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NOTES ON THE TYPIFICATION OF SOME SPECIES OF PENICILLIUM

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A number of so far not correctly typified species of the genus *Penicillium* were re-examined. The profiles of secondary metabolites in old type strains and fresh isolates were compared. The type culture of *P. implicatum* Biourge was found to be identical with *P. citrinum* Thom. The first available name for *P. implicatum* sensu Raper & Thom is *P. hispanicum* Ramírez & al. *Penicillium fellutanum* Biourge and *P. phaeojanthinellum* Biourge are both considered to be synonyms of *P. citrinum*. *Penicillium janthinellum* Biourge sensu Raper & Thom agrees with the iconotype of *Spicaria simplicissima* Oud., while *P. simplicissimum* (Oud.) Raper & Thom sensu Pitt is conspecific with *P. brasilianum* Batista & al. *Penicillium minioluteum* Dierckx is redefined, based on an authentic type strain (Biourge 60 = CBS 642.68). *Penicillium griseoroseum* Dierckx is the first available name for *P. chrysogenum* Westling, but the latter name is to be protected by conservation. *Penicillium olsonii* Bain. & Sartory is considered to be a distinct species because it has a specific profile of secondary metabolites, clearly different from *P. brevicompactum* Dierckx. *Penicillium solitum* Westling is regarded as the correct name for dark (bluish) green strains previously called *P. verrucosum* Dierckx var. *melanochlorum* Samson & al. and *P. mali* Gorlenko & Novobranova.

For many decades taxonomic studies on the genus *Penicillium* had been hampered by the lack of proper typification of the described taxa. One of the greatest merits of Pitt (1980) in his monograph is that he typified most anamorphic and teleomorphic species related to *Penicillium*. If no holotype was available, he designated neotypes in many cases. The morphological study of type cultures of many *Penicillium* species can be difficult, when the strains have degenerated after many years of preservation. The atypical strains no longer fit the orginal description and it is sometimes impossible to identify a species using the original strains. However, by analysing the profiles of secondary metabolites, the identity of a type strain with recent, well-developed isolates is often possible (Frisvad, 1986).

In this paper we report the results of the morphological and biochemical examination of some problematic taxa.

MATERIALS AND METHODS

The following species were examined: *P. minioluteum* Dierckx, *P. implicatum* Biourge, *P. paxilli* Bainier, *P. fellutanum* Biourge, *P. janthinellum* Biourge, *P. simplicissimum* (Oud.) Thom, *P. olsonii* Bainier & Sartory, *P. griseoroseum* Dierckx, *P. majusculum* Westling, and *P. solitum* Westling.

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All cultures 'ex-neotypes' and 'ex-holotypes' were inoculated on Czapek agar (CzA), Czapek yeast autolysate agar (CYA), Malt extract agar (MEA, as used by Raper & Thom, 1949) and 2% malt-extract agar, yeast extract-sucrose agar (YES), oatmeal agar (OA) and creatine-sucrose agar (for formulations see Samson & Pitt, 1985; Frisvad & Filtenborg, 1983; and Frisvad, 1985). The fungi were incubated on CYA at 37 °C and on all the other media listed at 25 °C for 14 days and examined after 5, 7, and 14 days morphologically, physiologically, and chemically. The fungi were examined for secondary metabolites using the methods of Frisvad & Filtenborg (1983) based on thin-layer chromatography (TLC), and some were also examined using high-performance liquid chromatography (HPLC) (Frisvad, 1987; Frisvad & Thrane, 1987).

Results and discussion

Penicillium citrinum Thom

Penicillium citrinum Thom *in* Bull. Bur. anim. Ind. US Dep. Agric. 118: 61. 1910. — Lectotype: IMI 92196ii (Pitt, 1980).
Penicillium fellutanum Biourge *in* Cellule 33: 262. 1923.
Penicillium implicatum Biourge *in* Cellule 33: 278. 1923.
Penicillium phaeojanthinellum Biourge *in* Cellule 33: 289. 1923.
For further synonyms see Pitt (1980), except *P. steckii* Zaleski.

Raper & Thom (1949) described *P. implicatum* as an often strongly coloured species producing strictly simple (monoverticillate) penicilli and ellipsoidal to globose conidia with finely roughened walls. They regarded NRRL 2061 as a characteristic isolate. Pitt (1980) suggested to neotypify *P. implicatum* with NRRL 2061, indicating that the original type, Biourge 76, was lost. The original type culture is, however, kept in the collection of the Centraalbureau voor Schimmelcultures (CBS) as CBS 232.38. This latter isolate is, in accordance with the protologue, quite typical of *P. citrinum*. Therefore, *P. implicatum* is a synonym of *P. citrinum*, and this is further confirmed by the fact that CBS 232.38 is still a good producer of citrinin. The first available name for *P. implicatum* sensu Raper & Thom (1949) and Pitt (1980) is *P. hispanicum* Ramírez & al. (1978) (IJFM 3223 = CBS 691.77). Other representative cultures for *P. hispanicum* are NRRL 2061 (= CBS 180.81) and NRRL 2054 (= CBS 337.48). All these cultures do not produce citrinin, but a series of specific secondary metabolites with distinct UV spectra.

Penicillium fellutanum Biourge

The species was described as producing simple to one-stage-branched penicilli with a terminal verticil of 2–5 metulae, thus resembling *P. citrinum*. Biourge listed his no. 177 as type and this is maintained at CMI as IMI 92229ii. This culture is in rather poor condition, but resembles *P. citrinum* and it is in agreement with the protologue. Weak production of citrinin by IMI 92229ii is a further confirmation of the synonymy of *P. fellutanum* with *P. citrinum*. The first available name for *P. fellutanum* sensu Pitt (1980) or Raper & Thom (1949) is *P. charlesii* G. Smith. The ex-type culture of this species does not produce citrinin, but a series of tetronic acids (carolic acid, carlosic acid, etc.).

Penicillium phaeojanthinellum Biourge

Another species described by Biourge (1923), *P. phaeojanthinellum,* also develops simple to one-stage-branched penicilli. The type culture IMI 92267 also produced citrinin on YES agar and therefore this taxon should also be regarded as a further synonym of *P. citrinum.*

Penicillium simplicissimum (Oud.) Thom

Spicaria simplicissima Oud. *in* Ned. kruidk. Archf, Ser. 2, 3: 763. 1903. — *Penicillium simplicissimum* (Oud.) Thom, The Penicillia: 335. 1930. — Neotype: IMI 40238 (Culture ex CBS 340.48 = NRRL 2016).
Penicillium glaucoroseum Demelius *in* Verh. zool.-bot. Ges. Wien 72: 72. 1922 ('1923').
Penicillium janthinellum Biourge *in* Cellule 33: 258. 1923.
Teleomorph: *Eupenicillium javanicum* (van Beyma) Stolk & Scott.

As pointed out by Stolk & Samson (1983), *P. janthinellum* sensu Raper & Thom (1949) and sensu Pitt (1980) cannot be delimited satisfactorily against *P. simplicissimum,* which they considered to be the anamorph of *Eupenicillium javanicum* (van Beyma) Stolk & Scott. Typification of *P. simplicissimum* is based on an iconotype of *Spicaria simplicissima* Oud. in herb. L, and a representative isolate is IMI 40238 (= CBS 340.48).

Penicillium simplicissimum produces variable, irregularly one- to two-stage-branched conidiophores with 2–4 metulae per verticil, slender phialides with a conspicuously narrowed neck and subglobose, very finely roughened conidia. This interpretation of *P. simplicissimum* is different from that by Pitt (1979), who based the species on a dried specimen (Herb. CUP 5921). Pitt also included *P. brasilianum* Batista & al. and its synonyms *P. paraherquei* Abe ex G. Smith and *Penicillium skrjabinii* Schmotina & Golovleva in his concept of *P. simplicissimum. Penicillium brasilianum* differs, however, from *P. janthinellum* by larger, more regular, biverticillate penicilli (3–6 metulae per verticil) and ellipsoidal to slightly fusiform, transversely striate conidia. Stolk & Samson (1983) regarded *P. brasilianum, P. paraherquei,* and *P. skjabinii* as synonyms of *P. ochrochloron* Biourge. However, these four species differ significantly from *P. simplicissimum* in their capacity to grow at 37 °C, their tolerance to high concentrations of copper sulphate and profiles of secondary metabolites. *Penicillium pulvillorum* Turfitt and *P. piscarium* Westling also included by Pitt (1979) in his concept of *P. simplicissimum* represent separate taxa.

Penicillium ochrochloron, as originally described by Biourge (1923), may indeed be identical with *P. brasilianum,* because it was described as having rough-walled conidia. *Penicillium ochrochloron* was neotypified by Pitt (1979) based on a copper-resistant isolate with smooth-walled conidia, and this concept fits that of the species in the taxonomical and biochemical literature for at least 50 years. We, therefore, suggest that this neotypification be accepted and that *P. brasilianum* be used for the species producing spirally roughened conidia, represented by CBS 253.55.

Penicillium chrysogenum Westling

Penicillium chrysogenum Thom *in* Bull. Bur. anim. Ind. US Dep. Agric. 118: 58. 1910. — Lectotype IMI 24314 (Pitt, 1980).
Penicillium griseoroseum Dierckx *in* Annls Soc. scient. Brux. 25: 89. 1901.
For further synonyms see Samson & al. (1977), Pitt (1980) and Cruickshank & Pitt (1987).

Based on morphology and profiles of secondary metabolites, it is obvious that *P. griseoroseum* Dierckx and *P. chrysogenum* are synonyms. Cruickshank & Pitt (1987) reached the same conclusion using profiles of isoenzymes as a taxonomic criterion. Hennebert (1985) pointed out that the ex-type culture of *P. griseoroseum* (IMI 92220i) survived as the only culture of those described by Dierckx (1901). Because of the long tradition for *P. chrysogenum* and its tremendous importance as the best penicillin producer, we support the proposal to conserve the name *P. chrysogenum* (compare also Frisvad & al., 1990). All isolates examined of *P. chrysogenum* and *P. griseoroseum* produce penicillin, roquefortine C, and meleagrin.

Penicillium brevicompactum Dierckx

Penicillium brevicompactum Dierckx, *in* Annls Soc. scient. Brux. 25: 88. 1901. — Neotype: IMI 40225.

For further synonyms, see Pitt (1980) except *P. volgaense*, which is a synonym of *P. olsonii*.

Penicillium olsonii Bain. & Sartory *in* Annls mycol. 10: 398. 1912. — Neotype: IMI 192502.

Penicillium brevicompactum Dierckx, *P. stoloniferum* Thom, and *P. paxilli* Bain. were included in the *P. brevicompactum* series by Raper & Thom (1949). We concur with Pitt (1980) that *P. stoloniferum* cannot be separated from *P. brevicompactum* and agree with their synonymy. *Penicillium paxilli* was placed by Pitt (1980) in *Penicillium* subgenus *Furcatum* and considered as closely related to *P. herquei*. However, the nomenclature of *P. paxilli* Bain., as adapted by Raper & Thom (1949) and Pitt (1980), is not in accordance with its protologue (Bainier, 1907) and its correct status will be discussed elsewhere (Frisvad & al., in prep.).

Penicillium olsonii is closely related to *P. brevicompactum*. Stolk & Samson (1985) found both species identical based on their morphological examination of the ex-neotype culture, while Bridge & al. (1989a and b) synonymized them on the basis of an integrated multidisciplinary investigation using biochemical, physiological, and morphological features. A type of *P. olsonii* was apparently not distributed, but the species had been neotypified with IMI 195502, originating from *Picea rhizosphere* in the Austrian Alps (Pitt, 1980). The isolate, which has been maintained for twenty years on agar is now deteriorated and has become morphologically close to *P. brevicompactum* (Figs. 1, 2a). However, it is chemically different from this taxon, and in all other characters duplicates the protologue and fresh cultures of *P. olsonii*. *Penicillium brevicompactum* consistently produces mycophenolic acid and other phenols (Fig. 3). In contrast, *P. olsonii* does not produce any known mycotoxins, but a series of unknown specific secondary metabolites.

Penicillium olsonii is characterized by very large conidiophores consisting of a long stipe and a compact, broad, multi-branched penicillus producing pear-shaped or broadly ellipsoidal, finely roughened conidia (Fig. 2). We have obtained numerous typical isolates of *P. olsonii* from glass house soil and plants (including isolates from bananas, the original substrate of the species). In addition, the species was repeatedly found as an airborne indoor contaminant (Verhoeff & al., 1988).

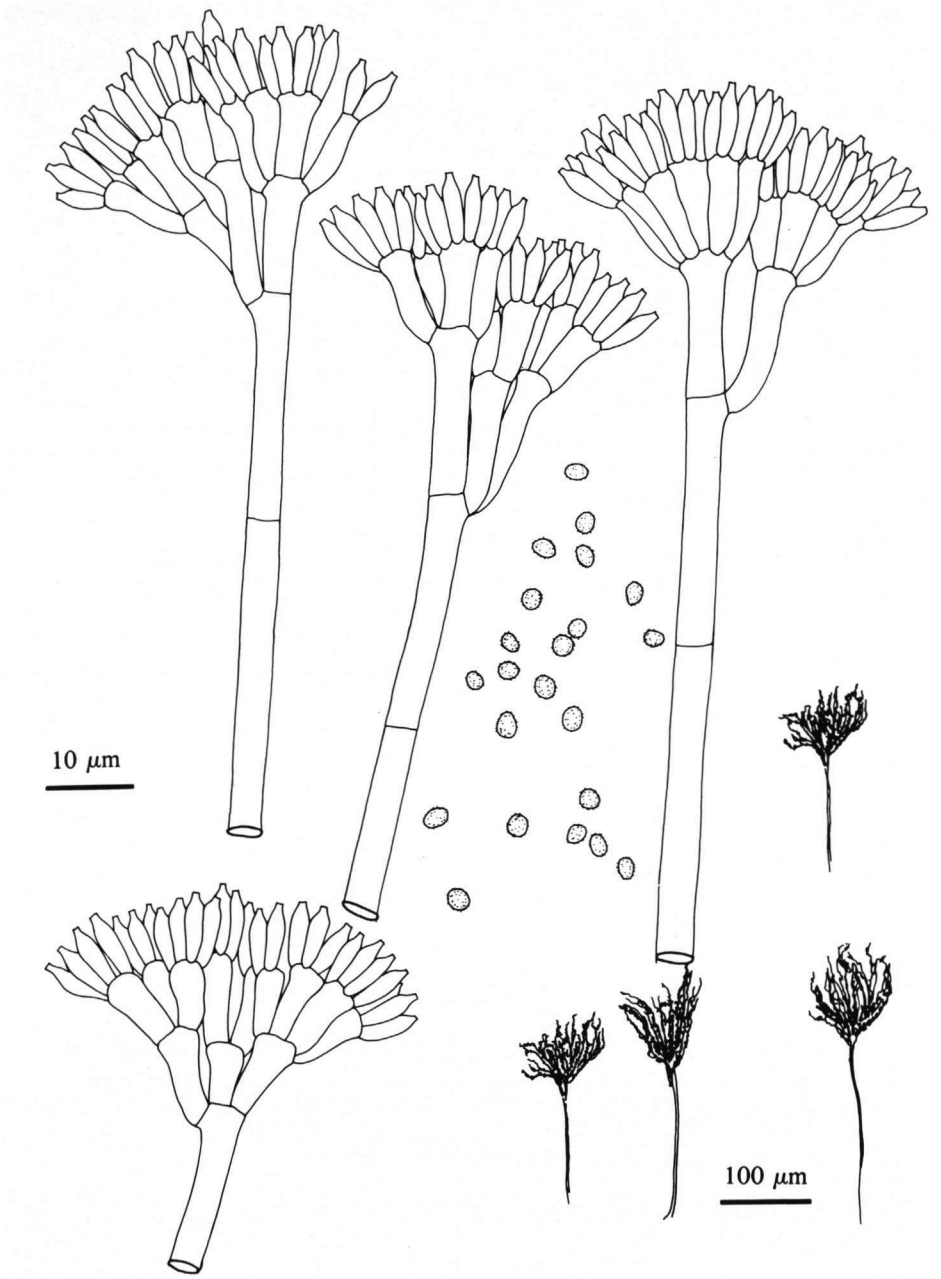

Fig. 1. Camera lucida drawings of *Penicillium brevicompactum*, conidiophores and conidia of CBS 210.28.

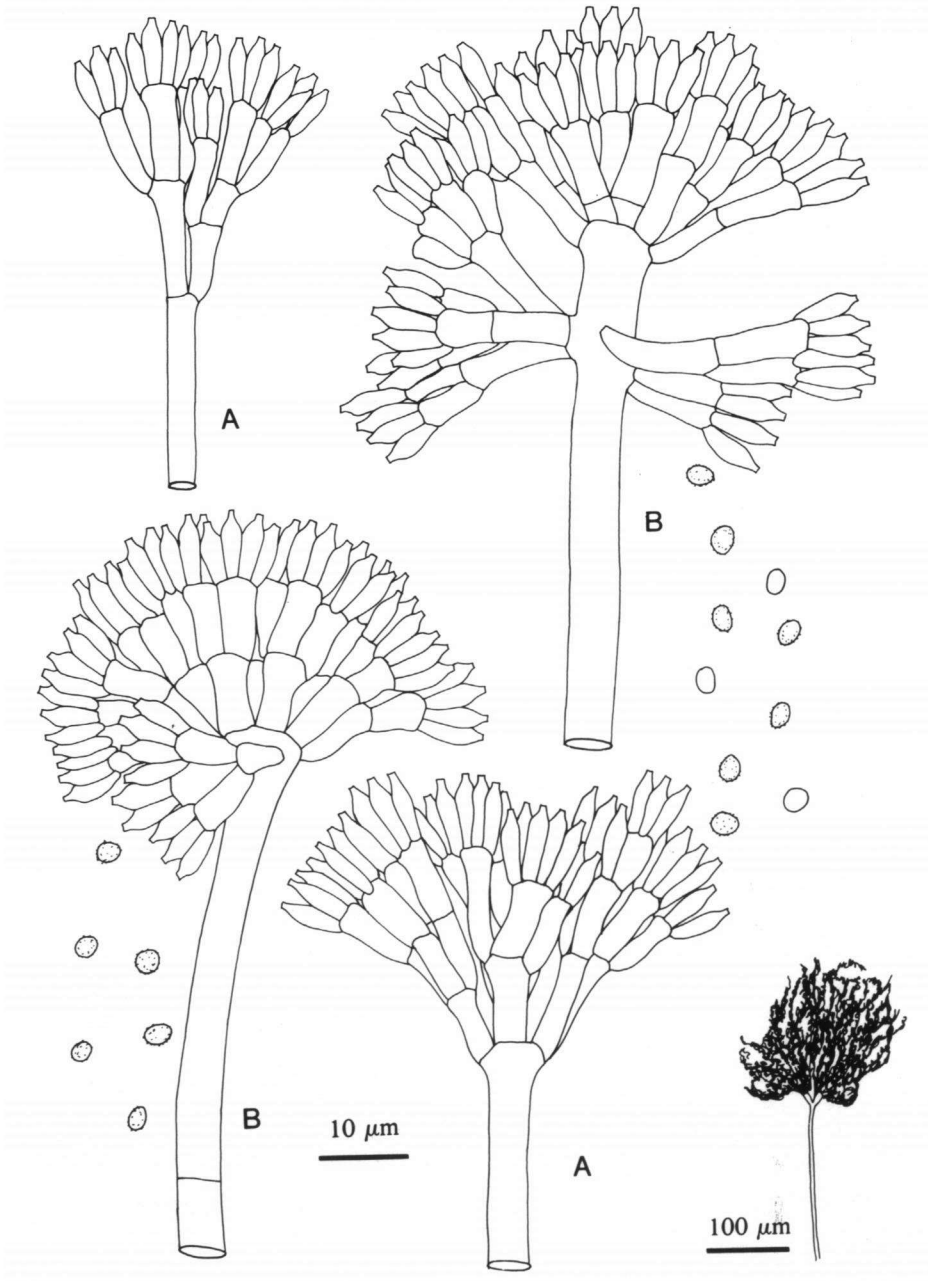

Fig. 2. Camera lucida drawings of *Penicillium olsonii*, conidiophores and conidia. — A. CBS 232.60 (neotype). — B. CBS 883.88.

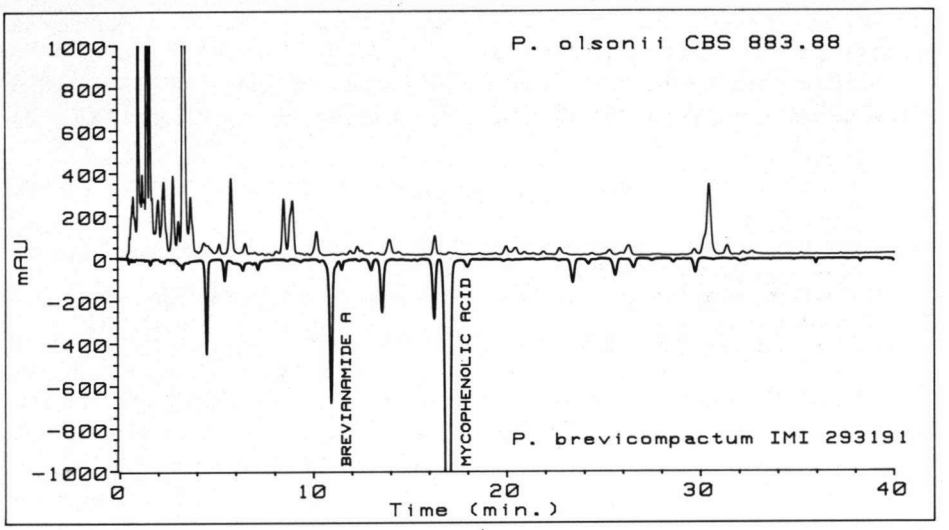

Fig. 3. Comparison of HPLC traces to show the difference between *P. brevicompactum* IMI 293191 and *P. olsonii* CBS 883.88. Note the absence of brevianamide A and mycophenolic acid in *P. olsonii*.

Penicillium minioluteum Dierckx

Penicillium minioluteum Dierckx *in* Annls Soc. scient. Brux. 25: 87. 1901. — Neotype IMI 89377 = CBS 642.68.

Penicillium gaditanum Ramírez & Martínez *in* Mycopathologia 74: 165. 1981.

Penicillium samsonii Quintanilla *in* Mycopathologia 91: 69. 1985.

The isolate Biourge 60 = IMI 89377 = CBS 642.68 considered by Biourge (1923) to represent *P. minioluteum*, is the most reliable material that can serve as neotype. This isolate is not the same as FRR 1714, the isolate on which Pitt (1980) based his concept of *P. minioluteum*. Biourge's isolate does not grow at 37 °C and it grows consistently more slowly than Pitt's FRR 1714 at 25 °C in one week: 6–13 mm diam. versus 20–40 mm on CYA, 10–22 mm versus 31–47 mm on MEA, 11–18 mm versus 22–40 mm on YES and 12–21 mm versus 31–41 mm on OA. The conidia of Biourge 60 are somewhat smaller than those of FRR 1714: 3–4 × 2–2.5 µm versus 4–5 × 2.5–3 µm. Furthermore the profiles of secondary metabolites are very different in the two isolates.

The specific epithet of *P. minioluteum* is particularly descriptive of the slow-growing strongly yellow strains such as CBS 642.68. A similar isolate, NRRL 1034 (from Pretoria, South Africa), was described by Raper & Thom (1949: 619) as 'producing restricted colonies on all substrata' and 'upon malt agar colonies are conspicuously tufted, funiculose, with sterile yellow mycelium' agreeing completely with e.g. CBS 642.68, but in sharp contrast to FRR 1714, which is 'fast growing', 'often low and velutinous' with 'conidiogenesis heavy' (Pitt, 1980: 420–421) on malt agar. The isolate FRR 1714, which Pitt (1980) designated as

the neotype of *P. minioluteum* can be best regarded in *P. rubrum* Stoll, although the exact taxonomic position is still under investigation.

Penicillium gaditanum Ramírez & Martínez and *P. samsonii* Quintanilla are synonyms of *P. minioluteum* (for a more detailed discussion see van Reenen-Hoekstra & al., 1990).

Penicillium solitum Westling

Penicillium solitum Westling *in* Ark. Bot. 11: 65. 1911. — Type: CBS 424.89 = NRRL 937.
Penicillium majusculum Westling *in* Ark. Bot. 11: 60, 1911.
Penicillium verrucosum Dierckx var. *melanochlorum* Samson, Stolk & Hadlok *in* Stud. Mycol. 11: 41. 1976 = *P. melanochlorum* (Samson & al.) Frisvad in Adv. *Penicillium Aspergillus* Syst.: 330. 1985.
For further synonyms see Pitt & Cruickshank (1990).

Westling's type culture of *P. solitum* was accessioned at CBS as CBS 288.36 (from Thom 275.2546) and in Peoria as NRRL 937. The CBS culture has smaller conidia than NRRL 937 and the former grows much faster than the latter on Czapek- and malt-based media and represent a taxon of *Penicillium* subgenus *Furcatum*. This seems to be caused by contamination and cannot be ascribed to degeneration. NRRL 937 is in perfect agreement with the protologue. NRRL 955 and NRRL 954 are Westling's original isolates of *P. majusculum* and they perfectly fit the protologue. Westling (1911) described *P. majusculum* with unusually large conidia, and indeed such conidia were found in both NRRL 954 and 955. However, such conidia were also observed in *P. solitum*, NRRL 937 and in *P. melanochlorum* (Samson & al.) Frisvad, CBS 487.75, even though they occurred in small proportions in all these isolates. Furthermore the four isolates mentioned above produced cyclopenin and some identical unidentified secondary metabolites and thus appear to represent the same species. *Penicillium solitum* was used by Raper & Thom (1949) and has recently been revived by Pitt (1988) and Pitt & Cruickshank (1990) and we follow this nomenclature.

The striking dark green conidial colour in fresh isolates of *P. melanochlorum* tends to get lost after prolonged cultivation on agar and the mycelial overgrowth often becomes more dominant. *Penicillium melanochlorum*, CBS 487.75 now has more greyish blue-green conidia.

Penicillium solitum is morphologically close to *P. commune* Thom, and both species also have a similar ecology (cheese and meat). By using pyrolysis gas chromatography, Soderstrom & Frisvad (1984) showed that an isolate of *P. commune* was different from three isolates of *P. solitum*, but Polonelli & al. (1987) found some relatedness between these taxa using serological techniques. The two taxa mainly differ by their profiles of secondary metabolites. *Penicillium solitum* produces viridicatin, cyclopenin, and compactin, while *P. commune* has cyclopiazonic acid, cyclopolic acid, rugulovasines, palitantin, and isofumigaclavine A. Isolates of *P. solitum* produce dark green conidia on Czapek-based media and orange-yellow mycelium and blue-green conidia on YES agar, while *P. commune* has greyish (blue) green conidia on Czapek-based media and cream-coloured mycelium on YES agar (the sporulation can be either poor or quite strong with green conidia on the latter medium).

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