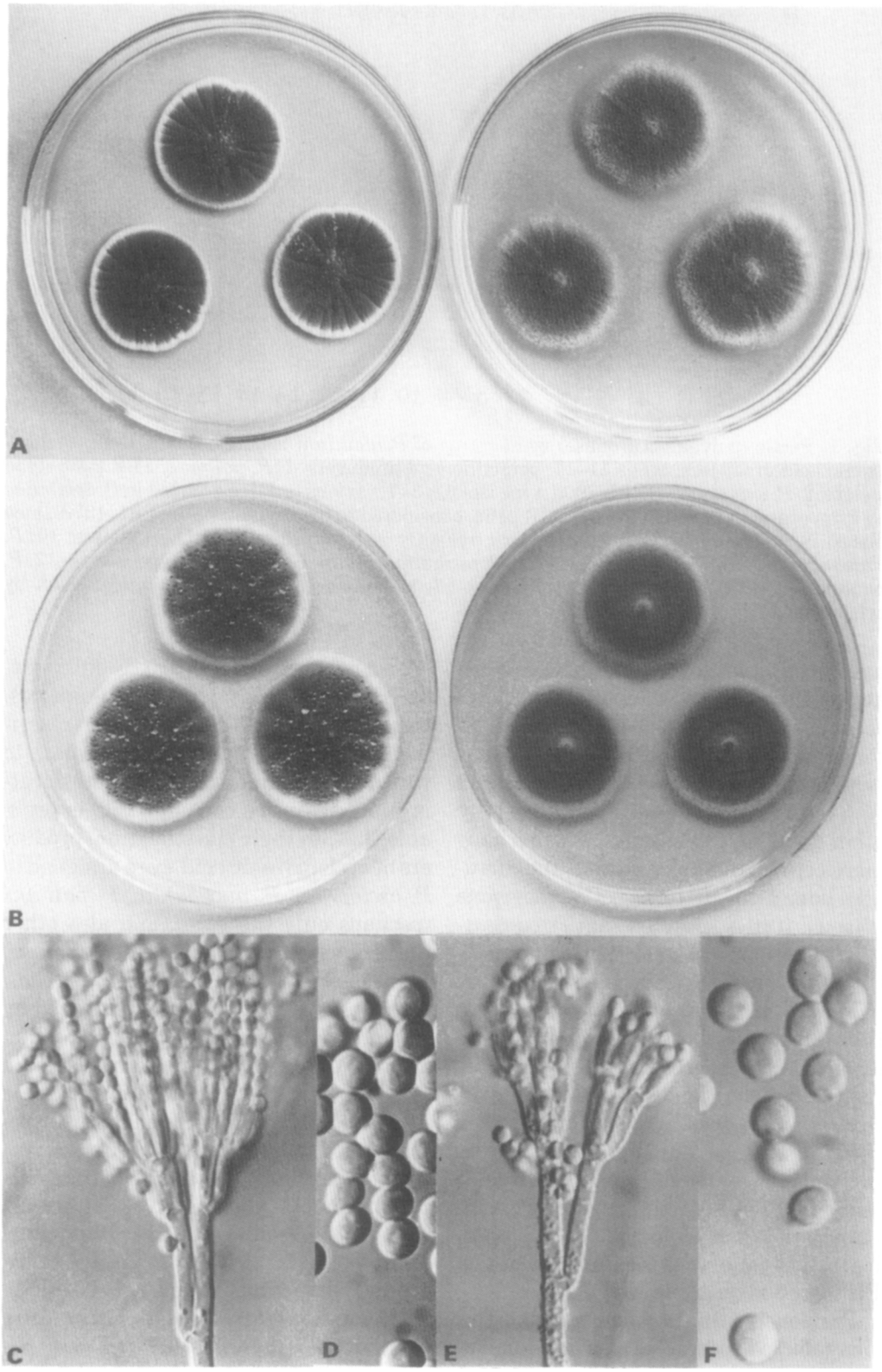

Fig. 2. *Penicillium aurantiogriseum*: (A) colonies on Czapek yeast extract agar and malt extract agar at 25°C, 7 days; (C) penicillus, ×750; (D) conidia, ×1875. *Penicillium commune*: (B) colonies on Czapek yeast extract agar and malt extract agar at 25°C, 7 days; (E) penicillus,

signed them to an enlarged *P. aurantiogriseum*.

More than 160 isolates known to produce cyclopiazonic acid, but not identifiable as *P. camembertii*, have been examined in this study. A high proportion of them, in excess of 80%, can be identified morphologically as a single species, now recognized as *P. commune*. This species is very similar morphologically to *P. aurantiogriseum*, usually differing from the latter by the features detailed in Table 1, although some degree of morphological overlap occurs. *P. commune* also resembles *P. viridicatum*: *P. viridicatum* produces brighter green conidial colours and more delicate penicilli. A small number of isolates have been examined which morphologically are indistinguishable from *P. viridicatum*, but which produce cyclopiazonic acid. In our present state of knowledge it is considered preferable to maintain the morphological basis to this species, and to accept that a minority of *P. viridicatum* isolates produce cyclopiazonic acid rather than to attempt to 'force' such isolates into *P. commune*.

Descriptions of *P. aurantiogriseum*, as now emended, and *P. commune*, as now revived, follow. Both species are illustrated in Fig. 2.

Penicillium aurantiogriseum Dierckx Annls Soc. Sci. Brux. 25: 88, 1901.

Penicillium puberulum Bainier, Bull. trimest. Soc. mycol. Fr. 23: 16, 1907.

Penicillium cyclopium Westling, *op cit.* 11: 90, 1911.

Penicillium aurantiovirens Biourge, Cellule 33: 119, 1923.

Penicillium martensii Biourge, *op. cit.* 33: 152, 1923.

Penicillium lanoso-coeruleum Thom, Penicillia: 322, 1930.

Penicillium verrucosum var. *cyclopium* (Westling) Samson et al., Stud. Mycol., Baarn 11: 37, 1976.

Penicillium commune Thom Bull. Bur. Anim. Ind. US Dep. Agric. 118: 56, 1910.

Penicillium palitans Westling, Ark. Bot. 11: 83, 1911.

Penicillium lanosum Westling, *op. cit.* 11: 97, 1911.

Penicillium lanosogriseum Thom, Penicillia: 327, 1930.

Colonies on CYA 30–37 mm diam, radially sulcate, moderately deep, texture velutinous to fasciculate; mycelium white, usually inconspicuous; conidiogenesis moderate to heavy, Greyish Turquoise to Dull Green (24-25D–E3-4); exudate usually conspicuous, clear or pale brown; soluble pigment produced by some isolates, brown to reddish brown; reverse pale, light to brilliant orange, or reddish to violet brown. Colonies on MEA 24–37 mm diam, plane or rarely radially sulcate, low and relatively sparse, surface texture velutinous to fasciculate; mycelium usually subsurface, occasionally conspicuous and then bright yellow; conidiogenesis usually moderate to heavy, Greyish Turquoise to Dull Green (24-25D–E4-5); soluble pigment sometimes produced, yellow brown to reddish brown; reverse pale, orange, or reddish brown. Colonies on G25N 18–24 mm diam, usually radially sulcate, moderately deep, dense, velutinous to fasciculate; reverse pale, yellow or brown. At 5°, colonies 2–5 mm diam, of white mycelium. No growth at 37°.

Conidiophores borne singly or in fascicles, mostly from subsurface hyphae, stipes 200–400 μm long, or of indeterminate length in fascicles, with walls smooth to finely roughened, only rarely rough, bearing terminal terverticillate or less commonly biverticillate penicilli; rami 15–25(–30) μm long; metulae 10–15(–18) μm long; phialides slender, ampulliform, mostly 7–10 μm long; coni-

dia spherical to subspheroidal, less commonly ellipsoidal, usually 3·0–4·0 μm long, with smooth walls, mostly borne in long, well defined columns.

Colonies on CYA 30–37 mm diam, radially sulcate, usually fasciculate, less commonly velutinous; mycelium white, usually inconspicuous; conidiogenesis moderate, of variable colour, Greyish Turquoise to Dull Green (24-27D–F3-5); exudate usually present, clear to pale brown; soluble pigment not produced; reverse usually pale, occasionally yellow, brown or purple. Colonies on MEA 23–30 mm diam, plane or lightly sulcate, low and dense, surface velutinous or lightly fasciculate; mycelium inconspicuous, white; conidiogenesis moderate, Dull Green (26-27D–E3-4); exudate and soluble pigment absent; reverse usually uncoloured. Colonies on G25N 18–22 mm diam, plane, sulcate or wrinkled, low to moderately deep, dense, usually fasciculate; mycelium white to yellowish; reverse pale to orange brown. At 5°, at least microcolony formation; typically colonies of 2–4 mm diam formed. No growth at 37°.

Conidiophores borne singly or in fascicles, mostly from subsurface hyphae, stipes 200–400 μm long or of indeterminate length in fascicles, with walls finely to conspicuously roughened, typically bearing terminal terverticillate penicilli; rami 15–20(–30) μm long; metulae 10–15(–18) μm long; phialides ampulliform, 9–11 μm long; conidia spherical, uncommonly subspheroidal, 3·5–4·0(-5) μm diam, smooth walled, borne in disordered chains.

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