Available online at www.sciencedirect.com
SCIENCE DIRECT®
ELSEVIER
Systematic and Applied Microbiology 28 (2005) 442–453
SYSTEMATIC AND APPLIED MICROBIOLOGY
www.elsevier.de/syapm

Taxonomic comparison of three different groups of aflatoxin producers and a new efficient producer of aflatoxin B_1, sterigmatocystin and 3-*O*-methylsterigmatocystin, *Aspergillus rambellii* sp. nov.

Jens C. Frisvad[a], Pernille Skouboe[b], Robert A. Samson[c,*]

[a]*Center for Microbial Biotechnology, BioCentrum-DTU, Technical University of Denmark, DK-2800 Kgs. Lyngby, Denmark*
[b]*Bioneer A/S, Kogle Allé 2, DK-2970 Hørsholm, Denmark*
[c]*Centraalbureau voor Schimmelcultures, 3508 AD Utrecht, The Netherlands*

Received 9 February 2005

Abstract

Accumulation of the carcinogenic mycotoxin aflatoxin B_1 has been reported from members of three different groups of Aspergilli (4) *Aspergillus flavus, A. flavus* var. *parvisclerotigenus, A. parasiticus*, *A. toxicarius*, *A. nomius*, *A. pseudotamarii*, *A. zhaoqingensis*, *A. bombycis* and from the ascomycete genus *Petromyces* (*Aspergillus* section *Flavi*), (2) *Emericella astellata* and *E. venezuelensis* from the ascomycete genus *Emericella* (*Aspergillus* section *Nidulantes*) and (3) *Aspergillus ochraceoroseus* from a new section proposed here: *Aspergillus* section *Ochraceorosei*. We here describe a new species, *A. rambellii* referable to *Ochraceorosei*, that accumulates very large amounts of sterigmatocystin, 3-*O*-methylsterigmatocystin and aflatoxin B_1, but not any of the other known extrolites produced by members of *Aspergillus* section *Flavi* or *Nidulantes*.

G type aflatoxins were only found in some of the species in *Aspergillus* section *Flavi*, while the B type aflatoxins are common in all three groups. Based on the cladistic analysis of nucleotide sequences of ITS1 and 2 and 5.8S, it appears that type G aflatoxin producers are paraphyletic and that section *Ochraceorosei* is a sister group to the sections *Flavi, Circumdati* and *Cervini*, with *Emericella* species being an outgroup to these sister groups. All aflatoxin producing members of section *Flavi* produce kojic acid and most species, except *A. bombycis* and *A. pseudotamarii,* produce aspergillic acid. Species in *Flavi,* that produce B type aflatoxins, but not G type aflatoxins, often produced cyclopiazonic acid. No strain was found which produce both G type aflatoxins and cyclopiazonic acid. It was confirmed that some strains of *A. flavus* var. *columnaris* produce aflatoxin B_2, but this extrolite was not detected in the ex type strain of that variety. *A. flavus* var. *parvisclerotigenus* is raised to species level based on the specific combination of small sclerotia, profile of extrolites and rDNA sequence differences. *A. zhaoqingensis* is regarded as a synonym of *A. nomius,* while *A. toxicarius* resembles *A. parasiticus* but differs with at least three base pair differences. At least 10 *Aspergillus* species can be recognized which are able to biosynthesize aflatoxins, and they are placed in three very different clades.

Keywords: Aflatoxin; Sterigmatocystin; *Aspergillus rambellii* sp. nov.; *Aspergillus ochraceoroseus*; *Aspergillus* section *Ochraceorosei* sect. nov.; *A. parvisclerotigenus* comb. nov.

*Corresponding author. Tel.: +31 30 2122656; fax: +31 30 2512097.
E-mail address: samson@cbs.knaw.nl (R.A. Samson).

0723-2020/$ - see front matter © 2005 Elsevier GmbH. All rights reserved.
doi:10.1016/j.syapm.2005.02.012

Introduction

Aflatoxins are the most carcinogenic natural products known and for many years their producers have been thought to be only species in section *Flavi* in *Aspergillus* [4], despite some early reports of aflatoxin production in other taxa, even outside *Aspergillus* and its teleomorphs. Aflatoxin production has been reported from *A. flavus*, *A. parasiticus*, *A. nomius*, *A. zhaoqingensis*, *A. pseudotamarii*, and *A. bombycis* [22,29,36,49] and the two varieties *A. flavus* var. *parvisclerotigenus* and var. *columnaris* [9,41,54]. In 1997 at the Third International Workshop on *Penicillium* and *Aspergillus* in Baarn, we reported that *Aspergillus ochraceoroseus* and *Emericella venezuelensis* produces aflatoxin B_1 [16]. Aflatoxin production by *A. ochraceoroseus* was later confirmed by Klich et al. [24,25]. In *Emericella* aflatoxin production has recently been reported in *E. astellata* [17] and in *E. venezuelensis* [12].

We wanted to test the hypothesis that aflatoxin accumulation is a character that has evolved several times during evolution, by analysing sequence data from the ribosomal DNA (ITS1, 5.8 S and ITS2) genes of representatives of each taxon in combination with their extrolite production. In addition to report a new species related to *A. ochraceoroseus* that produces aflatoxin B_1.

Materials and methods

Strains examined

The strains of *Aspergillus* were examined are listed in Table 1. Both strains identified as *A. ochraceoroseus* are from soil from the Taï National Forest, Ivory Coast. The former strain is the ex type culture deposited by A. Rambelli, the other is a strain later deposited by A. Bartoli. All strains of *Emericella astellata* are from South Seamur Island, Galapagos, Ecuador. A number of strains in *Aspergillus* subgenus *Cervini* were examined in order to compare those with *A. ochraceoroseus*.

The new taxa are registered with a Mycobank accession number (www.mycobank.org).

Growth media

The strains were grown on Czapek agar (Cz), Czapek yeast autolysate (CYA) agar, Yeast extract sucrose (YES) agar, malt extract autolysate (MEA) agar and oat meal (OAT) agar at 25 °C and on CYA at 37 °C for 1 week (for medium formulations see Samson et al. [45]. Three agar plugs were cut out of the colonies and analyzed using HPLC with diode array detection (DAD) according to Frisvad and Thrane [15] as modified by Smedsgaard [48]. All species were also inoculated on AFPA (Aspergillus flavus parasiticus agar) to test for production of aspergillic acid [37].

Analysis of extrolites [44]

Media from CBS 550.77 and CBS 101887 cultures were extracted and analyzed for extrolites seven times independently each using the methods described by Frisvad and Thrane [15], and Smedsgaard [48] and as described below. CBS 550.77 was analyzed three times and CBS 101887 twice using the method of Frisvad and Thrane [15] using a full extraction of a combination of the media CYA, MEA and YES. The other analyses were performed using three agar plugs on individual media (CYA, YES, MEA, OAT) and analyzed independently.

All strains were analyzed according the HPLC-DAD method of Frisvad and Thrane [14,15] and repeated later using the method of Smedsgaard [48] and Nielsen and Smedsgaard [32] with some modifications. HPLC-DAD analysis was performed on a HP-1100 high performance liquid chromatograph equipped with a diode array detector (Agilent, Germany) and a fluorescence detector (Agilent, Germany). Three UV spectra were collected per second from 200 to 600 nm along with chromatographic traces at 210 and 280 nm, all with a 4 nm resolution. Fluorescence signals were collected using an excitation at 230 nm and measuring emission at 333 and 450 nm. Furthermore emission spectra were collected from 300 to 700 nm with two spectra per second at a resolution of 10 nm. Separation was done on a 100 mm × 2 mm i.d. Luna C_{18} column (Phenomenex, USA) with a 10 × 2 mm i.d. Superspher RP-18 guard column (Agilent, USA) at a flow rate of 0.4 mL/min. The eluents used were water and acetonitrile both added 50 μL/L (v/v) triflouracetic acid. A linear gradient going from 15% acetonitrile to 100% acetonitrile in 20 min was used. Hundred percent acetonitrile was held at 5 min before the gradient was returned to starting conditions in 3 min followed by 5 min equilibration. The column temperature was 40 °C. All chemicals used were of analytical grade, acetonitrile (HPLC grade) was from Scanlab, triflouracetic acid was from Merck and the water was Milli-Q grade (Waters). A homologous series of alkylphenones was analyzed as external retention time references and used to calculate a bracketed retention inden (RI) for each detected compound. The production of aflatoxin was confirmed by HPLC-MS using electrospray ionisation (a $M+1$ ion at 313 confirmed the presence of aflatoxin B_1, the $M+1$ ion at 325 sterigmatocystin production, and the $M+1$ ion at 339 3-*O*-methylsterigmatocystin production) on a Hewlett Packard HP 1100 LC/MSD instrument. Aflatoxin production was confirmed by TLC analysis using the agar plug method and eluted in toluene/ethylacetate/

Table 1. Aflatoxin and non-aflatoxin producers in the genus *Aspergillus*: Strains included in this study (^{T} designates a culture ex type)

Fungal species	Strains used	EMBL/GenBank accession numbers
A. ochraceoroseus	CBS 550.77^{T} (= ATCC 38873 = IMI 223071 = IBT 21922)	AJ874115
A. ochraceoroseus	CBS 101887 (= ATCC 42001 = IBT14580)	AJ874116
Emericella astellata	CBS 135.55 (= WB 2397 = NRRL 2397 = IBT 21993)	
Emericella astellata	CBS 261.93^{T} (= IBT 21902)	AJ874123
Emericella astellata	WB 2396^{T} (IBT 22589 = CBS 134.55)	AJ874124
E. venezuelensis	CBS 868.97^{T} = IBT 20956 = IBT 24595	AJ874119
Emericella sp.	IBT 21903	AJ874125
A. pseudotamarii	CBS 766.97^{T} (= NRRL 25517 = IBT 21092)	
A. pseudotamarii	CBS 765.97 (= NRRL 443)	AF004931
A. caelatus	CBS 793.97^{T} (= NRRL 25528 = IBT 21091)	
A. caelatus	CBS 764.97 (= NRRL 25404)	
A. bombycis	NRRL 26010^{T} (= IBT 23536)	AJ874120
A. bombycis	NRRL 25593 (= IBT 23535)	AJ874122
A. bombycis	NRRL 29253 (= IBT 23537)	AJ874121
A. toxicarius	CBS 822.72^{T} (= IBT 4377)	AJ874126
A. toxicarius	CBS 561.82 (= IBT 3591)	AJ874127
A. zhaoqingensis	CBS 399.93^{T} (= IBT 14647)	
A. flavus	CBS 100927^{T}	AF138287
A. flavus var. *columnaris*	CBS 485.65^{T} (= IBT 3657)	
A. flavus var. *columnaris*	CBS 242.65 (= IBT 3660)	
A. flavus var. *columnaris*	ATCC 44310 (= IBT 13084)	
A. flavus var. *columnaris*	NRRL 5821 (= IBT 3640)	
A. flavus var. *columnaris*	IBT 12654	
A. flavus var. *parvisclerotigenus*	CBS 121.62 (= NRRL A-11612 = IBT 3651 = IBT 3850)	
A. flavus var. *parvisclerotigenus*	IBT 16808 (ex sesame seed, Mexico)	AJ874128
A. flavus var. *parvisclerotigenus*	NRRL 3251 (= IBT 3597 = IBT 3618)	
A. parasiticus	CBS 100926^{T} (= IMI 015957vi = IBT 3607)	AF027862
A. nomius	CBS 260.88^{T} (= NRRL 13137 = IBT 3656 = IBT 4966)	
A. nomius	NRRL 25393	AF0027864
A. togoensis	CBS 272.89^{T} (= IBT 21943)	AJ874113
A. coremiiformis	CBS 553.77^{T} (= IBT 21944)	AJ874114
A. cervinus	WB 5026^{T} (= CBS 196.64 = IBT 22044)	
A. cervinus	CBS 194.64 (= IBT 22044)	AJ874118
A. cervinus	CBS 410.64 (= IBT 22086)	
A. cervinus	CBS 537.65 (= IBT 22087)	
A. kanagawaensis	CBS 538.65^{T} (= IBT 22077)	
A. kanagawaensis	CBS 413.64 (= IBT 22082)	
A. kanagawaensis	CBS 423.68 (= IBT 22080)	
A. kanagawaensis	CBS 424.68 (= IBT 22081)	
A. kanagawaensis	WB 2161 (= IBT 22041)	
A. kanagawaensis	WB 4220 (= IBT 22039)	
A. kanagawaensis	WB 5023 (= IBT 22040)	
A. kanagawaensis	WB 5027 (= IBT 22042)	
A. nutans	CBS 121.56^{T} (= IBT 22083)	
A. nutans	CBS 122.56 (= IBT 22043)	AJ874117
A. nutans	CBS 411.64 (= IBT 22076)	
A. nutans	WB 4897 (= IBT 22043)	
A. nutans	IMI 343732 (= IBT 23735)	
A. parvulus	CBS 136.61^{T} (= IBT 22085)	
A. parvulus	CBS 262.67 (= IBT 22079)	
A. parvulus	CBS 298.71 (= IBT 22088)	
A. parvulus	WB 4994 (= IBT 22046)	
A. parvulus	WB 5028 (= IBT 22045)	
Petromyces albertensis	UAMH 2476^{T} (= IBT 14317)	AJ005673
Neopetromyces muricatus.	IMI 368521a^{T} (= CBS 112808 = IBT 19374)	AJ005674

ATCC: American Type Culture Collection, Manassas, VA, USA; CBS is Centraalbureau voor Schimmelcultures, Utrecht, the Netherlands; IBT is the collection of Center for Microbial Biotechnology, BioCentrum-DTU, Kgs. Lyngby, Denmark; IMI is from CABI International, Egham, United Kingdom; NRRL: Northern Center for Agricultural Utilization Research (NCAUR), Peoria, IL, USA, UAMH: University of Alberta Microfungus Collection and Herbarium, Edomonton, Alberta, Canada; WB: Wisconsin Bacteriology collection, now at NCAUR.

formic acid (6:3:1) and chloroform/acetone/2-propanol (85:15:20) [11,14]. Aflatoxin B_1, B_2, G_1, G_2, sterigmatocystin, 3-*O*-methylsterigmatocystin, kojic acid, and cyclopiazonic acid (Sigma) were used as analytical standards.

Isolation of total DNA

For the RAPD analysis and rDNA sequencing all strains were grown for 5–7 days at 25 °C on CYA agar and the DNA extracted using the FastDNA®SPIN Kit for Soil (BIO 101, Carlsbad, New Mexico, USA) according to the manufacturers instructions (homogenization for 45 s at speed 6.0).

RAPD analysis

The two strains originally identified as *A. ochraceoroseus* (CBS 550.77 and CBS 101887) were compared using RAPD. The primers used were selected for their differentiation ability in *Penicillium*: OPB1 (5′ GTTTCGCTCC 3') and OPB8 (5′ GTCCACACGG 3′), both from Operon Technologies (Alameda, CA) [30]. DNA amplification reactions were performed in total volumes of 50 µL each containing 1 × reaction buffer (10 mM Tris; pH 8.3, 50 mM KCl), 2.5 mM $MgCl_2$, 3 µM RAPD primer, 0.2 mM each of dNTP (Pharmacia Biotech, Uppsala, Sweden), 5.0 units of Ampli*Taq* DNA polymerase (Perkin–Elmer Corp., Norwalk, CT, USA), and 5 µL of genomic DNA (2–10 ng). Amplifications were performed in a GeneAmp PCR system 2400 model (Perkin–Elmer Corp., Norwalk, CT, USA) programmed for an initial denaturation at 94 °C for 1 min followed by 35 cycles of 94 °C for 15 s, 35 °C for 1 min, 72 °C for 1 min and a final 10 min elongation step at 72 °C. After PCR, 12 µL of each amplified sample were subjected to electrophoresis in a 2% agarose gel (Saveen Agarose) at 90 mA for 3.5–4 h and visualized by staining with ethidium bromide. The two *Aspergillus* strains were compared to a series of ochratoxin A producing isolates of *Penicillium verrucosum* (IBT 23126 to IBT 23131, IBT 23133 to IBT 23135, IBT 21149 to IBT 21154).

PCR amplification and DNA sequencing

PCR amplification and DNA sequencing of the ribosomal internal transcribed spacers (ITS 1 and ITS 2) and the 5.8S rRNA gene was carried out as described by Parenicová et al. [34] and Skouboe et al. [47]. *Aspergillus* strains sequenced in this study are listed in Table 1. In addition, few of the rDNA sequences included in Fig. 1 have been obtained directly from the EMBL/GenBank sequence databases and these accession numbers are listed in the ITS cladogram. *Aspergillus ochraceus* MZ14 has EMBL/GenBank no. AJ270057 and *Emericella nidulans* (Glasgow strain) has no. L76746.

Results and discussion

Aflatoxin producers

Aflatoxin B_1 production was confirmed in strains of *A. flavus* (except the ex type culture), *A. nomius*, *A. parasiticus*, *A. toxicarius*, *A. bombycis*, *A. pseudotamarii*, *A. flavus* var. *parvisclerotigenus* in section *Flavi*, *A. ochraceoroseus* and in *E. venezuelensis* and *E. astellata* in section *Nidulantes*. Aflatoxin B_2 was found as a minor extrolite in all aflatoxin B_1 producing species, but as the only type of aflatoxin in *A. flavus* var. *columnaris* NRRL 5821 and IBT 12654 and in *A. zhaoqingensis* CBS 399.93. On the other hand three cultures of *A. flavus* var. *columnaris* CBS 485.65 (ex type), CBS 242.65 and ATCC 44310 did not produce any aflatoxins. *A. zhaoqingensis* produced kojic acid, aspergillic acid, one aflatoxin (B_2), and tenuazonic acid like most strains of *A. nomius* (unpublished data). However, typical strains of *A. nomius* produced all known aflatoxins and synonymy of *A. zhaoqingensis* with *A. nomius* needs further investigation. The aflatoxin B_2 accumulating taxa should be examined further to elucidate their proper taxonomic status. Aflatoxins G_1 and G_2 were found in *A. parasiticus*, *A. nomius*, *A. bombycis* and *A. toxicarius*. 3-*O*-methylsterigmatocystin was found in all aflatoxin producers in this study, while sterigmatocystin was only accumulated in the Emericella and related Aspergilli and in both strains of *A. ochraceoroseus*.

Aflatoxin production by *A. ochraceoroseus* CBS 550.77 and CBS 101887

The two strains of *A. ochraceoroseus* CBS 550.77 and CBS 101887 produced aflatoxin B_1 on all media tested in seven independent experiments. Sterigmatocystin and 3-*O*-methylsterigmatocystin were also produced in all experiments and on all media, with one exception. In one HPLC run based on the medium OAT only aflatoxin B_1 was detected from CBS 101887. YES agar was the best medium for aflatoxin production by the two strains, followed by CYA, MEA and OAT. This production pattern is similar to that of *Emericella venezuelensis* [12], but in contrast to *E. astellata* that produces most aflatoxin B_1 on OAT and very small amounts, if any, on YES, CYA and MEA [17]. When compared within HPLC runs performed the same day, the production of aflatoxin B_1 on CYA was 23–38% of that on YES, on MEA production was 4–6% of that on YES and on OAT it was from 0.5% to 0.7% compared to the amounts produced on YES agar. Members of

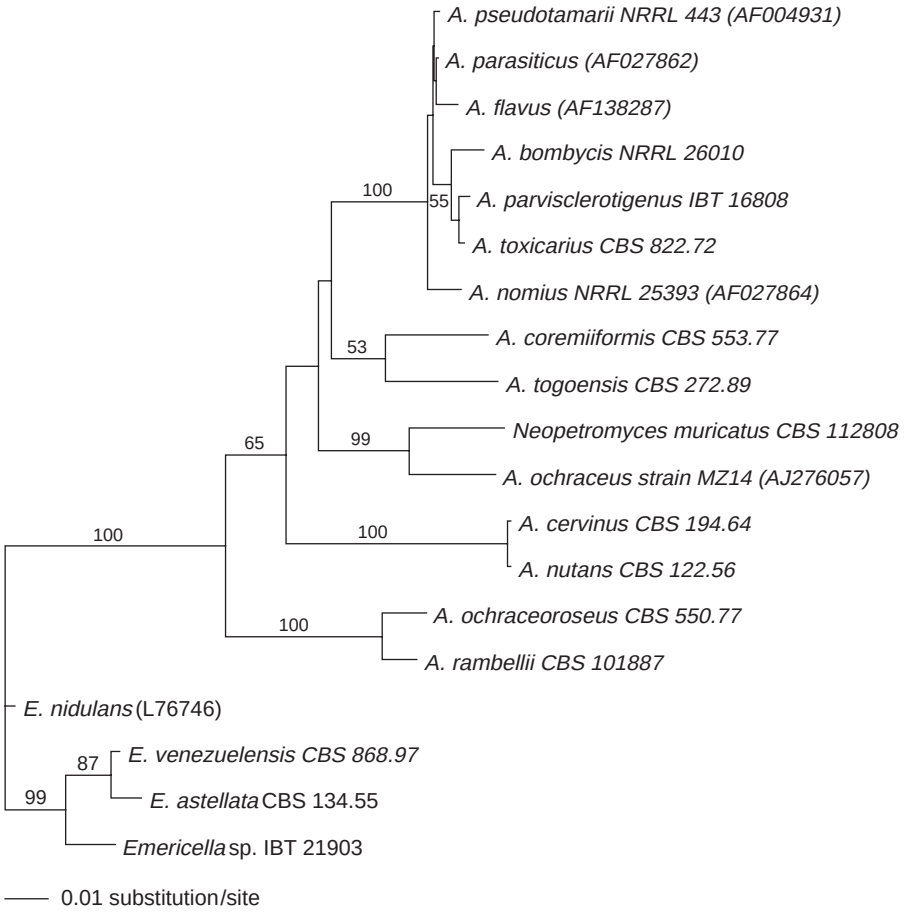

Fig. 1. Cladogram of aflatoxin producers and fungi claimed to be related to *A. ochraceoroseus* and *A. rambellii* based on sequences of the ITS1 and ITS2 and 5.8S regions of rDNA. The cladogram was based on neighbour joining and was bootstrapped 1000 times. Values above 50% are shown on the branches. Three strains of *A. bombycis,* the two strains of *A. toxicarius* and the two strains of *E. astellata* has the same sequences and only one strain is shown on the cladogram (see Material and methods). A heuristic search using parsimony gave a strict consensus tree of 158 retained trees of the same appearance as the neighbour joining tree shown (CI = 0.705; RI = 0.796; RC = 0.561; HI = 0.295).

Flavi also produced most aflatoxin on YES agar compared with the other media, but only produced traces of (or no) sterigmatocystin.

A. ochraceoroseus produced more aflatoxin B_1 than *E. venezuelensis* and *E. astellata*, but less than members of section *Flavi*. CBS 101887 produces the largest amounts of aflatoxin B_1 we have ever observed, even more than the best producers in section *Flavi* (*A. parasiticus*, *A. flavus* var. *parvisclerotigenus* and *A. nomius*). It should be noted, however, that our method is only semi-quantitative, so a more accurate extraction and detection method should be used in order to substantiate such comparisons. In *A. ochraceoroseus* strains extrolites of the aflatoxin biosynthetic family were the dominant peaks in the HPLC chromatograms, while in section *Flavi* other extrolites were also produced in very high amounts, i.e. kojic acid and cyclopiazonic acid. In *Emericella* species aflatoxins were always minor components compared to other extrolites, i.e. shamixanthones, desertorins, etc.

Classification of aflatoxin producers

Aflatoxin producing members of *Aspergillus* section *Flavi* have yellow green to dark green or golden brown conidia, grow very fast at 37 °C and, with the exception of *A. bombycis* and *A. pseudotamarii*, have a cadmium orange reverse on AFPA. The aspergilla were typical of *Aspergillus* subgenus *Circumdati* with a high proportion of metulae with phialides covering the entire vesicle, and some aspergilla with only phialides only covering the upper part of the vesicle. *A. ochraceoroseus* also produced aspergilla typical of subgenus *Circumdati*, but the yellow to light orange colours of their conidia is rather similar to colours seen in section *Circumdati* (the *Aspergillus ochraceus* group) and in *Petromyces alliaceus* and *P. albertensis* in section *Flavi* [13,24]. Furthermore they did not produce a cadmium orange reverse on AFPA. The major difference between species from section *Flavi* and the two strains of *A. ochraceoroseus* is the inability to grow at 37 °C.

Bartoli and Maggi [3] placed *A. ochraceoroseus* in section *Circumdati* but Christensen [7] mentioned a morphological resemblance of *A. ochraceoroseus* with members of the section *Cervini*. They were first placed in subgenus *Fumigati* and then in subgenus *Aspergillus* (see [40]). A comparison of members of *Cervini* showed that none of those species grew on Czapek agar in contrast to the other species considered here. Furthermore as described by Raper and Fennell [39], the species in *Cervini* grew slowly with fawn coloured conidia and do not produce metulae.

Within the subgenus *Nidulantes*, the aflatoxin producing species *Emericella astellata* and *E. venezuelensis* produce the typical short brown to yellow brown conidiophores and small vesicles covered with metulae with phialides in their upper part. Like *E. bicolor*, *E. foeniculicola*, *E. pluriseminata* and *E. spectabilis*, *E. astellata* and *E. venezuelensis* grow rather slowly or not at all at 37 °C (0 and 0–9 mm after one week of incubation, respectively) in contrast to most other species of *Emericella*. None of the *Emericella* species tested produced the orange reaction on AFPA.

Thus *A. ochraceoroseus* does not show an obvious morphological similarity to any known section in *Aspergillus* and a new section *Ochraceorosei* is proposed (see taxonomic conclusions below).

Considering the extrolite production, members of *Aspergillus* section *Flavi* that produce aflatoxin B_1 also produce kojic acid and, except for *A. bombycis* and *A. pseudotamarii*, aspergillic acid. Species examined that produced the G type aflatoxins do not produce cyclopiazonic acid and vice versa (Table 2) in agreement with Takahashi et al. [50]. Members of *Flavi* produce different combinations of aflatoxins, kojic acid, cyclopiazonic acid and aspergillic acid and only share the aflatoxins (B type) with species in the sections *Ochraceorosei* and *Nidulantes*. Furthermore, none of the strains that produced aflatoxins in *Aspergillus* section *Flavi* produced detectable amounts of sterigmatocystin, while sterigmatocystin was always accumulated together with aflatoxin B_1 in *Emericella venezuelensis* and *E. astellata* [12,17] and in *A. ochraceoroseus* [16,24]. Members of *Aspergillus* section *Cervini* (*A. cervinus, A. kanagawaensis, A. parvulus, A. nutans*) do not produce aflatoxins or any other extrolites in common with the other sections listed above.

Both strains of *A. ochraceoroseus* differ from *Emericella* species and *Aspergillus* section *Flavi* species by inability to grow at 37 °C, and by producing other extrolites than those found in *Aspergillus* section *Flavi* or in *Emericella*. Furthermore *A. ochraceoroseus* shares no extrolites with neither the sections *Circumdati* nor *Aspergillus* section *Cervini.* Several extrolites of unknown structure were produced by the Aspergilli examined and one of these, produced by *A. ochraceoroseus*, had the same UV spectrum as a common extrolite in species of the genus *Emericella*. Both strains of *A. ochraceoroseus* produce extrolites with kotanin chromophores, but these metabolites have also been detected in *Aspergillus* sections *Flavi*, *Nidulantes*, *Clavati* and *Nigri*. Section *Ochraceorosei*, *Flavi* and *Nidulantes* are very different and only share few taxonomic features. From their phenotypic features it appears that section *Ochraceorosei* is closer to section *Nidulantes* than to *Flavi*, *Circumdati*, or *Cervini*.

Cladification of aflatoxin producers

The aflatoxin producers in *Aspergillus* section *Flavi, A. flavus, A. parasiticus, A. nomius, A. parvisclerotigenus,*

Table 2. Aflatoxin producers and their production of chelating extrolites

Species	Aflatoxin		Cyclopiazonic acid	Kojic acid	Aspergillic acid
	B_1	G_1			
Aspergillus section *Flavi* (*Petromyces*)					
A. bombycis	+	+	–	+	–
A. flavus	+	–	+	+	+
A. nomius	+	+	–	+	+
A. parasiticus	+	+	–	+	+
A. parvisclerotigenus	+	±	+	+	+
A. pseudotamarii	+	–	+	+	—
A. toxicarius	+	+	–	+	+
Aspergillus subgenus *Nidulantes* (*Emericella*)					
E. astellata	+	–	–	–	–
E. sp. IBT 21903	+	–	–	–	–
E. venezuelensis	+	–	–	—	—
Aspergillus section *Ochraceorosei*					
A. ochraceoroseus	+	–	–	–	–
A. rambellii	+	–	–	–	–

A. bombycis and *A. pseudotamarii* have been shown to be phylogenetically closely related and related to the ascomycete genus *Petromyces* [4,10,13,18,19,36,55] which is also supported by phenotypic characters. Most isolates of these species produce black sclerotia, kojic acid and aspergillic acid, they all grow fast at 37 °C and they all accumulate aflatoxins, but not sterigmatocystin [13]. We predict that the genes required for aflatoxin accumulation is very alike in all six species. Interestingly the only two known species with a teleomorph in this group, *Petromyces alliaceus* and *P. albertensis*, accumulate ochratoxin A, not aflatoxins [13]. Data from Geiser et al. [19] and Peterson et al. [36] indicates that both *A. flavus* and *A. nomius* isolates can recombine sexually (cryptic speciation) yet they often retain their ability to accumulate extrolites.

A. toxicarius Murakami was synonymized with *A. flavus* by Samson [43] and regarded as closely related to *A. parasiticus*, yet distinct by Christensen [6]. Unfortunately the ex type culture of *A. parasiticus* was not included in the study of Christensen [6] and of the two subcultures derived from the same original culture IMI 091019b, one subculture (NRRL 2999) was placed in *A. toxicarius* and another (WB 5013) in *A. parasiticus. A. toxicarius* had *A. parvisclerotigenus* as a sister group. In contrast to earlier results [40,55], we found that the ex type culture of *A. toxicarius* had at least three sequence differences in the ITS regions as compared to four strains of *A. parasiticus*. Usually three or more sequence differences in ITS indicate different species. On the other hand, and in agreement with ITS sequencing data of Rigo et al. [40] and Nikkuni et al. [33], the profile of extrolites in *A. toxicarius* is very similar to that of *A. parasiticus*, and thus our ITS based cladification is not consistent with the similar profiles of extrolites in *A. parasiticus* and *A. toxicarius* (Table 2).

Even though kojic acid is produced by all species in the *Flavi* clade, the G type aflatoxin producers are not in the same subclade (Fig. 1). The three subclades represented by (1) *A. nomius*, (2) *A. flavus*, *A. parasiticus* and *A. pseudotamarii* and (3) *A. toxicarius*, *A, parvisclerotigenus* and *A. bombycis* all contain some aspergillic acid producers and the latter two subclades contain some cyclopiazonic acid producers, so most of the extrolite features appear to be homoplasies, at least as based on the ITS cladogram.

A. flavus var. *parvisclerotigenus* is a common taxon often identified as a microsclerotial variant of *A. flavus* [8,41,42,56]. In agreement with Geiser et al. [18] we also found that the microsclerotial strains differ from *A. flavus* (Fig. 1) and therefore we follow Hesseltine et al. [20] in regarding this as representing a separate taxon different from *A. flavus*. We propose to raise this variety to species level (see taxonomic conclusions below). Our strains of *A. parvisclerotigenus* only produced B type aflatoxins, and it is possible that the B and G type aflatoxin producers with small sclerotia mentioned by Geiser et al. [18] are referable to *A. toxicarius*, but this should be investigated further. According to our data *A. parvisclerotigenus* produces type B aflatoxins and cyclopiazonic acid, while the B and G type aflatoxin producers *A. toxicarius* (and *A. parasiticus*, *A. nomius* and *A. bombycis*) do not produce cyclopiazonic acid. According to the mycotoxin profiles one would have expected the cyclopiazonic acid and aflatoxin B_1 producers to be in one clade, and the aflatoxin $B_1 + G_1$ producers to be in another clade, but this is not reflected in the ITS rDNA based cladification in Fig. 1.

Emericella species are phylogenetically related to the sections *Nidulantes, Versicolores* and *Usti* [35], but are not to *Petromyces* and *A. ochraceoroseus* or *A. rambellii*. Again this is supported by phenotypic characters. The aflatoxin producers in *Emericella* accumulate both aflatoxin B_1 and sterigmatocystin and produce ascomata with Hülle cells.

In the cladogram (Fig. 1) it is seen that phylogenetically *A. ochraceoroseus* and *A. rambellii* form a strongly supported clade that has subgenus *Circumdati* and section *Cervini* as sister clade and *Emericella* species as outgroup. This is in not entirely in agreement with the results of Varga et al. [55] and Klich et al. [25]. They found that *A. ochraceoroseus* was a sister group to *Emericella*. Our inclusion of *A. rambellii* and section *Cervini* and two other tropical species (*A. togoensis* and *A. coremiiformis*) may have changed the overall cladification. The aflatoxin producing *A. ochraceoroseus* and *A. rambellii* are monophyletic and not members of the two other clades including aflatoxin producing species. A clade including all aflatoxin producers would be polyphyletic. Aflatoxin biosynthesis thus seems to have evolved at least three times. Sterigmatocystin has also been reported in *A. togoensis* [57], but we have not been able to confirm this. If this is also confirmed, production of sterigmatocystin may have evolved independently four times in *Aspergillus* and related teleomorphs. Sterigmatocystin production seems to have evolved at least seven times as is also occurs in the phylogenetically unrelated genera *Monocillium* [2], *Chaetomium* spp. [27,46,52,53], *Humicola* [23] and *Bipolaris* [30,31,38]. As the strains of *Farrowia* and *Achaetomiella* [21] reported to produce sterigmatocystin are regarded to belong to *Chaetomium* [1,5,51] sterigmatocystin may have evolved only once in *Chaetomium*, but this is unlikely as seven species have been reported to produce sterigmatocystin in *Chaetomium*: *C. caprinum, C. gracile, C. longicolleum, C. tetraspermum, C. thielavioideum, C. udagawa* and *C. virescens*.

The two strains of *A. ochraceoroseus* were compared using the RAPD technique with the primers OPB1 and OPB8 (Fig. 2). Isolates of *P. verrucosum* were quite alike in the banding pattern, whereas the two *Aspergillus* strains had no RAPD bands in common at all. This

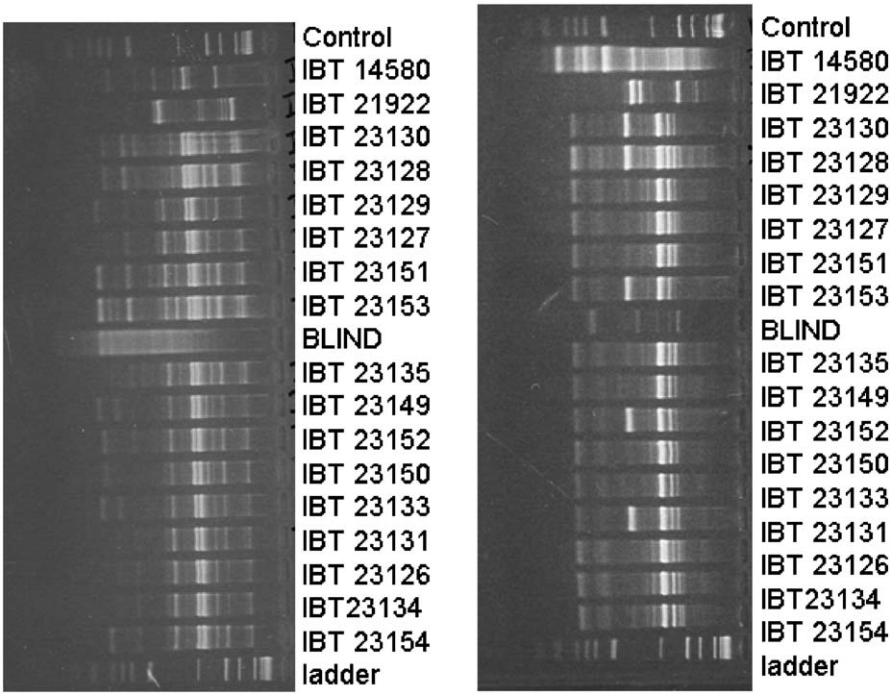

Fig. 2. RAPD patterns using the primers OPB1 and OPB8. From bottom to top: 1 kb ladder, *Aspergillus ochraceoroseus* CBS 101887 (= IBT 14530), CBS 550.77 (= IBT 21922), *P. verrucosum* IBT 23130, 23128, 23129, 23127, 23151, 23153, blind sample, 23135, 23149, 23152, 23150, 23133, 23131, 23126, 23134, 23154, 1 kb ladder.

indicated they were different species, in agreement with phenotypic differences. Because of both morphological, extrolite, sequence and RAPD differences we are proposing CBS 101887 as a new species *Aspergillus rambellii* (see taxonomic conclusions).

Taxonomic conclusions

***Aspergillus rambellii* Frisvad & Samson sp. nov. (Fig. 3)**

Coloniae in agaro CYA 38–53 mm, una hebdomade, 25 °C; in agaro CYA 0 mm, 37 °C; mycelium floccoso, albo, conidia griseoflava in massa (Methuen 4-B-C-4) [26], reverso aurantioaco, conidiophorae erectae orientis e substrato imis cellulis conspicuis, stipites 750–1000 × 9–11 µm, parietibus levibus, vesiculae globosae, 25–65 µm diam., fertilis in tota superficie, metulae 7–10 × 4–4.5 µm, levibus; phialides 7–10 × 2.5–3.5 µm; conidia ellipsoidea vel pyriformia, parietibus levibus, 3.5–4 × 4.5–5.5 µm, aflatoxinum B_1 et sterigmatocystinum producuntur.

Mycobank MB 500164
Holotypus: CBS 101887
Culture ex type ATCC 42001 = IBT 14580 = IBT 24753 = CBS 101887.

Etymology: named after Prof. A. Rambelli for his contribution to the taxonomy of *Aspergillus*.

Conidial heads biseriate consisting of both metulae and phialides, conidiophore stipes are long 750–1000 µm, smooth-walled, with well developed foot-cells and swollen globose vesicles, diam. 25–65 µm and swollen metulae, 7–10 × 4–4.5 µm, phialides, 7–10 × 2.5–3.5 µm, phialide collula relatively long, conidia are ellipsoidal, with few being pyriform, smooth-walled, 3.5–4 × 4.5–5.5 µm, with few conidia being longer, conidia coloured greyish yellow *en masse* (Methuene 4-B-C-4). Exudate droplets were not observed.

Colonies after 1 week at 25 °C on CYA 38–53 mm, reverse orange to cayenne, MEA: (8–)22–36 mm, colony reverse weakly yellow, YES 62–72 mm, reverse yellowish orange to orange red, OAT: 24–45 mm, CREA: 12–25 mm, thin colonies and no acid production, no growth on CYA at 37 °C.

Extrolites produced (Fig. 4): Aflatoxin B_1, aflatoxin B_2, sterigmatocystin, 3-*O*-methylsterigmatocystin, versicolorins, averufin, norsolorinic acid (all in the aflatoxin biosynthetic family), 18 different chromophore families were detected by HPLC-DAD, but none of those have yet been structure elucidated. One had a chromophore suggesting kotanins or desertorins, another chromophore was close to that of wortmannin, a third chromophore suggested emerin or xanthocillin and a fourth chromophore had an UV spectrum suggesting an indole extrolite. These latter four chromophore families were in common with those found in *A. ochraceoroseus*,

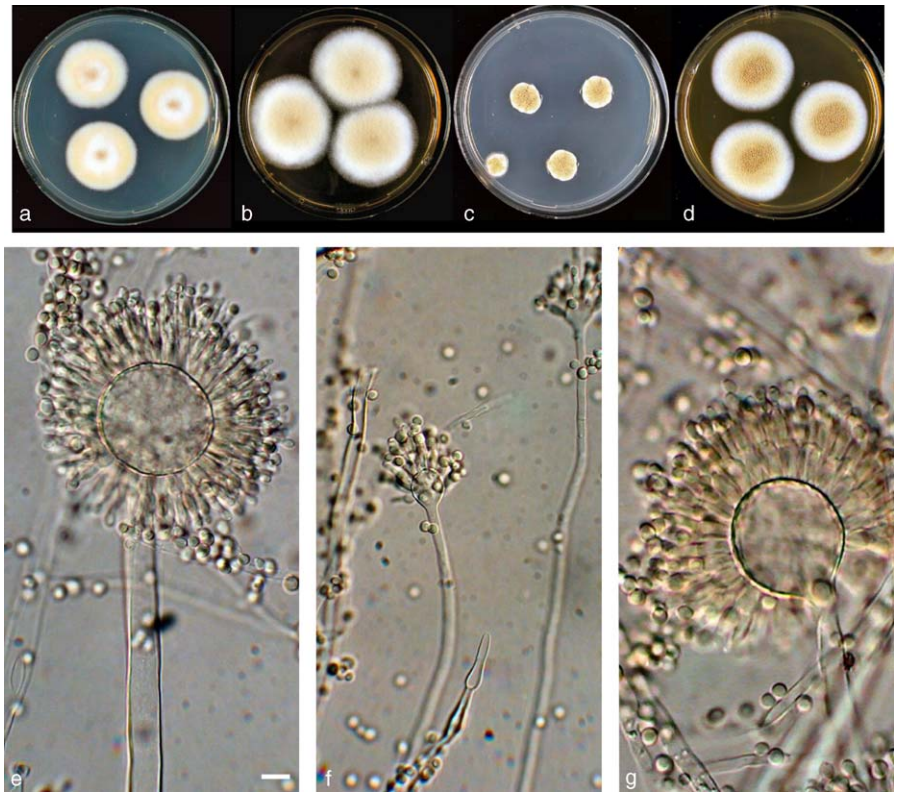

Fig. 3. a–b. *A. rambellii* on Czapek agar and malt extract agar. c–d. *A. ochraceoroseus* on Czapek agar and malt extract agar. e–f. Conidiophores of *A. rambellii.* g. Conidiophores of *A. ochraceoroseus*. Scale bar is 10 µm.

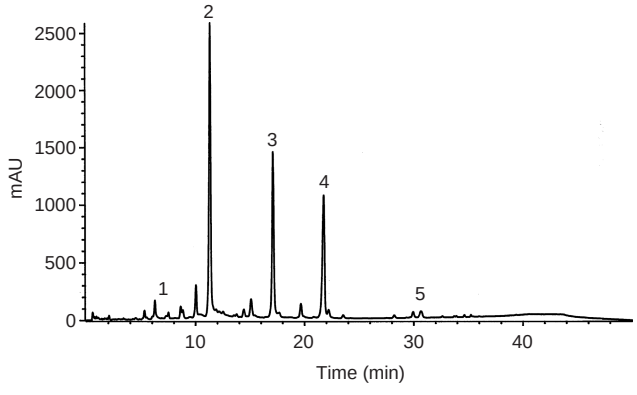

Fig. 4. HPLC trace of an extract of *Aspergillus rambellii* analyzed according to Frisvad and Thrane [15]. Note the large amounts of aflatoxin B_1 (peak 2), sterigmatocystin (peak 4), 3-*O*-methylsterigmatocystin (peak 3). Aflatoxin M_1 (peak 1) and averufin (peak 5) are also marked. The amount of aflatoxin B_1 is comparable to the amount of aflatoxin B_1 production by the best producers in *A. toxicarius, A. parvisclerotigenus*, *A. nomius*, *A. parasiticus* and *A. pseudotamarii*.

even though the number of metabolites in each chromophore family was higher in *A. ochraceoroseus* than in *A. rambellii*. 13 types of UV spectra have until now only been found in *A. rambellii*.

Aspergillus ochraceoroseus Bartoli and Maggi

This species was described by Bartoli and Maggi [3] and has since been characterized by Samson [24], Christensen [7], Kozakiewicz [28] and Klich et al. [11]. We have made some further observations on the morphology of this species: The species has smooth conidia of varying shapes, but most conidia are ellipsoidal and subglobose with few conidia being very long. The long sterile metulae mentioned by Bartoli and Maggi [3] are often observed and occasionally small conidial heads as in *A. rambellii* are seen (Fig. 3). As mentioned by Frisvad et al. [9] and Klich et al. [11] this species does not grow at 37 °C. Colonies after one week of growth at 25 °C: CYA: 17–21 mm, MEA: (9–)17–33 mm, YES: 43–58 mm, OAT: 22–42 mm, CREA: poor growth and no acid production.

Extrolites produced: Aflatoxin B_1 and B_2, sterigmatocystin, 3-*O*-methylsterigmatocystin, some fluorescing metabolites with some resemblance to aflatoxin, not seen in other aflatoxin producers, kotanin-like metabolites, wortmannin-like metabolites, some indole-alkaloids and a yellow metabolite also found in *Emericella* spp., and one further extrolite family in common with *A. rambellii*. Of these extrolites families two are unique to *A. ochraceoroseus* as compared to *A. rambellii*.

Aspergillus section *Ochraceorosei* Frisvad & Samson, sect. nov.

Sectio in subgenere *Circumdati* cum speciebus aflatoxinum producuntur, conidia flava in massa, stipitibus levibus et 37 °C non crescentes.

Mycobank MB 500165

Section in *Aspergillus* subgenus *Circumdati* containing species not able to grow at 37 °C, producing yellow ellipsoidal conidia, biseriate conidial heads, long conidiophore stipes that are smooth and producing aflatoxin and sterigmatocystin

Type species *A. ochraceoroseus* Bartoli & Maggi

Aspergillus parvisclerotigenus (Saito and Tsuruta) Frisvad and Samson, comb. nov.

Basionym *Aspergillus flavus* var. *parvisclerotigenus* Saito & Tsuruta, Proc. Jpn. Assoc. Mycotoxicol. 37: 32 (1993).

Mycobank MB 500166

Neotype designated here CBS 121.62, as the original type culture and herbarium specimen is unavailable (CBS 121.62 = NRRL A-11612 = IBT 3651 = IBT 3851, ex *Arachis hypogea*, Nigeria). Other representative strains NRRL 3251, IBT 16808.

Extrolites produced: Aflatoxin B_1, B_2, G_1, G_2, parasiticol, cyclopiazonic acid, kojic acid, 3-*O*-methylsterigmatocystin, versicolorins, α and β aflatrem, paspalinine,paspaline, aflavarin and A-30461.

Acknowledgements

Financial support to Program for Predictive Biotechnology from Danish Technical Research Council is acknowledged. We thank Flemming Lund and Anni Bech Nielsen for help with the RAPD analyses and Dorthe Lauritsen for help with the ITS sequencing.

References

[1] J. A von Arx, J. Guarro, M.J. Figueras, The ascomycete genus *Chaetomium*, Beihefte zur Nova Hedwigia 84 (1986) 1–162.

[2] W.A. Ayer, L. Pena-Rodriguez, J.C. Vederas, Identification of sterigmatocystin as a metabolite of *Monocillium nordinii*, Can. J. Microbiol. 27 (1981) 846–847.

[3] A. Bartoli, O. Maggi, Four new species of *Aspergillus* from Ivory Coast soil, Trans. Br. Mycol. Soc. 71 (1978) 383–394.

[4] J.W. Bennett, K.E. Papa, The aflatoxigenic *Aspergillus* spp, Adv. Plant Pathol. 6 (1988) 263–280.

[5] P.F. Cannon, A revision of Achaetomium, Achaetomiella and Subramaniula, and some similar species of Chaetomium, Trans. Br. Mycol. Soc. 87 (1986) 45–76.

[6] M.A. Christensen, Synoptic key and evaluation of species in the *Aspergillus flavus* group, Mycologia 73 (1981) 1056–1084.

[7] M.A. Christensen, The *Aspergillus ochraceus* group: two new species from Western soils and a synoptic key, Mycologia 74 (1982) 210–225.

[8] P.J. Cotty, Virulence and cultural characteristics of two *Aspergillus flavus* strains on cotton, Phytopathology 79 (1989) 805–814.

[9] I. El-kady, S. El-Maraghy, A.-N. Zihri, Mycotoxin producing potential of some isolates of *Aspergillus flavus* and *Eurotium* groups from meat products, Microbiol. Res. 149 (1994) 297–307.

[10] T.P. Feibelman, P.J. Cotty, M.A. Doster, T.J. Michailides, A morphologically distinct strain of *Aspergillus nomius*, Mycologia 90 (1998) 618–623.

[11] O. Filtenborg, J.C. Frisvad, J.A. Svendsen, Simple screening method for molds producing intracellular mycotoxins in pure cultures, Appl. Environ. Microbiol. 45 (1983) 581–585.

[12] J.C. Frisvad, R.A. Samson, Emericella venezuelensis, a new species with stellate ascospores producing sterigmatocystin and aflatoxin B_1,, System. Appl. Microbiol. 27 (2004) 672–680.

[13] J.C. Frisvad, R.A. Samson, *Neopetromyces* gen. nov. and an overview of teleomorphs of *Aspergillus* subgenus *Circumdati,*, Stud. Mycol. (Baarn) 45 (2000) 201–207.

[14] J.C. Frisvad, U. Thrane, Application of high-performance liquid chromatography, In: V. Betina (Ed.), Chromatography of Mycotoxins: techniques and Applications, J. Chromatograp. Lib., 54 (1993) 253-372.

[15] J.C. Frisvad, U. Thrane, Standardized high-performance liquid chromatography of 182 mycotoxins and other fungal metabolites based on alkylphenone retention indices and UV-VIS spectra (diode array detection), J. Chromatograph. 404 (1987) 195–214.

[16] J.C. Frisvad, J. Houbraken, R.A. Samson, *Aspergillus* species and aflatoxin production: a reappraisal, In: A.C.J. Tuijtelaars, R.A. Samson, F.M. Rombouts, S. Notermans (Eds.), Food Microbiology and Food Safety into the Next Millennium, Foundation Food Micro '99, Zeist, 1999, pp. 125–126.

[17] J.C. Frisvad, J. Smedsgaard, R.A. Samson, *Emericella astellata*, a new producer of aflatoxin B_1, B_2 and sterigmatocystin, Lett. Appl. Microbiol. 38 (2004) 440–445.

[18] D.M. Geiser, J.W. Dorner, B.W. Horn, J.W. Taylor, The phylogenetics of mycotoxin and sclerotium production in *Aspergillus flavus* and *Aspergillus oryzae*, Fung. Genet. Biol. 31 (2000) 169–179.

[19] D.M. Geiser, J.I. Pitt, J.W. Taylor, Cryptic speciation and recombination in the aflatoxin-producing fungus *Aspergillus flavus*, Proc. Natl. Acad. Sci. USA 95 (1998) 388–393.

[20] C.W. Hesseltine, O.T. Shotwell, M. Smith, J.J Ellis, E. Vandegraft, G. Shannon, Production of various aflatoxins by strains of the *Aspergillus flavus* series, In: Proceedings of the first US-Japan Conference on Toxic Microorganisms, UJNR Joint Panels on Toxic Microorganisms and

the US Department of the Interior, Washington DC, 1970. pp. 202–210.

[21] C.W. Holzapfel, I.H.F. Purchase, P.S. Steyn, L. Gouws, The toxicity and chemical assay of sterigmatocystin, a carcinogenic mycotoxin, and its isolation from two new fungal sources, South African Med. J. 40 (1966) 1100–1101.

[22] Y. Ito, S.W. Peterson, D.T. Wicklow, T. Goto, *Aspergillus pseudotamarii*, a new aflatoxin producing species in *Aspergillus* section *Flavi*, Mycol. Res. 105 (2001) 233–239.

[23] B.K. Joshi, .J.B. Gloer, D.T. Wicklow, Bioactive natural products from a sclerotium-colonizing isolate of *Humicola fuscoatra*, J. Nat. Prod. 65 (2002) 1734–1737.

[24] M. Klich, E.J. Mullaney, C.B. Daly, J.W. Cary, Molecular and physiological aspects of aflatoxin and sterigmatocystin biosynthesis by *Aspergillus tamarii* and *A. ochraceoroseus*, Appl. Microbiol. Biotechnol. 53 (2000) 605–609.

[25] M.A. Klich, J.W. Cary, S.B. Beltz, J.A. Bennett, Phylogenetic and morphological analysis of *Aspergillus ochraceoroseus*, Mycologia 95 (2003) 1252–1260.

[26] A. Kornerup, J.H. Wanscher, Methuen handbook of colour, third ed, Eyre Methuene, London, 1978.

[27] K. Koyama, K. Takahashi, S. Natori, S. Udagawa, Production of mycotoxins by *Chaetomium* species, Maikotokishin (Tokyo) 33 (1991) 40–43.

[28] Z. Kozakiewicz, *Aspergillus* species on stored products, Mycol. Papers 161 (1989) 1–188.

[29] C.P. Kurtzman, B.W. Horn, C.W. Hesseltine, Aspergillus nomius, a new aflatoxin- producing species related to Aspergillus flavus and Aspergillus parasiticus, Antonie van Leeuwenhoek 53 (1987) 147–158.

[30] F. Lund, P. Skouboe, Identification of *Penicillium caseifulvum* and *P. commune* isolates related to specific cheese and rye bread factories using RAPD fingerprinting, J. Food Mycol. 1 (1998) 131–139.

[31] C.M. Maes, P.S. Steyn, Polyketide-derived fungal metabolites from *Bipolaris sorokiniana* and their significance in the biosynthesis of sterigmatocystin and aflatoxin B_1, J. Chem. Soc. Perkin Trans. I (1984) 1140–1937.

[32] K.F. Nielsen, J. Smedsgaard, Fungal metabolite screening: database of 474 mycotoxins and fungal metabolites for dereplication by standardised liquid chromatography-UV-mass spectrometry methodology, J. Chromatograp. A 1002 (2003) 111–136.

[33] S. Nikkuni, H. Nakajima, S. Hoshina, M. Ohno, C. Suzuki, Y. Kashiwagi, K. Mori, Evolutionary relationships among *Aspergillus oryzae* and related species based on the sequences of 18S rRNA genes and internal transcribed spacers, J. General Appl. Microbiol. 44 (1998) 225–230.

[34] L. Parenicova, P. Skouboe, J.C. Frisvad, R.A. Samson, L. Rossen, M. ten Hoor-Suykerbuyk, J. Visser, Combined molecular and biochemical approach identifies *Aspergillus japonicus* and *A. aculeatus* as two species, Appl. Environ. Microbiol. 67 (2001) 521–527.

[35] S.W. Peterson, Phylogenetic relationships in Aspergillus based on rDNA sequence analysis, In: R.A. Samson, J.I. Pitt (Eds.), Integration of Modern Taxonomic Methods for *Penicillium* and *Aspergillus* Classification, Harwood Academic Publishers, Amsterdam, 2000, pp. 323–355.

[36] S.W. Peterson, Y. Ito, B.W. Horn, T. Goto, *Aspergillus bombycis*, a new aflatoxigenic species and genetic variation in its sibling species, *A. nomius*, Mycologia 93 (2001) 689–703.

[37] J.I. Pitt, A.D. Hocking, D.R. Glenn, An improved medium for detection of *Aspergillus flavus* and *A. parasiticus*, J. Appl. Bacteriol. 54 (1983) 109–114.

[38] C.J. Rabie, A. Lübben, M. Steyn, Production of sterigmatocystin by *Aspergillus versicolor* and *Bipolaris sorokiniana* on semisynthetic liquid and solid media, Appl. Environ. Microbiol. 32 (1976) 206–208.

[39] K.B. Raper, D.I. Fennell, The Genus Aspergillus, Williams and Wilkins, 1965.

[40] K. Rigó, J. Varga, B. Tóth, J. Téren, À. Mesterházy, Z. Kozakiewicz, Evolutionary relationships within Aspergillus section Flavi based on sequences on the intergeneric transcribed spacer regions and the 5.8S rRNA gene, J. General Appl. Microbiol. 48 (2002) 9–16.

[41] M. Saito, O. Tsuruta, A new variety of *Aspergillus flavus* from tropical soil in Thailand and its aflatoxin productivity, Proc. Jpn. Assoc. Mycotoxicol. 37 (1993) 31–36.

[42] M. Saito, O. Tsuruta, P. Siriacha, S. Kawasugi, M. Manabe, Atypical strains of *Aspergillus flavus* isolated in maize fields– Aflatoxin-producing ability and distribution in Thailand, J. Agri. Res. Quart. 23 (1989) 151–154.

[43] R.A. Samson, A compilation of the Aspergilli described since 1965, Stud. Mycol. (Baarn) 18 (1979) 1–38.

[44] R.A. Samson, J.C. Frisvad, *Penicillium* subgenus *Penicillium*: new taxonomic schemes, mycotoxins and other extrolites, Studies in Mycology 49 (2004) 1–257.

[45] R.A. Samson, E.S. Hoekstra, J.C. Frisvad (Eds.), Introduction to Food- and Airborne Fungi, Seventh ed, Centraalbureau voor Schimmelcultures, Utrecht, 2004.

[46] S. Sekita, K. Yoshihira, S. Natori, S. Udagawa, Y. Sugiyama, H. Kurata, M. Umeda, The bioproduction of mycotoxins by *Chaetomium* species, Maikotokishin (Tokyo) 13 (1981) 46–48.

[47] P. Skouboe, J.C. Frisvad, D. Lauritsen, M. Boysen, J.W. Taylor, L. Rossen, Nucelotide sequences from the ITS region of *Penicillium* species, Mycol. Res. 103 (1999) 1873–1881.

[48] J. Smedsgaard, Micro-scale extraction procedure for standardized screening of fungal metabolite production in cultures, J. Chromatograp. A 760 (1997) 264–270.

[49] Sun, Zeng-Mei, Qi. Zu-tong, A new aflatoxin producing species of sect. *Flavi* of *Aspergillus*, Acta Mycolog. Sin. 10 (1991) 22–26.

[50] H. Takahashi, H. Kamimura, M. Ichinoe, Distribution of aflatoxin-producing *Aspergillus flavus* and *Aspergillus parasiticus* in sugarcane fields in the southernmost islands of Japan, J. Food Protect. 67 (2004) 90–95.

[51] S. Udagawa, New or noteworthy ascomycetes from Southeast Asian soils. I, Trans. Mycol. Soc. Japan 21 (1980) 17–34.

[52] S. Udagawa, T. Muroi, H. Kurata, S. Sekita, K. Yoshihira, S. Natori, The production of chaetoglobosins, sterigmatocystin, O-methylsterigmatocystin and

chaetocin by *Chaetomium* spp. and related fungi, Can. J. Microbiol. 25 (1979) 170–177.
[53] S. Udagawa, T. Muroi, H. Kurata, S. Sekita, K. Yoshihira, S. Natori, *Chaetomium udagawae*, a new producer of sterigmatocystin, Trans. Mycol. Soc. Japan 20 (1979) 475–480.
[54] W. Van Waalbeek, P.M. Scott, F.S. Thatcher, Mycotoxins from some food-borne fungi, Can. J. Microbiol. 14 (1968) 131–137.
[55] J. Varga, K. Rigó, J. Tóth, J. Téren, Z. Kozakiewicz, Evolutionary relationships among *Aspergillus* species producing economically important mycotoxins, Food Technol. Biotechnol. 41 (2003) 20–36.
[56] Z.-G. Wang, T. Zhe, S.Y. Cheng, L.-M. Cong, Study of pectinase and sclerotium producing abilities of two kinds of *Aspergillus flavus* isolates from Zhejiang, Mycopathologia 121 (1993) 163–168.
[57] D.T. Wicklow, R.F. Vesonder, C.E. McAlpin, R.J. Cole, M. Roquebert, Examination of *Stilbothamnium togoense* for *Aspergillus flavus* group mycotoxins, Mycotaxon 34 (1989) 249–252.