*Antonie van Leeuwenhoek* **57**: 179–189, 1990.


# Chemotaxonomy of the genus *Talaromyces*

J.C Frisvad[1], O. Filtenborg[1], R.A. Samson[2] & A.C Stolk[2]
[1] *Department of Biotechnology, Food Technology, the Technical University of Denmark, DK-2800 Lyngby, Denmark;* [2] *Centraalbureau voor Schimmelcultures, 3740 AG Baarn, the Netherlands*

Received 30 June 1989; accepted 1 November 1989

*Key words:* heat-resistant moulds, chemotaxonomy, HPLC, TLC, mycotoxins

**Abstract**

Species of the ascomycetous genus *Talaromyces* have been examined for profiles of secondary metabolites on TLC. The greatest number of specific metabolites were produced on oatmeal-, malt extract- and yeast-extract sucrose agars. Profiles of intracellular secondary metabolites produced on oatmeal agar were specific for each species and provided a means of simple differentiation of the taxa. Examination of the most important species using high performence liquid chromatography (HPLC) allowed to solve some taxonomic problems. Known mycotoxins are produced by *T. stipitatus* (duclauxin, talaromycins, botryodiploidin), *T. stipitatus* chemotype II (emodin), *T. panasenkoi* (spiculisporic acid), *T. trachyspermus* (spiculisporic acid), *T. macrosporus* (duclauxin) and *T. wortmannii* (rugulosin). Wortmannin is produced by an atypical strain of *T. flavus* but not *T. wortmannii.* Several other secondary metabolites were discovered for the first time in the following species: Glauconic acid is produced by *T. panasenkoi, T. ohiensis* and *T. trachyspermus;* vermiculine by *T. ohiensis;* duclauxin by *T. flavus* var. *macrosporus* and the mitorubrins by *T. flavus* and *T. udagawae.* The profiles of secondary metabolites support the established taxonomy of the species based on morphology, showing the genetic stability of profiles of secondary metabolites in *Talaromyces.* Two new taxa are proposed: *T. macrosporus* comb. nov. (stat. anam. *Penicillium macrosporum* stat. nov.), and *Penicillium vonarxii,* sp. nov. for the anamorph of *T. luteus.*

## Introduction

Since the monographic treatment by Stolk & Samson (1972) the species delimitation in the ascomycetous genus *Talaromyces* has been generally accepted, except for the classification of certain species in either *Talaromyces, Hamigera* or *Byssochlamys* (Stolk & Samson 1971 and 1972; Samson & Abdel-Fattah 1978; Pitt 1980; Pitt & Hocking 1979; von Arx 1986). These genera contain species with heat-resistant ascospores (Hocking & Pitt 1984) and some species are known mycotoxin producers (Frisvad 1986). The stability of *Talaromyces* taxonomy contrasts with the different views in *Eupenicillium* and *Penicillium* taxonomy (Stolk & Samson 1983; Pitt 1980; Ramirez 1982; Frisvad 1986). Species of *Talaromyces* should therefore provide good test material for the hypothesis that profiles of secondary metabolites are fundamental taxonomic criteria in filamentous fungi.

In this paper the results of the analysis of secondary metabolites of the genus are presented and the taxonomic implications discussed.

## Materials and methods

Isolates in good condition of all species in *Talaro-*

*myces* (Table 2) were grown on Czapek yeast-extract agar (CYA), malt extract agar (MEA), yeast-extract sucrose agar (YES) agar, and oatmeal agar (OA) (for formulations see Samson & Pitt 1985; Frisvad & Filtenborg 1983). Trace metals were added to all media (Frisvad & Filtenborg 1983) and the yeast extract and peptone ingredients were from Difco. The fungi were incubated at 25° C and 30° C in the dark and examined after 1 and 2 weeks.

Representative and authentic strains of all species in Talaromyces were examined for secondary metabolites by simple thin-layer chromatographic techniques (Filtenborg & Frisvad 1980; Filtenborg et al. 1983): Extracellular metabolites were determined by applying of superimposed agar plugs of YES and MEA agar; intracellular metabolites were determined by applying of superimposed extracted mycelial plugs of YES plus CYA agar, MEA plus OA or either MEA or OA agar alone on silica gel plates. The plates were developed in toluene: ethylacetate: 90% formic acid (5/4/1, v/v/v) (TEF) and chloroform: acetone: 2-propanol (85/15/20, v/v/v) (CAP) with griseofulvin as an external standard. All elutions were allowed to migrate 15 cm from the application line. Plates eluted in TEF were treated with cold 48% sulphuric acid and afterwards with anisaldehyde spray (Frisvad & Filtenborg 1983) and heated at 130° C for 8 minutes. Plates eluted in CAP were treated with cerium sulphate spray (Filtenborg et al. 1983). The plates were examined in daylight and under UV light (254 and 366 nm) before and after all treatments. Liquid chromatography (HPLC) was performed according to Frisvad & Thrane (1987) using a Hewlett Packard diode array detector (DAD) to obtain UV-VIS spectra of all eluting compounds and an alkylphenone retention index system. A series of standards were used to confirm the identity of some of the secondary metabolites detected. These standards are listed in Table 1. For HPLC analysis the contents of five 9 cm diam Petri dishes each of MEA and OA, with 14 day old cultures grown at 25° C, were placed in a plastic bag together with 100 ml chloroform/methanol (2:1, v/v) and extracted for 3 min in a Colworth Stomacher 400. After filtration through a hydrophobic filter (Whatman 1PS), the remaining agar and mycelium was reextracted with 100 ml ethyl acetate containing 1 ml 85% phosphoric acid. The filtered water free organic phases were combined and evaporated in vacuo. Further details of the procedure are described by Frisvad & Thrane (1987).

## Results and discussion

### *Production of secondary metabolites*

All species in *Talaromyces* produced high numbers of secondary metabolites making a direct identification of each species based on TLC profiles of coloured spots possible. The HPLC results showed that each species produced chromophore families

*Table 1.* Retention indices for secondary metabolites standards in the study of *Talaromyces*.

| Secondary metabolite | Retention index (RI) |
|---|---|
| Alternariol | 937 |
| Alternariol monomethylether | 1070 |
| Catenarin | 1191 |
| Chrysophanol | 1240 |
| Citreoviridin | 1028 |
| Dipicolinic acid | 670 |
| Duclauxin | 1133 |
| Emodin | 1130 |
| Erythroglaucin | 1439 |
| Flavoglaucin | 1538 |
| Glauconic acid | 910 |
| Helminthosporin | 1325 |
| Islandicin | 1354 |
| Luteoskyrin | 1240 |
| Mitorubrin | 1083 |
| Mitorubrinic acid | 924 |
| Mitorubrinol | 928 |
| Mitorubrinolacetate | 1052 |
| Monorden | 917 |
| Naphthalic anhydride | 1432 |
| Purpurogenone | 1184 |
| Rubratoxin B | 1071 |
| Rugulosin | 1123 |
| Rugulovasine A | 716 |
| Secalonic acid D | 1165 |
| Skyrin | 1333 |
| Stipitatic acid | 676 |
| Vermiculine | 834 |
| Vermicelline | 962 |
| Wortmannin | 938 |

specific for one taxon and some chromophore families which were shared by two or more taxa.

All species of *Talaromyces* produced many yellow, orange and red metabolites. Some of these metabolites were members of the known anthraquinone chemosyndrome (biosynthetic family) centered around emodin, skyrin, rugulosin, catenarin and erythroglaucin (*T. wortmannii* and chemotype II of *T. stipitatus*). The mitorubrin chemosyndrome was restricted to *T. flavus, T. macrosporus, T. udagawae, T. mimosinus* and *T. wortmannii*. The variety of members of the mitorubrin biosynthetic family differed from species to species and were most diverse in *T. wortmannii*. Other chromophore families with UV-vis spectra containing strong absorptions from 400 to 450 nm were major coloured products of species like *T. helicus* and *T. luteus*, so the macroscopically visible yellow pigments in the mycelium and ascomata of these species are caused by different biosynthetic families of compounds. Weakly coloured secondary metabolites such as duclauxin are present in only two taxa: *T. stipitatus* and *T. flavus* var. *macrosporus*, while the closely related bacillosporins were only present in another heat-resistant fungus *T. bacillisporus*. These three heat-resistant species characteristically contained these closely related phenalenones in large amounts and as dominant secondary metabolites, indicating that they may be a major protective principle in heat resistance.

While stipitatic acid was only produced by *T. stipitatus*, glauconic acid was detected for the first time in *T. panasenkoi, T. ohiensis* and *T. trachyspermus* indicating a close chemotaxonomic relationship between the latter three species. This confirms the morphological observations by Stolk and Samson (1972), who found that these species produce similar ascomatal initials. Vermiculine was found in *T. ohiensis* and the production of this secondary metabolite was confirmed for *T. flavus*, but the production of vermiculine in *T. wortmannii* (Jones et al. 1984) could not be confirmed. All the secondary metabolites mentioned above have also been found in one or more species of *Penicillium* subgenus *Biverticillium* (Turner 1971; Turner & Aldridge 1983; Frisvad 1986; Mantle 1987), and this supports the close taxonomic relationship between *Talaromyces* and the anamorphic *Penicillium* subgenus *Biverticillium*.

The profiles of intracellular metabolites produced on OA, as seen on TLC plates using the agar plug method, were specific and consistent for each taxon, but with TLC alone it is difficult to assess whether these metabolites represent one or more biosynthetic families in each taxon. *T. stipitatus* and *T. wortmannii* (Table 2, see also Turner 1971; Turner & Aldridge 1983) are at least 4 to 5 known biosynthetic families and our HPLC results strongly indicate that all *Talaromyces* taxa produce at least four, often eight to ten chromophore families on MEA and OA. The profiles of intracellular metabolites produced on MEA were less specific as evaluated using TLC, but the technique was useful in showing which metabolites were common in different taxa. The most conspicous of these metabolites was a yellow coloured (both in daylight and UV light before and after spraying with sulphuric acid, relative Rf value to griseofulvin 0.98) substance produced by *T. flavus, T. macrosporus, T. mimosinus, T. udagawae, T. wortmannii* and several species in *Penicillium* subgenus *Biverticillium*. A partial spectroscopic characterisation (UV-vis and NMR) showed that this metabolite was mitorubrinic acid and this was confirmed by comparison with an authentic standard. *T. rotundus, T. trachyspermus, T. assiutensis* and *T. ohiensis* also have some metabolites in common, viz. glauconic and glaucanic acid seen on TLC (Table 2 and 3).

Mycotoxin production by *Talaromyces* species is of interest because these species may occur in pasteurized fruit juices (Hocking & Pitt 1984; Baggerman & Samson 1988) and other food products. *T. flavus* could be a potential producer of wortmannin in food, but this species is much less heat-resistant than *T. flavus* var. *macrosporus*, a producer of the mycotoxin duclauxin, Wortmannin, however, is also reported from food-borne species such as *Penicillium funiculosum, Myrothecium roridum* and *Fusarium oxysporum* (Abbas & Mirocha 1988). Very little is known on the toxicity of the metabolites of the other species in *Talaromyces* but emodin, cited as a toxin by Wells et al. (1975) is produced by an atypical strain of *T. stipitatus* (see discussion under Taxonomic implications). Other

*Table 2.* Species in *Talaromyces*, anamorph names, isolates examined and secondary metabolite production.

| Teleomorph / anamorph | Isolates | Secondary metabolites |
|---|---|---|
| *T. flavus* (Klocker) Stolk & Samson / | NRRL 2098 (NT) (b) | Mitorubrin (+ acid) (a), Vermicellin, vermiculine |
| *P. dangeardii* Pitt | CBS 261.55 | Mitorubrin (+ acid), Vermicellin, vermiculine |
| | CBS 284.58 | Mitorubrin (+ acid), Vermicellin, vermiculine |
| | CBS 582.72A | Mitorubrin (+ acid), Vermicellin, vermiculine |
| | CBS 387.47 | Mitorubrin (+ acid), Vermicellin, vermiculine |
| | Fravel 282.3 | Mitorubrin (+ acid), Vermicellin, vermiculine |
| *T. macrosporus* (Stolk & Samson) Frisvad, | CBS 317.63 (T) | Mitorubrin (+ acid), Duclauxin |
| Stolk & Samson chemotype I / | CBS 117.72 | Mitorubrin (+ acid), Duclauxin |
| *P. macrosporum* Frisvad, Stolk & Samson | CBS 353.72 | Mitorubrin (+ acid), Duclauxin |
| | CBS 580.72 | Mitorubrin (+ acid), Duclauxin |
| | CBS 130.89 | Mitorubrin (+ acid), Duclauxin |
| *T. macrosporus* chemotype II | CBS 350.72 | |
| *T. helicus* (Raper & Fennell) C.R. Benjamin / | CBS 335.48 (T) (poor) | |
| *P. spirillum* Pitt | CBS 137.65 | |
| | CBS 760.68 | |
| | CBS 550.72B | |
| | CBS 562.72 | |
| | CBS 585.72 | |
| *T. stipitatus* (Thom) C.R. Benjamin | CBS 375.48 (T) | Stipitatic acid, Duclauxin |
| chemotype I / *P. emmonsii* Pitt | CBS 292.53 | Stipitatic acid, Duclauxin |
| | CBS 189.72 | Stipitatic acid, Duclauxin |
| | CBS 227.72 | Stipitatic acid, Duclauxin |
| *T. stipitatus* chemotype II | CBS 349.72 | Catenarin, emodin, erythroglaucin |
| *T. panasenkoi* Pitt / *P. panasenkoi* Pitt | CBS 583.72C | Glauconic acid |
| | NRRL 2103 | Glauconic acid |
| *T. luteus* (Zukal) C.R. Benjamin / | CBS 348.51 (NT) | |
| *P. vonarxii* Frisvad & Samson | CBS 533.59 | |
| | CBS 865.71 | |
| | CBS 750.74 | |
| *T. udagawae* Stolk & Samson / *P. udagawae* | CBS 579.72 (T) | Mitorubrin (+ acid), mitorubrinol acetate |
| Stolk & Samson | | |
| *T. wortmannii* (Klocker) C.R. Benjamin / | CBS 391.48 (T) | Mitorubrin (+ acid), mitorubrinol (+ acetate) |
| *P. kloeckeri* Pitt | | Rugulosin, skyrin |
| | CBS 235.38 | Mitorubrin (+ acid), mitorubrinol (+ acetate) |
| | | Rugulosin |
| | CBS 293.63 | Mitorubrin (+ acid), mitorubrinol (+ acetate) |
| | | Rugulosin, skyrin |
| | CBS 319.63 | Mitorubrin (+ acid), mitorubrinol (+ acetate) |
| | | Rugulosin, skyrin |
| | CBS 387.67 | Mitorubrin (+ acid), mitorubrinol (+ acetate) |
| | | Rugulosin, skyrine |
| | CBS 533.72 | Mitorubrin (+ acid), mitorubrinol (+ acetate) |
| | | Rugulosine, skyrine |
| *T. rotundus* (Raper & Fennell) | CBS 369.48 (T) | |
| C.R. Benjamin / P. sphaerum Pitt | CBS 587.72 | |
| *T. trachyspermus* (Shear) Stolk & Samson / | CBS 373.48 (T) | Glauconic acid |
| *P. lehmanii* Pitt | CBS 346.54 | Glauconic acid |
| | CBS 282.58 | Glauconic acid |
| | CBS 112.64 | Glauconic acid |
| *T. assiutensis* Samson & Abdel-Fattah | CBS 147.78 (T) | Glauconic acid |
| (= *T. gossypii* Pitt) / *P. assiutense* | CBS 645.80 | Glauconic acid |
| Samson & Abdel-Fattah | | |
| *T. ohiensis* Pitt / *P. ohiense* Huang & Smith | CBS 162.67 (T) | Vermiculine, Glauconic acid |

*Table 2.* Continued.

| Teleomorph / anamorph | Isolates | Secondary metabolites |
|---|---|---|
| *T. galapagensis* Samson & Mahoney / *P. galapagense* Samson & Mahoney | CBS 751.74 (T) | |
| *T. mimosinus* Hocking / *P. mimosinum* Hocking | CBS 659.80 (T) | Mitorubrin (+ acid) |
| *T. intermedius* (Apinis) Stolk & Samson / *P. intermedium* Stolk & Samson | CBS 152.65 (T) | |
| *T. derxii* Takada & Udagawa / *P. derxii* Takada & Udagawa | NHL 2981<br>NHL 2982 | |
| *T. bacillisporus* (Swift) C.R. Benjamin / *G. swiftii* Pitt | CBS 296.48 (T) | |
| *T. byssochlamydoides* Stolk & Samson / *Paecilomyces byssochlamydoides* Stolk & Samson | CBS 413.71 (T)<br>CBS 533.71 | |
| *T. emersonii* Stolk / *Penicillium emersonii* Stolk | CBS 393.64 (T)<br>CBS 814.70<br>IMI 116825ii<br>CBS 204.75<br>CBS 373.75 | |
| *T. thermophilus* Stolk / P. dupontii Griffin & Maublanc | CBS 236.58 (T)<br>CBS 116.72 | |
| *T. purpureus* (E. Miller & Pacha-Aue) Stolk & Samson / *P. purpureum* Stolk & Samson | CBS 475.71 (T) | |
| *T. leycettanus* Evans et Stolk / *Paecilomyces leycettanus* (Evans & Stolk) Samson & Evans | CBS 398.68 (T) | |

(a) T = type culture, NT = neotype culture
(b) (+ acid) = mitorubrinic acid, (+ acetate) = mitorubrinol acetate.

mycotoxin producers in *Talaromyces* are *T. stipitatus*, producing duclauxin (Mantle 1987) talaromycins (Phillips et al. 1987), and botryodiploidin (Fuska et al. 1988), *T. macrosporus* producing duclauxin (this paper) and *T. trachyspermus* producing spiculisporic acid (Fujimoto et al. 1988)

Strains of *T. flavus* have been applied as biological control agents of *Verticillium* wilt of potato (Davis et al. 1986; Fravel et al. 1986). One of the isolates (Fravel 282.3) was examined and found to produce mitorubrin, mitorubrinic acid, and vermicellin, typical products of *T. flavus*. Fig. 1 shows a HPLC trace of this strain grown on MEA and OA. Kim et al. (1988) identified glucose oxidase as a metabolite that mediated inhibition of *Verticillium dahliae*. However, other secondary metabolites detected by us could play also a role (vermicellin). Another metabolite (retention time 28.6 min), produced in quite large amounts on MEA and OA shown in Fig. 1 seems to agree well with talaron, a fungal inhibitor (Mizuno et al. 1974).

All known species in *Talaromyces* are homothallic, with the exception of a recently described heterothallic taxon, *T. derxii* Takada & Udagawa (1988). The extract of this fungus was green, (like that of *T. bacillisporus*), and both A and a strains and mated A + a strains produced the same characteristic profile of secondary metabolites. The major products in this fungus has not been found in any other species of *Talaromyces* or the related anamorphs *Penicillium* of subgenus *Biverticillium*.

### *Taxonomic implications*

Table 3 lists the species accepted in *Talaromyces*, their anamorphic states and representative cultures chosen for the profile of secondary metabolites.

Raper & Thom (1949) placed NRRL 2103 hesistantly in *T. luteus*. The TLC trace of this strain was typical of *T. panasenkoi* and our morphological reexamination of NRRL 2103 showed that this

*Table 3.* Biosynthetic families of secondary metabolites produced by species of *Talaromyces*.

| Species | Specific profile of spots on TLC plates (a) | Production of secondary metabolites including mycotoxins |
|---|---|---|
| *T. flavus* | ++ | I: Mitorubrin (c,d), mitorubrinic acid (c,d)<br>II: Vermicellin (Fuska et al. 1979) (c)<br>III: Vermiculine (Fuska et al. 1972) (c)<br>IV: Vermistatine (Fuska et al. 1986)<br>V: Talarone (Mizuno et al. 1974)<br>VI: Wortmannin, deoxywortmannin (Simpson et al. 1979; MacMillan et al. 1972), wortmannolone (only seen in IMI 44277) |
| *T. helicus* | ++ | |
| *T. macrosporus* chemotype I | ++ | I: Mitorubrin (c,d), mitorubrinic acid (c,d)<br>II: Duclauxin (c,d) |
| *T. macrosporus* chemotype II | ++ | |
| *T. stipitatus* chemotype I | ++ | I: Stipitatic acid (c), stipitatonic acid (c), stipitalide, ethylstipitate, stipitaldehydic acid (Dewar 1945; Segal 1959; Divekar et al. 1961; Bryant & Light 1974)<br>II: 3-methyltriacetic acid lactone, triacetic acid lactone, tetraacetic acid lactone (Acker et al. 1966; Bentley & Zwitkovitz 1967; Scott et al. 1971)<br>III: Botryodiploidin (Fuska et al. 1988)<br>IV: Duclauxin (c) (Kuhr et al. 1973)<br>V: Talaromycins (Phillips et al. 1987) |
| *T. stipitatus* chemotype II | ++ | I: Catenarin (c), emodin (c), erythroglaucin (c) (van Eijk 1973) |
| *T. panasenkoi* | + | I: Spiculisporic acid (Fujimoto et al. 1988)<br>II: Glauconic acid (c,d) |
| *T. luteus* | ++ | I: Luteic acid (Turner & Aldridge 1983) |
| *T. udagawae* | ++ | I: Mitorubrin (c,d), mitorubrinic acid (c,d), mitorubrinol acetate (c,d) |
| *T. wortmannii* | ++ | I: Mitorubrin (c), mitorubrinol (c), mitorubrinic acid (c), mitorubrinol acetat (c), wortmin (Turner & Aldridge 1983)<br>II: Flavomannin (Atherton et al. 1968), skyrin (c), chrysophanol (c) (Turner 1971)<br>III: Rugulosin (c) (Breen et al. 1955) |
| *T. rotundus* | ++ | |
| *T. trachyspermus* | ++ | I: Spiculisporic acid, (−) decyl citric acid (Gatenbeck & Mahlen 1968; Tabuchi et al. 1977)<br>II: Glauconic acid (c,d) |
| *T. assiutensis* | ++ | II: Glauconic acid (c,d) |
| *T. ohiensis* | ++ | I: Vermiculine (c,d)<br>II: Glauconic acid (c,d) |
| *T. galapagensis* | + | |
| *T. mimosinus* | ++ | I: Mitorubrin (c,d), mitorubrinic acid (c,d) |
| *T. intermedius* | + | |
| *T. purpureus* | ++ | |
| *T. thermophilus* | ++ | |
| *T. derxii* | ++ | |
| *T. bacillisporus* | ++ | I: Bacillosporin A, B & C (Turner & Aldridge 1983)<br>II: Pinselin, pinselic acid (Turner & Aldridge 1983) |
| *T. emersonii* | + | |
| *T. byssochlamydoides* | ++ | |
| *T. leycettanus* | ++ | |

(a) ++: A very distinct pattern of more than 5 spots before spraying on a TLC plate developed in TEF, +: 2–5 spots on the TLC plates before and after spraying with sulphuric acid and heating.
(b) The known and newly found secondary metabolites were ordered in biosynthetic families and given roman numbers.
(c) Confirmed by TLC and HPLC-DAD and using authentic standards (see table 1).
(d) New record.

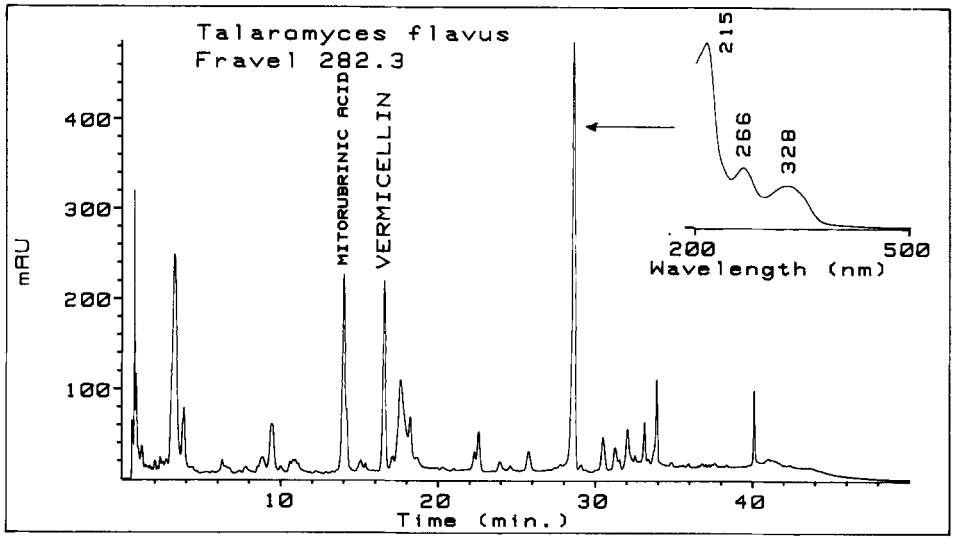


*Fig. 1.* HPLC traces of an extract of *T. flavus* (Fravel 282.3). Note the production of mitorubrinic acid, vermicellin and possibly talaron (see UV spectrum in upper right corner).

strain was indeed *T. panasenkoi.* The TLC traces also showed that *T. helicus* var. *major* is not sufficiently distinct from var. *helicus.*

Stolk & Samson (1972) recognized two varieties in *T. flavus:* var. *flavus* and var. *macrosporus.* The strains of both varieties only had one metabolite family in common (the mitorubrins), but both taxa produced a conspicous profile of blue, violet, yellow and orange metabolites in TLC plates. This observation was confirmed by HPLC of cultures grown on MEA and OA (Fig. 3). Three isolates of var. *macrosporus* (CBS 353.72, CBS 317.63, and CBS 130.89) all produced great amounts of duclauxin and other specific metabolites, while

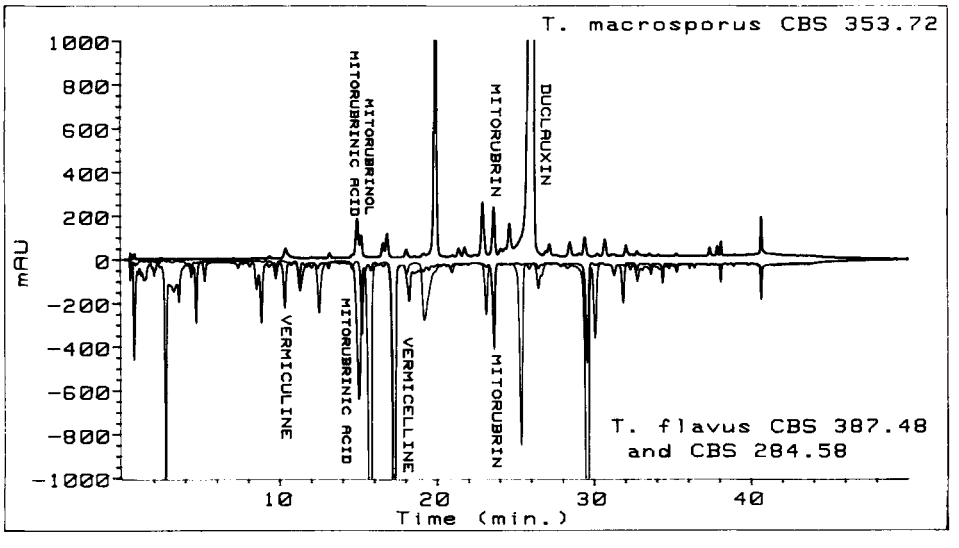


*Fig. 2.* HPLC traces of *Talaromyces macrosporus* CBS 353.72 and two isolates of *T. flavus* (CBS 387.48 and CBS 284.58). Members of the mitorubrinic biosynthetic family were the only common metabolites.

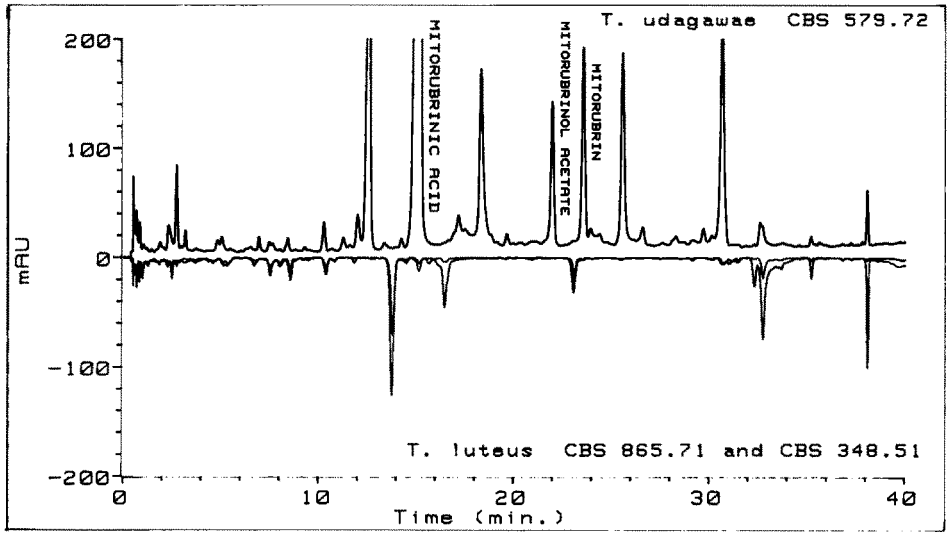


*Fig. 3.* HPLC traces of *Talaromyces udagawae* (CBS 579.72) and two isolates of *T. luteus* (CBS 865.71 and 348.51) to show the different profiles of secondary metabolites.

strains of *T. flavus* produced vermiculine, vermicelline and other unknown secondary metabolites not found in var. *macrosporus*. Beuchat (1988) compared several isolates of *T. flavus* and found that small-spored strains are less heat-resistant than large-spored strains. Among the large-spored strains he included CBS 317.63, the type culture of *T. flavus* var. *macrosporus*, isolated as a heat resistant strain from pasteurized apple juice by Van der Spuy et al. (1975). Based on these differences in ascospore size, heat-resistance and profile of secondary metabolites we propose that the two taxa should be considered as separate species.

***Talaromyces macrosporus* (Stolk & Samson) Frisvad, Samson & Stolk, comb. nov.**

Basionym: *Talaromyces flavus* (Klöcker) Stolk & Samson var. *macrosporus* Stolk & Samson, Stud. Mycol., Baarn 2: 15, 1972.

Status conidialis *Penicillium macrosporum* stat. nov.

Descriptio stat. anam. in Stolk & Samson, Stud. Mycol., Baarn, 2: 15, 1972. Metabolica: Duclauxin, mitorubrin, mitorubrinic acid. Typus: CBS 317.63.

Stolk & Samson (1972) separated *T. udagawae* and *T. luteus* on the basis of different ascoma initials and smaller ascopores with different ornamentation. Pitt (1980) found that these differences were not sufficient and synonymized both taxa. Our TLC and HPLC results, however, showed that *T. udagawae* is a well-defined species, and that the two taxa have no secondary metabolites in common (metabolites having the same retention time in the two species were different as they had different UV-VIS spectra (Fig. 3).

In recognizing *T. luteus* and *T. udagawae* as two different species, there is no name available for the anamorph of *T. luteus*, as *P. luteum* was described inclusive of the teleomorph. We therefore propose the following name for the anamorph of *T. luteus:*

***Penicillium vonarxii* Frisvad & Samson, sp. nov.**

Status anamorphosis *Talaromycetis lutei.* Conidiophora portata ex hyphis aeriis, stipites 15–35 (–100) × 2.0–3 μm, parietibus levibus, interdum incrustatis, penicilli biverticillati et monoverticillati, metulae 10–20 × 2–3 μm, phialides acerosae, 9–15 × 2–3 μm, collulis longis, apicibus interdum viridibus, conidia ellipsoidea vel pyriformia,

2.5–4 × 2–3.2 μm, parietibus levibus, viridibus. In agaro CYA, coloniae aetate unius hebdomadis 15–20 mm diam. Metabolica absorb. max. 232 nm, 281 nm, 320 nm, 330 nm, 404 nm. Typus: CBS 579.72.

Samson & Abdel-Fattah (1978) described *T. assiutensis*. A morphological and chemical comparison of the type cultures of *T. assiutensis* and *T. gossypii* Pitt (1980) showed that these species are conspecific.

In their monograph Stolk & Samson (1972) mentioned deviating strains of *T. flavus* var. *macrosporus* (CBS 350.72) (with more ellipsoidal ascospores, showing more variation in size than other isolates of var. *macrosporus*) and *T. stipitatus* (CBS 349.72) (with slightly larger ascospores and different mycelial colours than *T. stipitatus*on all media) respectively. In our studies of the secondary metabolites these strains produce completely different profiles of secondary metabolites and they might represent new taxa in *Talaromyces*. As neither of the two aberrant strains have any metabolites in common with the other species of the genus they cannot be regarded as mutants or varieties of those species. However, before warranting their description as new species we would like to examine more than one isolate. For the time being we call them *T. macrosporus* chemotype II for CBS 350.72 and *T. stipitatus* chemotype II for CBS 349.72, following the recommendations by Pitt & Hawksworth (1985) for naming chemical variants.

Our comparative studies of the strains of *Talaromyces* and *Penicillium* subgenus *Biverticillium* showed that *T. wortmannii* and the anamorph *P. variabile* have many secondary metabolites in common, which may indicate that the latter may be the anamorph of the former fungus. This is further supported by similar growth rates and the fact that Raper & Thom (1949) reported the production of a few ascomata in *P. variabile* NRRL 2025 with ascospores like those of *T. wortmannii*. Both *T. wortmannii* and *P. variabile* have, however, each some specific secondary metabolites too and at present we prefer to keep these species separate.

The results reported here indicate the great value of profiles of secondary metabolites as taxonomic characters in *Talaromyces*. While the morphological treatment of the genus *Talaromyces* by Stolk & Samson (1972) and Pitt (1980) is upheld, the simple TLC technique can also help in solving problems where morphological characters alone leave uncertainty. The profiles of secondary metabolites in *Talaromyces* and *Penicillium* subgenus *Biverticillium* indicate a close relationship between these taxa, but no *Biverticillium* taxa could be regarded as anamorphs of any *Talaromyces* species.

## Acknowledgements

The authors thank the NATO Scientific Affairs Division (Brussel, Belgium) for the research grant (216/86) for international collaboration. Dr G.W. van Eijk provided helpful suggestions during the preparation of the manuscript.

## References

Abbas HK & Mirocha CJ (1988) Isolation and purification of a hemorrhagic factor (wortmanin) from *Fusarium oxysporum* (N17B). Appl. Environ. Microbiol. 54: 1268–1274

Acker TE, Brenneisen PE & Tanenbaum SW (1966) Isolation, structure and radiochemical synthesis of 3,6-dimethyl-4-hydroxy-2-pyrone. J. Amer. Chem. Soc. 88: 834–837

Arx JA von (1986) On *Hamigera*, its *Raperia* anamorph and its classification in the Onygenaeae. Mycotaxon 26: 119–123

Atherton J, Bychroft BW, Roberts JC, Roffey P & Wilcox ME (1968) Studies in mycological chemistry. 23. The structure of flavomannin, a metabolite of *Penicillium wortmanni*. J. Chem. Soc. C 1968: 2560–2564

Baggerman W & Samson RA (1988) Heat resistance of fungal spores. In: Samson RA & van Reenen-Hoekstra ES Introduction to Food-borne Fungi (pp 262–267), 3rd edn. Centraalbureau voor Schimmelcultures, Baarn

Bentley R & Zwitkowits PM (1967) Biosynthesis of tropolones in *Penicillium stipitatum*. VII. The formation of polyketide lactones and other nontropolone compounds as a result of ethionine inhibition. J. Amer. Chem. Soc. 89: 676–685

Beuchat LR (1988) Influence of organic acids on heat resistance characteristics of *Talaromyces flavus* ascospores. Int. J. Food Microbiol. 6: 97–105

Breen J, Dacre JC, Raistrick H & Smith G (1955) Studies in the biochemistry of microorganisms. 95. Rugulosin, a crystalline colouring matter of *Penicillium rugulosum* Thom. Biochem. J. 60: 618–626

Bryant RW & Light RJ (1974) Stipitatonic acid biosynthesis: incorporation of (formyl-14C) -3-methylorcylaldehyde and

(14C) stipitaldehydic acid, a new tropolone metabolite. Biochemistry 13: 1516–1522

Davis JR, Fravel DR, Marois JJ and Sorensen LH (1986) Effect of soil fumigation and seed piece treament with *Talaromyces flavus* on wilt incidence and yield. 1983. Biol. Cult. Tests Cont. Pl. Dis. 1: 18

Dewar MJS (1945) Structure of stipitatic acid. Nature (London) 155: 50–51

Divekar PV, Brenneisen PE & Tannenbaum SW (1961) Stipitatic acid ethyl ester: a naturally occuring tropolone derivative. Biochim. Biophys. Acta 50: 588–589

Filtenborg O & Frisvad JC (1980) A simple screening method for toxigenic moulds in pure cultures. Lebensm. Wiss. Technol. 13: 128–130

Filtenborg O, Frisvad JC & Svendsen JA (1983) Simple screening method for molds producing intracellular mycotoxins in pure cultures. Appl. Environ. Microbiol. 45: 581–585

Fravel DR, Davis JR & Sorensen LH (1986) Effect of *Talaromyces flavus* and metham on *Verticilliumwilt* incidence and potato yield. 1984–1985. Biol. Cult. Tests Cont. Pl. Dis. 1: 17

Frisvad JC (1986) Taxonomic approaches to mycotoxin identification In: Cole RJ (Ed) Modern methods in the analysis and structural elucidation of mycotoxins (pp 415–457) Academic Press, New York and London

Frisvad JC & Filtenborg O (1983) Classification of terverticillate penicillia based on profiles of mycotoxins and other secondary metabolites. Appl. Environ. Microbiol. 46: 1301–1310

Frisvad JC & Thrane U (1987) Standardized high-performance liquid chromatography of 182 mycotoxins and other fungal metabolites based on alkylphenone indices and UV-VIS spectra (diode array detection). J. Chromatogr. 404: 195–214

Fujimoto H, Jisai Y, Horie Y, Yamazaki M (1988) On the isolation of spiculisporic acid, a toxic metabolite from *Talaromyces panasenkoi.* Proc. Jpn. Assoc. Mycotoxicol. 27: 15–19

Fuska J, Uhrin D, Proksa B, Voticky Z & Ruppelt J (1986) The structure of vermistatin a new metabolite from *Penicillium vermiculatum.* J. Antibiot. 39: 1605–1608

Fuska J, Nemec P & Fuskova A (1979) Vermicillin, a new metabolite from *Penicillium vermiculatum* inhibiting tumor cells in vitro. J. Antibiot. 32: 667–669

Fuska J, Nemec P & Kuhr I (1972) Vermiculine, a new antiprotozoal antibiotic from *Penicillium vermiculatum.* J. Antibiot. 25: 208–211

Fuska J, Proksa B & Uhrin D (1988) The antibiotic PSX-1 produced by *Penicillium stipitatum* is identical with botryodiploidin. Folia Microbiol. 33: 238–240

Gatenbeck S & Mahlen A (1968) A metabolic variation in *Penicillium spiculisporum* Lehman. I. Production of (+) and (−)-decylcitric acids. Acta Chem Scand. 22: 2613–2616

Hocking AD & Pitt JI (1984) Food spoilage fungi. II. Heat-resistant fungi. CSIRO Food Res. Q. 44: 73–82

Jones D, Andersen HA, Russell JD, Fraser AR & Onions AHS (1984) Vermiculine, a metabolic product from *Talaromyces wortmannii.* Trans. Brit. Mycol. Soc. 83: 718–721

Kuhr I, Fuska J, Sedmera P, Podojil M, Vokoun J & Vanek Z (1973) Antitumor antibiotic produced by *Penicillium stipitatum:* its identity with duclauxin. J. Antibiot. 26: 535–536

Kim KK, Fravel DR & Papavizas GC (1988) Identification of a metabolite produced by *Talaromyces flavus* as glucose oxidase and its role in the biocontrol of *Verticillium dahliae.* Phytopathology 78 488–492

MacMillan J, Vanstone AE & Yeboah SK (1972) Fungal products III. Structure of wortmannin and some hydrolysis products. J. Chem. Soc. Perkin Trans. I: 2898–2903

Mantle PG (1987) Secondary metabolites of *Penicillium* and *Acremonium.* In: Peberdy JF (Ed) *Penicillium* and *Acremonium* (pp 161–243) Plenum, New York and London

Mizuno K, Yagi A, Takada M, Matsuura K, Yamaguchi K & Asano K (1974) A new antibiotic, talaron. J. Antibiot. 27: 560–563

Phillips NJ, Cole RJ & Lynn DG (1987) Talaromycins C, D and E. Tetrahedron Lett. 28: 1619–1622

Pitt JI (1980) The genus *Penicillium* and its teleomorphic states *Eupenicillium* and *Talaromyces.* Academic Press, New York and London

Pitt JI & Hawksworth DL (1985) The naming of chemical variants in *Penicillium* and *Aspergillus.* In: Samson RA and Pitt JI (Eds) Advances in *Penicillium* and *Aspergillus* systematics (pp 89–91) Plenum Press, New York and London

Pitt JI & Hocking AD (1979) *Merimbla* gen. nov. for the anamorphic state of *Talaromyces avellaneus.* Can. J. Bot. 57: 2394–2398

Ramirez C (1982) Manual and atlas of the penicillia. Elsevier, Amsterdam

Raper KB & Thom C (1949) Manual of the penicillia. Williams and Wilkins, Baltimore

Samson RA & Abdel-Fattah HM (1978) A new species of *Talaromyces* and a discussion of some recently described taxa. Persoonia 9: 501–504

Samson RA & Pitt JI (Eds) (1985) Advances in *Penicillium* and *Aspergillus* systematics. Plenum, New York and London

Scott AI, Philips GT & Kircheis U (1971) Biosynthesis of polyketides: synthesis of 6-methylsalicylic acid and triacetic acid lactone in *Penicillium patulum.* Bioorg. Chem. 1: 380–399

Segal W (1959) Stipitatonic acid: a new mould tropolone from *Penicillium stipitatum* Thom. J. Chem. Soc. 1959: 2847–2851

Simpson TJ, Lunnon MW & MacMillan J (1979) Fungal products 21. Biosynthesis of the fungal metabolite, wortmannin, from (1,2 13C2)-acetate. J. Chem. Soc. Perkin Trans 1: 931–934

Stolk AC & Samson RA (1971) Studies on *Talaromyces* and related genera I. *Hamigera* gen. nov. and *Byssochlamys.* Persoonia 6: 341–357

Stolk AC & Samson RA (1972) Studies on *Talaromyces* and related genera II. The genus *Talaromyces.* Stud. Mycol. (Baarn) 2: 1–67

Stolk AC & Samson RA (1983) The ascomycete genus *Eupenicillium* and related *Penicillium* anamorphs. Stud. Mycol. (Baarn) 23: 1–149

Tabuchi T, Nakamura I & Kobayashi T (1977) Accumulation of

the open-ring acid of spiculisporic acid by *Penicillium spiculisporum*. J. ferment. technol. 5: 37–49

Takada M & Udagawa S-I (1988) A new species of heterothallic *Talaromyces*. Mycotaxon 31: 417–425

Turner WB (1971) Fungal metabolites. Academic Press, London and New York

Turner WB & Aldridge DC (1983) Fungal Metabolites II. Academic Press, London and New York

Van Eijk GW (1973) Anthraquinones in the fungus *Talaromyces stipitatus*. Experientia 29: 522–523

Van der Spuy JE, Matthee FN & Crafford DJA (1975) The heat resistance of moulds *Penicillium vermiculatum* Dangeard and *Penicillium brefeldianum* Dodge in apple juice. Phytophylactica 7: 105–108

Wells JM, Cole RJ & Kirksey J (1975) Emodin a toxic metabolite of *Aspergillus wenti* isolated from weevil-damaged chestnuts. Appl. Microbiol. 30: 26–28