

## ***Penicillium discolor*, a new species from cheese, nuts and vegetables**

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### **Abstract**

The new species *Penicillium discolor*, frequently isolated from nuts, vegetables and cheese is described. It is characterised by rough, dark green conidia, synnemateous growth on malt agar and the production of the secondary metabolites chaetoglobosins A, B and C, palitantin, cyclopenin, cyclopenol, cyclopeptin, dehydrocyclopeptin, viridicatin and viridicatol. It also produces the mouldy smelling compounds geosmin and 2-methyl-isoborneol, and a series of specific orange to red pigments on yeast extract sucrose agar, hence the epithet *discolor*. *P. discolor* resembles *P. echinulatum* morphologically but on basis of the secondary metabolites is also related to *P. expansum*, *P. solitum* and *P. crustosum*.

### **Introduction**

The mycotoxin chaetoglobosin C was first described from *Chaetomium globosum* (Sekita et al. 1973), but was later also found in fungi identified as *Penicillium aurantiovirens*, isolated from weevil-damaged pecans in Georgia (Springer et al. 1976). Frisvad and Filtenborg (1983) observed that these *Penicillium* strains have rough conidia and were similar to a strain isolated by them from an Israeli radish, which because of the morphological resemblance to *P. hordei* Stolk (rough conidia, synnemata formation) was first allocated to a provisional subgroup IV of *P. hirsutum*. This placement was made due to its good growth on creatine-sucrose agar, dark green conidia, orange red reverse colours on yeast extract sucrose agar and the specific profile of secondary metabolites (Frisvad & Filtenborg 1983). The strain also shows strong resemblance to *P. echinulatum* based on the dark rough conidia and it was later allocated to that species as chemotype II (Frisvad & Filtenborg 1989). A large number of isolates of this taxon have since been found especially on wal-

nuts, hazelnuts, acorns, onions, and hard cheese and the fungus is described here as a new species.

### **Materials and methods**

Isolates studied (Table 1) were three point inoculated on Czapek agar (Cz), Czapek yeast autolysate (CYA) agar, malt extract agar (MEA), 2% malt extract agar (MA), oat meal agar (OA), creatine-sucrose agar (CREA), yeast extract sucrose (YES) agar for one week at 25 °C, but also on CYA for one week at 5 and 37 °C (for media formulations, see Samson et al. 1995). Colony diameters were recorded after one week of growth, development of orange red pigments were examined from day 5 to day 14 after inoculation.

All isolates were examined by TLC using the agar plug method (Filtenborg et al. 1983) in two different eluents. The secondary metabolites were visualized by anisaldehyde spray and 48% sulphuric acid spray (Frisvad & Filtenborg 1983; Cole & Cox 1981). Authentic standards of chaetoglobosin A (Jim Gloer, University of Iowa, USA), chaetoglobosin C (Sigma Chemical

Table 1. Isolates examined

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CBS 474.84 = IMI 285515 = LHR9 = IBT 5738 = IBT 14440 (ex type): ex Radish, Israel
CBS 221.92 = IBT 14439 ex cheese, the Netherlands
CBS 183.88 = IBT 3904 = IBT 4231 = IBT 5734 = IBT 5920 ex <i>Corylus avellana</i> , Denmark
CBS 547.95 = DS 18342, isolated from cheese, Italy
CBS 548.95 = DS 19932, isolated from cheese, The Netherlands
CBS 549.95 = DS 19962, isolated from air in cheese factory, Belgium
CBS 550.95 = DS 19965, isolated from cheese, Austria
CBS 551.95 = DS 20033, isolated from cheese, France
ATCC 32000 = IBT 4232 = IBT 5733 ex weevil damaged pecans, Georgia, USA
ATCC 32002 = IBT 3088 = IBT 4229 = IBT 5730 ex weevil damaged pecans, Georgia, USA
IBT 3086 = 4224 = 5744 ex <i>Helianthus tuberosus</i> , Denmark
IBT 3089 = 4223 = 5731 ex onion, Denmark
IBT 3090 = SA1 ex cheese The Netherlands
IBT 3179 = IBT 3769 = IBT 5736 = IBT 4237 ex onion, Denmark
IBT 3185 = 4228 = 5740 ex pecan nut
IBT 3187 = IBT 4220 = IBT 5735 ex walnut, Denmark
IBT 3557 = 4227 = 5737 ex apple, Lyngby, Denmark
IBT 4512 = 4522 = 245,12 BMI
IBT 5740 walnut, India
IBT 11513, 11626 and 11511 ex acorn, Lyngby, Denmark
IBT 11699 ex walnut, France
IBT 11724 and 11725 ex cheese, Denmark
IBT 13022 ex citrus fruit, Korea
IBT 13945 = TM 92.103
IBT 14472 ex Lechuguilla cave, Carlsbad, New Mexico, USA
IBT 15185 103 K5, flower bulb, Denmark
IBT 15186 ex blue cheese, France
IBT 16126 = RMF 9950, IBT 16476 = IBT 16218 ex black walnut, North Carolina, USA

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Co.), palitantin, cyclophenin, cyclophenol, viridicatin, 3-methoxyviridicatin, cyclopeptin, dehydrocyclopeptin (from D.C. Aldridge, ICI, Macclesfield, Great Britain & A.G. Kozłowski, Puschino, Russia) were used to confirm identity.

All isolates were also analyzed by reversed phase HPLC with diode array detection according to Frisvad and Thrane (1987, 1993) with extracts from two plates of each of CYA, MEA, YES, YES made from Sigma Y-4000 yeast extract, and OA. Identity of the compounds was only accepted if the compounds detected had the same UV spectra and retention indices as authentic standards.

### Description

*Penicillium discolor* Frisvad and Samson, sp.nov. – Figures 1–2. Coloniae in agaro YES dicto post 7 dies 28–45 mm diam., pigmento laete aurantio vel rubro dif-

fundente. In agaro creatino et sucrosio confecto bene crescit, nec 37 °C crescere potest in ullo medio. In agaro CYA dicto 5 °C 3–6 mm diam. post 7 dies. Conidia globosa vel subglobosa, (3–)3.5–4 µm diam., obscure glauco-grisea vel viridi-grisea, aggregata raro ad colorem atrovenetum vertentia; conidiophora asperata, terverticillata, stipites 200–250 × 3.5–4 µm. Unus vel raro duo rami asymmetrice ad stipitem appressi; metulae cylindricae, asperatae, 12–15 × 3–3.5 µm; phialides lageniformes, 8–10 × 2.0–2.5 µm, collulo brevi praeditae. Chaetoglobosinum A et C, palitantinum, cyclopheninum, cyclophenolum, viridicatolum et alia viridicatina, geosminum, 2-methyl-isoborneolum producuntur.

Types Herb. IMI 285513, cultures ex type IMI 285513 = CBS 474.84 = FRR 2933 = IBT 5738, isolato e *Raphano sativo*, Israel.

*Diagnosis.* The species can be distinguished from other *Penicillia* by the combination of the following

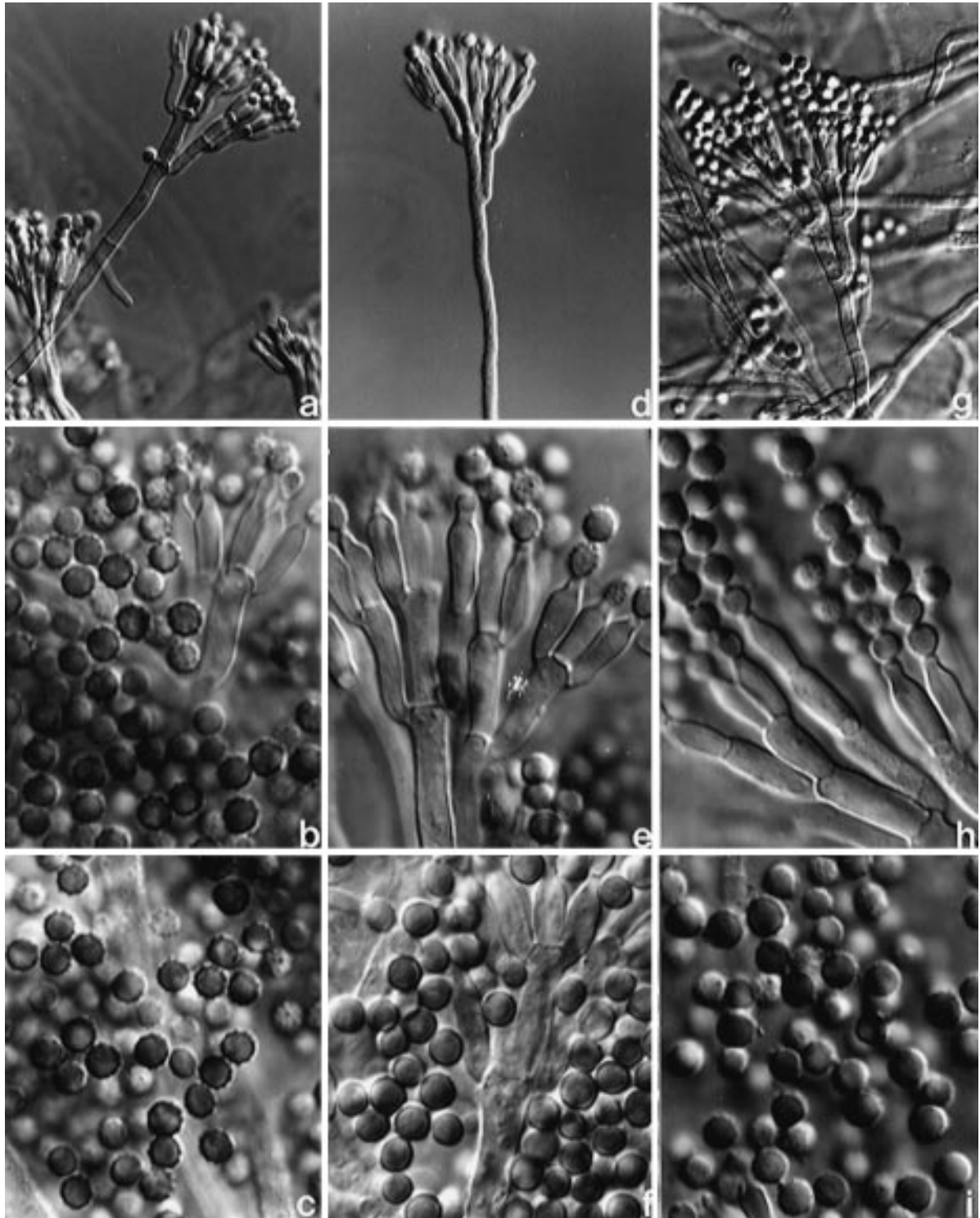


Figure 1. Nomarski phase contrast micrographs of three isolates of *Penicillium discolor*. a–c. CBS 474.84 (type isolate); d–f CBS 221.92; g–i. CBS 183.88. Magnifications: a, d and g. 600 ×, all others 1500 ×.

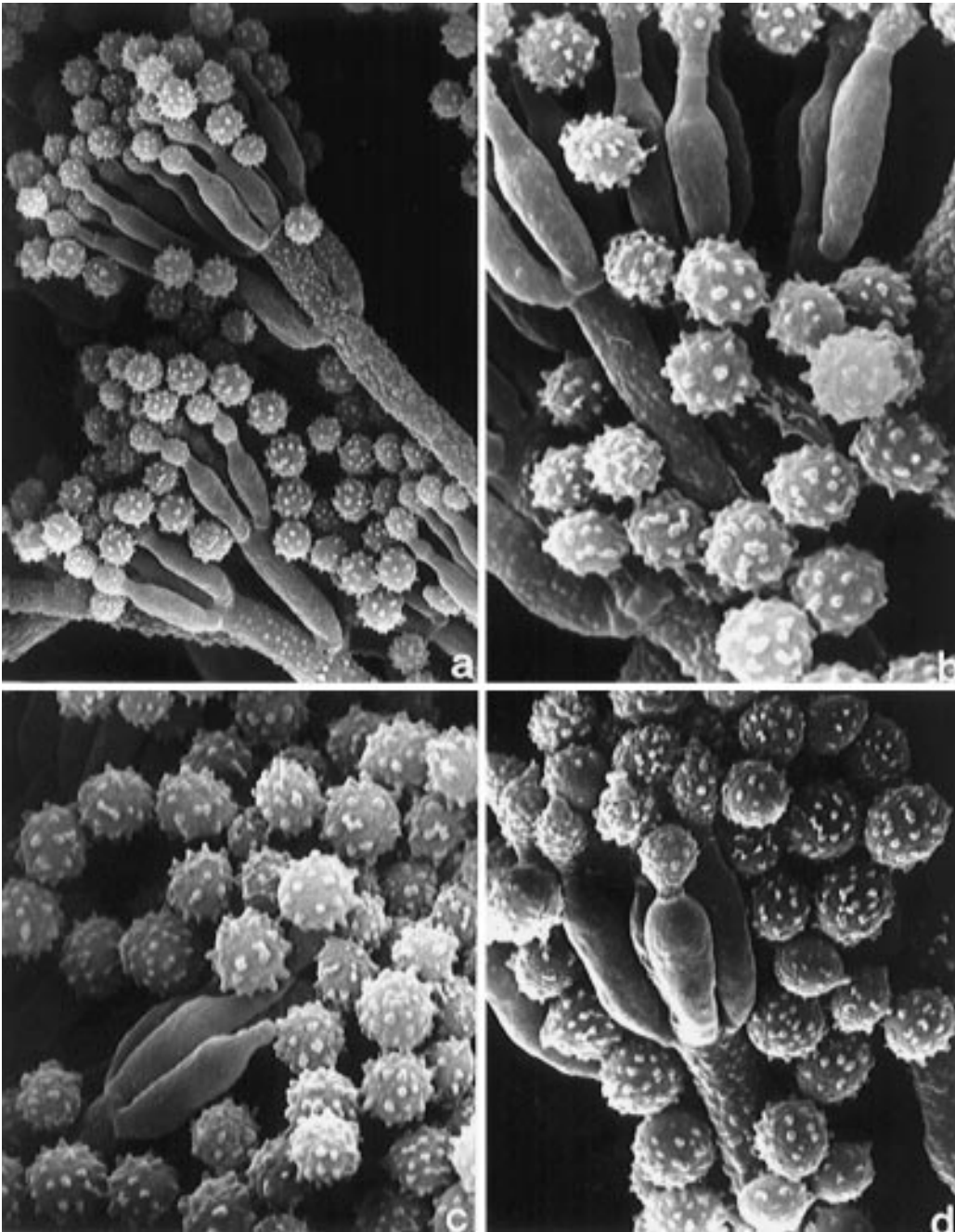


Figure 2. Scanning electron micrographs of critically point dried specimens a–c. CBS 474.84, a. 1900 ×, b., 4000 ×, c. 3500 ×; d. CBS 221.92, 3300 ×.

characters: globose to subglobose (3.5–4  $\mu\text{m}$ ) dark green rough walled conidia, terverticillate asymmetric penicilli with rough-walled stipes, synnemata production on malt agar (though not always pronounced), bright orange to red pigments diffusing on YES agar,

production of chaetoglobosin A and C and other chaetoglobosins, palitantin, cyclopinin, cyclopinol, viridicatol and other viridicatin, geosmin, 2-methylisoborneol, and good growth on creatine sucrose agar. Several unknown metabolites were detected and indi-

Table 2. Production of secondary metabolites by isolates of *P. discolor*, as identified by HPLC-DAD

CBS 474.84 (T):	Cha A, B, C, cyp, dec, col, cin, vol, vin, pal, or-met, met A, B, OY, XYX
IBT 15185:	Cha A, B, C, cyp, dec, col, cin, vol, vin, pal, or-met, met A, B, cf. austdiol,
IBT 15186:	Cha A, B, C, cyp, dec, col, cin, vol, vin, pal, or-met, met A, B, OY
IBT 13945:	Cha A, B, C, cyp, dec, col, cin, vol, vin, pal, or-met, met A, B, OY, a naphthopyrone
IBT 3187:	Cha A, B, C, cyp, dec, col, vol, vin, pal, or-met
IBT TM93045:	Cha A, B, C, cyp, dec, col, cin, vol, vin, pal, or-met, met A,
CBS 221.92:	Cha A, B, C, cyp, dec, col, cin, vol, vin, pal, or-met, met A, a naphthopyrone
IBT 14472:	Cha A, B, C, cyp, dec, col, cin, vol, vin, pal, or-met, met A2, XYX, CO
IBT 11513:	Cha A, B, C, cyp, dec, col, cin, vol, vin, pal, or-met, a naphthopyrone, met OY
IBT 11626:	Cha A, B, C, cyp, dec, col, cin, vol, vin, pal, or-met, a naphthopyrone, met OY
IBT acorn 13:	Cha A, B, C, cyp, dec, col, cin, vol, vin, pal, or-met, a naphthopyrone, met A2, OY
IBT 11724:	Cha A, B, C, pal, or-met, met OY, XYX
IBT 11725:	Cha A, B, C, cyp, dec, col, cin, vol, vin, pal, or-met, met OY, XYX
IBT 3089:	Cha A, B, C, cyp, dec, col, cin, vol, vin, pal, or-met, met XYX
IBT 3090:	Cha A, B, C, cyp, dec, col, cin, vol, vin, pal, or-met, met XYX
ATCC 32000:	Cha A, B, C, cyp, dec, col, cin, vol, vin, pal, or-met
ATCC 32002:	Cha A, B, C, cyp, dec, col, cin, vol, vin, pal, or-met
IBT 11459:	Cha A, B, C, cyp, dec, col, cin, vol, vin, pal, or-met, met OY
IBT 222-171:	Cha A, B, C, cyp, dec, col, cin, vol, vin, pal, or-met, met A2, OY,
IBT 4512:	Cha A, B, C, cyp, dec, col, cin, vol, vin, pal, or-met, met OY, a naphthopyrone
IBT 6289:	Cha A, B, C, cyp, dec, col, cin, vol, vin, pal, or-met, met OY, a naphthopyrone
IBT 6219:	Cha A, B, C, cyp, dec, col, cin, vol, vin, pal, or-met, met A2
IBT 6288:	Cha A, B, C, cyp, dec, col, cin, vol, vin, pal, or-met, a naphthopyrone, met A2, OY
IBT 6286:	Cha A, B, C, cyp, dec, col, cin, vol, vin, pal, or-met, a naphthopyrone, met A2, OY,
IBT 6287:	Cha A, B, C, cyp, dec, col, cin, vol, vin, pal, or-met, met A2, OY
IBT 3086:	Cha A, B, C, cyp, dec, col, cin, vol, vin, pal, or-met, met A, B, cf. austdiol
IBT 16218:	Cha A, B, C, cyp, dec, col, cin, vol, vin, pal, or-met, a naphthopyrone
IBT 16126:	Cha A, B, C, cyp, dec, col, cin, vol, vin, pal, or-met

Cha = Chaetoglobosin; cyp = cyclopeptin; dec = dehydrocyclopeptin; col = cyclophenol, cin = cyclophenin; vol = viridicatin; vin = viridicatin; pal = palitantin, or-met = orange metabolites, met = metabolite.

cated in Table 2 as *cf.* austdiol, a naphthopyrone and metabolites A, A2, B, OY and XYZ.

**Description.** Conidia globose to subglobose, (3–)3.5–4  $\mu\text{m}$ , dark glaucous-gray to artemisia green, or occasionally approaching nickel or pea green *en masse*, the conidiophores are rough-walled, terverticillate, stipes 200–250  $\mu\text{m}$   $\times$  3.5–4  $\mu\text{m}$ . One ramus or rarely two rami are placed asymmetrically and appressed to the main stipe. Metulae cylindrical, rough-walled, 12–15  $\mu\text{m}$   $\times$  3–3.5  $\mu\text{m}$ . Phialides flask-shaped with short necks, 8–10  $\mu\text{m}$   $\times$  2–2.5  $\mu\text{m}$ .

Colonies on Czapek agar (7 days, 25 °C) are 18–24 mm diam., velutinous with floccose mycelium in the centre, plane or sulcate. Good sporulation, mycelium white and conidia dark glaucous-gray or artemisia green *en masse*. Reverse cream coloured to light brown, exudate clear to orange, soluble pigment absent.

Colonies on CYA (7 days, 25 °C) are 15–33 mm diam., mostly velutinous, floccose or fasciculate, sometimes sulcate. Good sporulation, mycelium white and conidia artemisia green to nickel, dusk or pea green *en masse*. Reverse light brown with a dark centre, exudate orange or absent, soluble pigment absent. Clear to yellow exudate produced.

Colonies on MEA or 2% MEA (7 days, 25 °C) are 22–31 mm diam., velutinous to strongly fasciculate, flat not sulcate. Good sporulation, mycelium white and conidia celandine green or sage green *en masse*. Reverse light green with a yellowish centre, exudate and soluble pigment absent.

Colonies on YES (7 days, 25 °C) are 28–45 mm diam., velutinous to floccose, sulcate, good sporulation and a bright yellow reverse. In 6 to 12 days a bright orange to red diffusible pigment is produced.

Very good growth on creatine-sucrose agar with acid and subsequent base production. No growth on

Table 3. Differences and similarities between *P. discolor* and related species (from Frisvad & Filtenborg 1989; Svendsen & Frisvad 1994; Larsen & Frisvad 1995a; Lund 1995)

	<i>P. discolor</i>	<i>P. echinulatum</i>	<i>P. solitum</i>	<i>P. palitans</i>
Growth on creatine	++	++	++	++
Dark green conidia	+	++	+	+
Rough conidia	+ / ++	++	- / (+)	-
Palitantin	+	+	-	+ / -
Viridicatins	+	+	+	+ / -
Chaetoglobosins	+	-	-	-
Territrems	-	+	-	-
Penechins	-	+	-	-
Compactins	-	-	+	-
Cyclopiazonic acid	-	-	-	+
Fumigaclavines	-	-	-	+
Geosmin	+	+	-	-
2-methyl-isoborneol	+	-	+	+
Isopentanol	+	+	+	+
Orange metabolite	+	-	-	-

any media at 37 °C. Colonies 3–6 mm diam. on CYA at 5 °C after one week.

## Results and discussion

### Morphological differentiation

All isolates of *P. discolor* produce rough conidia, however occasionally the roughening of the conidia was delayed compared to *P. echinulatum* and/or less pronounced (Figures 1, 2). The conidiophore stipes are not always distinctly roughened on malt agars. Colonies are slightly to strongly fasciculate on malt extract agars. *P. discolor* shows a resemblance to several creatine positive *Penicillia*, especially *P. solitum*, *P. echinulatum*, *P. palitans*, *P. expansum*, and *P. crustosum*. Some of the differences between the creatine positive species with dark green conidia are listed in Table 3.

### Chemical differentiation

Isolates of *P. discolor* have been compared with all other terverticillate *Penicillia* using either profiles of secondary metabolites (Svendsen & Frisvad 1994) or profiles of volatiles (Larsen & Frisvad 1995a, b) and found to be distinct in both studies. *P. discolor* and *P. crustosum* are the only terverticillate *Penicillium* species that are able to produce geosmin and 2-methyl-isoborneol both volatiles known to have the musty/mouldy odours.

They also both produce isobutanol and isopentanol, although *P. crustosum* also produces dimethyldisulphide, styrene and several esters (Larsen & Frisvad 1995a). When the volatile profile of *P. discolor* was compared to the other terverticillate *Penicillia*, it shared most volatiles with *P. roqueforti* var. *carneum* (Larsen & Frisvad 1995b). However, if it was compared using non-volatile secondary metabolites (Svendsen & Frisvad 1994), it showed greatest similarity to *P. solitum* and *P. echinulatum*.

All isolates of *P. discolor* examined produced chaetoglobosin A and C (Table 2). This was confirmed by comparison to authentic standards in different TLC eluents using anisaldehyde as spray reagent (Frisvad & Filtenborg 1983) and by HPLC diode array detection (Frisvad & Thrane 1987). The production of chaetoglobosin A and C is only otherwise seen from *P. expansum* (Frisvad & Filtenborg 1989), another frequently occurring fungus on nuts. Chaetoglobosin C, however, appears to be one of the minor members of a whole series of chaetoglobosins in *P. discolor*. A comparison with an extract of *Chaetomium globosum* indicated that another major product in *P. discolor* was chaetoglobosin B, but no standard was available for this compound.

The production of palitantin, also confirmed by comparison with an authentic standard, was shared with *P. solitum*, *P. palitans*, *P. commune* and *P. echinulatum*. This secondary metabolite was originally

isolated from *P. palitans* but was also isolated from a strain identified as *P. frequentans* (Turner 1971).

The production of viridicatin is a feature of all strains of *P. discolor* examined, but these metabolites are quite common in the terverticillate *Penicillia* such as *P. crustosum*, *P. solitum*, *P. palitans*, *P. echinulatum*, *P. vulpinum*, *P. freii*, *P. polonicum*, *P. aurantiovirens*, *P. cyclopium*, *P. neoehinulatum* and *P. scabrosum* (Frisvad & Filtenborg 1989; Frisvad et al. 1990; Lund & Frisvad 1994). The most specific feature of *P. discolor* is the production of a number of yellow, orange-red metabolites with UV spectra not seen in any other *Penicillium* species. These metabolites are produced on media containing high concentrations of sugar, i.e. YES agar.

### Ecology

*P. discolor* is particularly common on nuts, both from commercial and non-commercial sources. It has been found on hazelnuts, walnuts, black walnuts, pecans, and acorns (Table 1). It has also been found frequently in rhizosphere soil of onions, Jerusalem artichokes, radishes, Iris bulbs, and other plants parts found under soil. It also occurs on hard cheese. Thus its preferred habitats are rich in protein and often also lipid rich. It has not yet been found on cereals or any other sources and until now it has been found in temperate and subtropical climates in Europe, Israel and USA. The isolates found to produce chaetoglobosin C by Springer et al. (1976) were from weevil-damaged pecans, so a connection to certain insects cannot be excluded. All strains of *P. discolor* produced cyclopenin and cyclophenol. A strain of *P. solitum*, NRRL 6233, also a good producer of cyclophenin and cyclophenol, was isolated from a pecan that had been injured by a Southern green stink bug (*Nezara viridula* L.) (Cutler et al. 1984). Cyclophenin and cyclophenol had a strong effect on wheat coleoptiles, but also on other plants and insects (Cutler et al. 1984; Paterson et al. 1990). The production by *P. discolor* of both geosmin, 2-methyl-isoborneol and several other terpenes, alcohols and esters plus several insect toxic metabolites may suggest different interactions with animals.

### Toxicology

Ethyl acetate extracts of the isolates of *P. discolor* from weevil-damaged pecans (ATCC 32000–32004), grown on wheat, were shown by Springer et al. (1976) to be toxic to rats when introduced orally. The major tox-

ic principle found was chaetoglobosin C, but toxicity tests with the pure compound were unsuccessful as the toxin was insoluble in water (Cole & Cox 1981). Chaetoglobosin A and the other chaetoglobosins have been shown to be cytotoxic (Sekita et al. 1973, 1976, 1977; Umeda et al. 1975) and acutely toxic to rats and mice (Ohtsubo et al. 1978). The consistent presence in extracts of both chaetoglobosin A and C and other chaetoglobosins, palitantin, cyclophenin, cyclophenol, viridicatin and viridicatol, all soluble in ethylacetate, indicates that the mixture of some of those compounds is toxic. No data are available on the production of these secondary metabolites on either nuts, vegetables nor cheese. None of the metabolites found are considered important mycotoxins (Cole & Cox 1981; Smith & Moss 1985).

The best substrate for chaetoglobosin production was CYA and YES agar. On those media chaetoglobosin A is the major metabolite in that biosynthetic family.

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