Polyphasic taxonomy of Aspergillus section Usti

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Abstract: Aspergillus ustus is a very common species in foods, soil and indoor environments. Based on chemical, molecular and morphological data, A. insuetus is separated from A. ustus and revived. A. insuetus differs from A. ustus in producing drimans and ophiobolin G and H and not producing usitic acid and austocystins. The molecular, physiological and morphological data also indicated that another species, A. keveii sp. nov. is closely related but distinct from A. insuetus. Aspergillus section Usti sensu stricto includes 8 species: A. ustus, A. punicicus, A. granulosus, A. pseudodefectus, A. caldoustus, A. insuetus and A. keveii together with Emericella heterothallica.

Taxonomic novelties: Aspergillus insuetus revived, Aspergillus keveii sp. nov.

Key words: actin, Aspergillus, β-tubulin, calmodulin, extrolite profiles, ITS, phylogenetics, polyphasic taxonomy.

INTRODUCTION

Aspergillus ustus is a very common filamentous fungus found in foods, soil and indoor air environments (Samson et al. 2002). This species is considered as a rare human pathogen that can cause invasive infection in immunocompromised hosts. However, A. ustus has been noted increasingly as causes of invasive aspergillosis in tertiary care centres in the US (Malani & Kaufman 2007). Up to date, 22 invasive aspergillosis cases have been reported to be caused by A. ustus (Verweij et al. 1999; Pavie et al. 2005; Panackal et al. 2006; Yildiran et al. 2008). Several studies indicate that A. ustus isolates are resistant to amphotericin B, echinocandins and azole derivatives (Verweij et al. 1999; Pavie et al. 2005; Gene et al. 2001; Garcia-Martos et al. 2005). Other species related to A. ustus can also cause human or animal infections. Aspergillus granulosus was found to cause disseminated infection in a cardiac transplant patient (Fakhil et al. 1995), while A. deflectus has been reported to cause disseminated mycosis in dogs (Robinson et al. 2000; Kahler et al. 1990; Jang et al. 1986).

A. ustus is a variable species. Raper & Fennell (1965) stated that "not a single strain can be cited as wholly representative of the species as described". Indeed, A. ustus isolates may vary in their colony colour from mud brown to slate grey, with colony reverse colours from uncoloured through yellow to dark brown (Raper & Fennell 1965; Kozakiewicz 1989). Molecular data also indicate that this species is highly variable; RAPD analysis carried out in various laboratories could be used to detect clustering of the isolates (Rath et al. 2002; Panackal et al. 2006), and sequence analysis of parts of the ribosomal RNA gene cluster also detected variability within this species (Henry et al. 2000; Peterson 2000; Hinikson et al. 2005).

We examined a large set of A. ustus isolates and related species originating from environmental and clinical sources to clarify the taxonomic status of the species, and to clarify the taxonomy of Aspergillus section Usti. The methods used include sequence analysis of the ITS region (intergenic spacer region and the 5.8 S rRNA gene of the RNA gene cluster), and parts of the β-tubulin, calmodulin and actin genes, analysis of extrolite profiles, and macro- and micromorphological analysis of the isolates.

MATERIALS AND METHODS

Morphological examination. The strains examined are listed in Table 1. Both clinical and environmental strains were grown as 3-point inoculations on Czapek yeast agar (CYA), malt extract agar (MEA), creatine agar (CREA) and yeast extract sucrose agar (YES) at 25 °C, and on CYA at 37 °C for 7 d (medium compositions according to Samson et al. 2004). For micro morphological examination light microscopy (Olympus BH2 and Zeiss Axioskop 2 Plus) was employed.

Extrolite analysis. Extrolites were analysed by HPLC using alkylphenone retention indices and diode array UV-VIS detection as described by Frisvad & Thrane (1987), with minor modifications as described by Smedsgaard (1997). Standards of ochratoxin A and B, aflavamine, asperazine, austamide, austdil, kotanin and other extrolites from the collection at BioCentrum-DTU were used to compare with the extrolites from the species under study.

Isolation and analysis of nucleic acids. The cultures used for the molecular studies were grown on malt peptone (MP) broth using 10 % (v/v) of malt extract broth (Brix 10) and 0.1 % (w/v) bacto peptone (Difco), 2 mL of medium in 15 mL tubes. The cultures were incubated at 25 °C for 7 d. DNA was extracted from the cells using the Masterpure™ yeast DNA purification kit (Epicentre Biotechnol.) according to the instructions of the manufacturer. Fragments containing the ITS region were amplified using primers ITS1 and ITS4 as described previously (White et al. 1990). Amplification of part of the β-tubulin gene was performed using the primers Bt2a and Bt2b (Glass 1995). Amplifications of the partial calmodulin and actin genes were set up as described previously (Hong et al. 2005). Sequence analysis was performed with the Big Dye Terminator...
Table 1. Isolates in Aspergillus section Usti and related species examined in this study.

| Species          | Strain No.   | Source                                                                                           |
|------------------|--------------|--------------------------------------------------------------------------------------------------|
| A. calidoustus   | CBS 112452   | Indoor air, Germany                                                                               |
| A. calidoustus   | CBS 113228   | ATCC 38849; IBT 13091                                                                             |
| A. calidoustus   | CBS 114380   | Wooden construction material, Finland                                                              |
| A. calidoustus   | CBS 121601   | Bronchoalveolar lavage fluid, proven invasive aspergillosis, Nijmegen, The Netherlands†           |
| A. calidoustus   | CBS 121602   | Bronchial secretion, proven invasive aspergillosis, Nijmegen, The Netherlands†                     |
| A. calidoustus   | CBS 121589   | Autopsy lung tissue sample, proven invasive aspergillosis, Nijmegen, The Netherlands†              |
| A. calidoustus   | CBS 121603   | Elevator shaft in hospital, Nijmegen, The Netherlands                                              |
| A. calidoustus   | CBS 121604   | Patient room, Nijmegen, The Netherlands                                                           |
| A. calidoustus   | CBS 121605   | Laboratory, Nijmegen, The Netherlands                                                             |
| A. calidoustus   | CBS 121606   | Sputum, Nijmegen, The Netherlands                                                                |
| A. calidoustus   | CBS 121607   | Feces, Nijmegen, The Netherlands                                                                  |
| A. calidoustus   | CBS 121608   | Bronchoalveolar lavage, Nijmegen, The Netherlands                                                  |
| A. calidoustus   | 7843         | Pasteur Institute, Paris, France                                                                  |
| A. calidoustus   | 8623         | Oslo, Norway                                                                                      |
| A. calidoustus   | 9331         | Mouth wash, Nijmegen, The Netherlands                                                              |
| A. calidoustus   | 9371         | Mouth wash, Nijmegen, The Netherlands                                                              |
| A. calidoustus   | 9420         | Bronchial secretion, Nijmegen, The Netherlands                                                     |
| A. calidoustus   | 9692         | Hospital ward, Nijmegen, The Netherlands                                                           |
| A. calidoustus   | V02-46       | Tongue swab, Nijmegen, The Netherlands                                                            |
| A. calidoustus   | V07-21       | Bronchial secretion, Nijmegen, The Netherlands                                                     |
| A. calidoustus   | V17-43       | Bronchial secretion, Nijmegen, The Netherlands                                                     |
| A. calidoustus   | V22-60       | Skin biopsy, Nijmegen, The Netherlands                                                             |
| A. calidoustus   | CBS 121609   | Post-cataract surgery endophthalmitis, Turkey                                                      |
| A. calidoustus   | 907          | Post-cataract surgery endophthalmitis, Turkey                                                      |
| A. calidoustus   | 908          | Post-cataract surgery endophthalmitis, Turkey                                                      |
| A. calidoustus   | 64           | Post-cataract surgery endophthalmitis, Turkey                                                      |
| A. calidoustus   | 67           | Post-cataract surgery endophthalmitis, Turkey                                                      |
| A. calidoustus   | CBS 121610   | Post-cataract surgery endophthalmitis, Turkey                                                      |
| A. calidoustus   | 351          | Osteorickets                                                                                      |
| A. calidoustus   | 482          | Post-cataract surgery endophthalmitis, Turkey                                                      |
| A. calidoustus   | CBS 121611   | Patient 4, Washington, U.S.A.                                                                     |
| A. calidoustus   | CBS 121616   | Environmental, Washington, U.S.A.                                                                 |
| A. calidoustus   | FH 165       | Patient 5b, Washington, U.S.A.                                                                    |
| A. calidoustus   | CBS 121614   | Patient 5a, Washington, U.S.A.                                                                    |
| A. calidoustus   | CBS 121615   | Patient 6, Washington, U.S.A.                                                                     |
| A. calidoustus   | CBS 121613   | Patient 2, Washington, U.S.A.                                                                     |
| A. calidoustus   | CBS 121612   | Patient 1, Washington, U.S.A.                                                                     |
| A. calidoustus   | FH 91        | Patient 1a, Washington, U.S.A.                                                                    |
| A. calidoustus   | NRRL 26162   | Culture contaminant, Peoria, U.S.A.                                                                |
| A. calidoustus   | NRRL 281     | Thom 5634                                                                                          |
| A. calidoustus   | NRRL 277     | Thom 5698.754, Green rubber                                                                       |
| A. granulosus    | CBS 588.65†  | Soil, Fayetteville, Arkansas, U.S.A.                                                                |
| A. granulosus    | CBS 119.58   | Soil, Texas, U.S.A.                                                                               |
| A. granulosus    | IBT 23478 = WB 1932 = IMI 017278iii = CBS 588.65         | Soil, Fayetteville, Arkansas, U.S.A.                                                                |
| A. inaeutus      | CBS 107.25†  | South Africa                                                                                      |
| A. inaeutus      | CBS 119.27   | Unknown                                                                                           |
| A. inaeutus      | CBS 102278   | Subcutaneous infection left forearm and hand of 77-year-old woman                                  |
| A. keveii        | CBS 209.92   | Soil, La Palma, Spain                                                                             |
| A. keveii        | CBS 561.65   | Soil, Panama                                                                                      |
| A. keveii        | IBT 10524 = CBS 113227 = NRRL 1254                   | Soil, Panama                                                                                      |
were performed by using CLUSTAL-X (Thompson software package Seqman from DNAStar Inc. Sequence alignments Data analysis. The sequence data was optimised using the
filtration through Sephadex G-50 (Amersham Pharmacia Biotech, Biosystems). All the sequencing reactions were purified by gel
sequences were aligned with the MT Navigator software (Applied
Cycle Sequencing Ready Reaction Kit for both strands, and the
sequences were deposited in the GenBank under accession numbers EU076344–EU76377.

Polyphasic taxonomy of Aspergillus section Usti

| Species       | Strain No.          | Source                                      |
|---------------|---------------------|---------------------------------------------|
| A. keveii     | IBT 16751 = DMG 153 | Galápagos Islands, Ecuador, D.P. Mahoney    |
| A. pseudodeflectus | CBS 596.65          | Sugar, U.S.A.                               |
| A. pseudodeflectus | CBS 756.74          | Desert soil, Egypt, Western Desert         |
| A. puniceus   | CBS 122.33          | Unknown                                     |
| A. puniceus   | 9377                | Mouth wash, Nijmegen, Netherlands           |
| A. puniceus   | V41-02              | Faeaces, Nijmegen, Netherlands              |
| A. puniceus   | NRRL 29173          | Indoor air, Saskatoon, Canada               |
| A. puniceus   | CBS 495.65          | Soil, Zarcero Costa Rica                    |
| A. puniceus   | CBS 128.62          | Soil, Louisiana, U.S.A.                     |
| A. ustus      | CBS 116057          | Antique tapestries, Krakow, Poland          |
| A. ustus      | CBS 114901          | Carpet, The Netherlands                     |
| A. ustus      | CBS 261.67          | Culture contaminant, U.S.A.                 |
| A. ustus      | CBS 133.55          | Textile buried in soil, Netherlands         |
| A. ustus      | CBS 239.90          | Man, biopsy of brain tumor, Netherlands      |
| A. ustus      | CBS 113233          | IBT 14495                                   |
| A. ustus      | CBS 113232          | IBT 14932                                   |
| A. ustus      | NRRL 285            | Soil, Iowa, U.S.A.                         |
| A. ustus      | NRRL 280            | Bat dung, Cuba                              |
| A. ustus      | NRRL 1609           | Bat dung, Cuba                              |
| A. ustus      | NRRL 29172          | Indoor air, Edmonton, Canada                |
| E. heterothallica | CBS 489.65          | soil, Costa Rica                           |
| E. heterothallica | CBS 488.65          | soil, Costa Rica                           |

†These samples were taken from the same patient (Verweij et al. 1999)

Cycle Sequencing Ready Reaction Kit for both strands, and the sequences were aligned with the MT Navigator software (Applied Biosystems). All the sequencing reactions were purified by gel filtration through Sephadex G-50 (Amer sham Pharmacia Biotech, Piscataway, NJ) equilibrated in double-distilled water and analyzed on the ABI PRISM 310 Genetic Analyzer (Applied Biosystems).

Phylogenetic analyses

RESULTS

For the molecular analysis, four genomic regions, the ITS region, and parts of the actin, calmodulin and β-tubulin genes were amplified and sequenced. Phylogenetic analysis of the data was carried out using the neighbour-joining technique and parsimony analysis. The trees obtained by the different approaches were identical, neighbour-joining trees based on the different data sets are shown in Figs 1–4. During analysis of part of the β-tubulin gene, 487 characters were analyzed, 111 of which were found to be parsimony informative. The topology of the tree is the same as that of one of the more than 104 maximum parsimony trees constructed by the PAUP program (length: 216 steps, consistency index: 0.8148, retention index: 0.9679). The calmodulin data set included 474 characters, with 172 parsimony informative characters (1 MP tree, tree length: 360, consistency index: 0.8083, retention index: 0.9550). The actin data set included 406 characters, with 161 parsimony informative characters (3 MP trees, tree length: 292, consistency index: 0.8870, retention index: 0.9633). The ITS data set included 482 characters, 26 of which were parsimony informative (>104 MP trees, tree length: 71, consistency index: 0.9155, retention index: 0.9781).

Molecular data revealed that Aspergillus section Usti consists of eight species: A. ustus, A. puniceus, A. granulosus, A. pseudodeflectus, A. calidoustus, A. insuetus and a new species including CBS 209.92 and some other isolates. We propose the name A. keveii sp. nov. for this set of isolates. The trees based on ITS, calmodulin and β-tubulin sequence data indicated that also E. heterothallica belongs to this section, although actin sequence data did not support this finding.
Fig. 1. Neighbour-joining tree based on β-tubulin sequence data of Aspergillus section Usti. Numbers above branches are bootstrap values. Only values above 70% are indicated.
Fig. 2. Neighbour-joining tree based on calmodulin sequence data of Aspergillus section Ust. Numbers above branches are bootstrap values. Only values above 70% are indicated.
Fig. 3. Neighbour-joining tree based on actin sequence data of *Aspergillus* section *Usti*. Numbers above branches are bootstrap values. Only values above 70% are indicated.
Fig. 4. Neighbour-joining tree based on ITS sequence data of *Aspergillus* section *Usti*. Numbers above branches are bootstrap values. Only values above 70 % are indicated.
**Table 2. Overview of morphological criteria to differentiate between the members of Aspergillus section Usti.**

| Species          | CYA37 (mm) | YES | Ehrlich reaction | Reaction on CREA | Conidial colour on MEA** |
|------------------|------------|-----|------------------|------------------|-------------------------|
| A. ustus         | No growth  | 43–49 | None             | Good growth, faint yellow mycelium | Hair brown |
| A. punicus       | No growth  | 48–53 | None             | Moderate to good growth, yellow mycelium | Olive brown |
| A. calidoustus   | 20–35      | 36–41 | Violet           | Weak to moderate growth, hyaline mycelium | Brownish grey |
| A. insuetus      | No growth  | 23–30 | Violet           | Good growth, hyaline mycelium | (Brownish) grey to light grey |
| A. keveii        | No growth  | 40–46 | Violet*          | Good growth, hyaline mycelium | Brownish grey / pinkish brown |
| A. pseudodeflectus | 15–20      | 20–30 | None             | Weak to moderate growth, hyaline mycelium | No sporulation |
| A. granulosus    | 30–35      | 35–40 | Violet           | Weak growth, hyaline mycelium | Buff to greyish brown |
| E. heterothallica| 5–10       | 38–42 | None             | Weak growth, bright yellow mycelium | No sporulation |

* All have violet reaction, except CBS 113227
** Colour according Methuen handbook of colours

**Morphological and physiological studies**

Phenotypic comparison of the different members of the section *Usti* showed that eight taxa could be distinguished. Various characters showed to be valuable for differentiation (see also Table 2). One of the main criteria is the growth rate on CYA at 37 °C. *A. calidoustus, A. pseudodeflectus* and *A. granulosus* had high growth rates at this temperature, while *E. heterothallica* only grew restrictedly. The other members of this section were unable to grow at 37 °C, which reduces the potential of these species to become opportunistic human pathogens. The growth rate and the mycelium colour on creatin agar (CREA) also proved to be a good tool to differentiate between the species examined. Some species, like *A. ustus, A. punicus, A. insuetus* and *A. keveii* have a good growth on this medium. Since sporulation on this medium is often inhibited, this medium was also useful to determine the colour of the mycelium. The colours varied from bright yellow by *A. punicus* and *E. heterothallica* to faint yellow in *A. ustus* to colourless in the other species. Another useful character was the use of the Ehrlich test to detect the presence of indol metabolites. This feature gave, with the exception of *A. keveii*, very clear-cut results. Besides these features, the colony diam on YES was also suitable to differentiate between *A. insuetus* and the other species.

**Extrolite profiles**

*Aspergillus ustus* has been claimed to produce a range of extrolites including austidiol (Vlieggaar et al. 1974), austocystins (Steyn & Vlieggaar 1974; Kfir et al. 1986), brevianamide A (Steyn 1973), sterigmatocystin (Rabie et al. 1977), austalides (de Jesus et al. 1987), austamide (Steyn 1971), dehydroaustin (Scott et al. 1986), pergillin (Cutler et al. 1980), dehydropergillin (Cutler et al. 1981), phenylahistin (Kanoh et al. 1997), ophiobolins G & H (Cutler et al. 1984), dimrains (Hayes et al. 1996), diacetoxyscirpenol (Tuomi et al. 2000) and ustic acid (Raistrick & Strickings 1951).

The mycotoxins and other extrolites found to be produced by the examined species in this study are listed in Table 3. Species assigned to section *Usti* could clearly be divided in three chemical groups based on the extrolites produced by them. *A. ustus, A. granulosus* and *A. punicus* produced ustic acids in common. *A. ustus* and *A. punicus* also produced austocystins and versicolorins. In the second chemical group, *A. pseudodeflectus* produced dimrains (Hayes et al. 1996) in common with the other species in this group, and also several unique unknown compounds. *A. calidoustus* isolates produced drimans and ophiobolins in common with *A. insuetus* and *A. keveii*, but also produced austins not identified in other species of section *Usti*. *A. insuetus* isolates also produced pergillin, while *A. keveii* together with some other isolates produced nidulol. In the third chemical group, *E. heterothallica* has been reported to produce emethallicins A–F (Kawahara et al. 1989, 1990a, 1990b), 5’-hydroxyaveranthin (Yabe et al. 1991), emetherone (Kawahara et al. 1988), emesterones A & B (Hosoe et al. 1998), 5’-hydroxyaveranthin (Yabe et al. 1991), Mer-NF8054X (Mizuno et al. 1995). This latter compound is an 18,22-cyclosterol derivative, and was also identified in an *A. ustus* isolate (Mizuno et al. 1995). Apart from this chemical similarity *Emericella heterothallica* appear to be quite different from the anamorphic species in section *Usti*, in agreement with actin sequencing data. Austamide, deoxybrevianamide E and austadiol could not be detected in any of the strains examined here and the strain producing these mycotoxins should be reexamined.

Comparing the extrolites profiles of section *Usti* with other sections within subgenus *Nidulantes*, nidulol and versicolorins are also produced by members of sections *Versicolores* and *Nidulantes* (Cole & Schweikert 2003). Interestingly, versicolorins and 5’-hydroxyaveranthin are intermediates of the aflatoxin biosynthetic pathway and also produced by species assigned to *Aspergillus* section *Flavi* and *Ochraceorosei* (Yabe et al. 1991; Frisvad et al. 2005). However, while the versicolorins are precursors of sterigmatocystin in section *Ochraceorosei, Versicolores* and *Nidulantes*, they are precursors of austocystins in section *Usti*.

Section *Usti* contains the only *Aspergillus* species known to produce pergillins, ophiobolins, austins, austocystins, ustic acids, dimrains, Mer-NF8054X, austalides, deoxybrevianamides and austamide and thus this section is chemically unique. We have not examined the species for production of emethallicins, emesterones and emeheterones, as standards of these compounds were not available.

**DISCUSSION**

Raper and Fennell (1965) classified *A. ustus* in the *Aspergillus ustus* group together with four other species: *A. panamensis, A. punicus, A. conjunctus* and *A. deflectus*. Later, Kozakiewicz (1989) revised the taxonomy of the group, and included *A. ustus,*
Table 3. Extrolites produced by species assigned to Aspergillus section Usti.

| Species                  | Extrolites produced                                                                 |
|--------------------------|------------------------------------------------------------------------------------|
| Chemical group I         |                                                                                     |
| A. ustus                 | Ustic acids, austostacins (and versicolorins), austalides, a compound related to sterigmatocystin, nidulol |
| A. granulosus            | Ustic acids, a compound resembling sterigmatocystin, nidulol, drimans                |
| A. purniceus             | Ustic acids, austostacins (and versicolorins), phenylahistin, a compound related to sterigmatocystin, nidulol |
| Chemical group II        |                                                                                     |
| A. pseudodeflectus       | Drimans, unknown compounds                                                           |
| A. calidoustus           | Drimans, ophiobolins G and H, austins                                              |
| A. insuetus              | Drimans, ophiobolins G and H, pergillin-like                                         |
| A. keveii                | Drimans, ophiobolins G and H, nidulol                                               |
| Chemical group III       |                                                                                     |
| E. heterothallica        | Emethallicins A, B, C, D, E & F, emeheterone, emesterones A & B, 5’-hydroxyaveranthin, Mer-NF8054X, sterigmatocystin, versicolorins |

A. pseudodeflectus, A. conjunctus, A. purniceus, A. panamensis and A. granulosus into the A. ustus species group, and established the A. deflectus group including A. deflectus, A. pulvinus and A. silvaticus based on morphological studies. Klich (1993) treated A. granulosus as member of section Versicillia, and found that A. pseudodeflectus is only weakly related to this section based on morphological treatment of section Versicillia. Peterson (2000) transferred most species of section Usti to section Nidulantes based on sequence analysis of part of the 28S rRNA gene. On his cladogram, A. ustus, A. pseudodeflectus, A. granulosus and A. purniceus form a well-supported branch closely related to A. versicolor and its allies, while A. deflectus is on another branch related to A. elongatus and A. lucknowensis. Peterson (2000) transferred A. conjunctus, A. funiculosus, A. silvaticus, A. panamensis and A. anthodesmis to section Sparsi. Recently Varga et al. (submitted) studied large numbers of isolates from clinical and other sources using molecular, morphological and physiological approaches. Phylogenetic analysis of partial β-tubulin, calmodulin, actin and ITS sequences indicated that none of the clinical isolates recognised previously as A. ustus belong to the A. ustus species. All but two of these isolates formed a well-defined clade related to A. pseudodeflectus based on sequence analysis of protein coding regions. Morphological and physiological examination of the isolates indicated that they are able to grow above 37 °C, in contrast with A. ustus isolates, and give a positive Ehrlich reaction, in contrast with related species including A. granulosus, A. ustus, and A. pseudodeflectus. These isolates were described as A. calidoustus.

Aspergillus ustus (Bainier) Thom & Church was redescribed by Thom & Church (1926) based on Sterigmatocystis ustus Bainier. In this manual, A. insuetus (Bainier) Thom & Church was also accepted based on S. insuta (Bainier) Thom & Chuch (1926), but later A. insuetus was abandoned (Thom and Raper, 1945) and included in the broad description of A. ustus in Raper and Fennell (1965). Our studies clarified that A. insuetus is a valid species which can be distinguished from A. ustus and other species assigned to Aspergillus section Usti. A. insuetus could be separated from the other members of the section Usti by various phenotypic characters. The most important one is the slower growth rate on YES agar and clear differences in extrolite profiles (Table 2). This finding was supported by all the different data sets used to characterise section Usti. The molecular data showed that this species is more related to A. calidoustus and A. pseudodeflectus than A. ustus. Also different extrolite patterns were observed. There were many differences between A. ustus and A. insuetus, and, like the molecular data, this species was mostly related to A. calidoustus and A. pseudodeflectus. The main difference between the latter species was the production of a pergillin-like compound by A. insuetus (Table 3).

Our polyphasic taxonomic approach revealed that Aspergillus section Usti includes eight species: A. ustus, A. purniceus, A. granulosus, A. pseudodeflectus, A. calidoustus, A. insuetus and A. keveii sp. nov. The phylogenetic trees based on ITS, calmodulin and β-tubulin sequence data indicated that E. heterothallica also belongs to this section. This species has similar morphology of the conidiophores and Hülle cells. In our study we were not able to observe ascospores by crossing the two mating strains but these are described by Raper and Fennell (1965: 502–503).
**Aspergillus calidoustus** Varga et al. Eukaryotic Cell submitted. Fig. 5.

**Type:** CBS 121604 from human, Netherlands

**Other no. of the type:** strain 677

**Description strain**
Colony diam, 7 d, in mm: CYA25 27–32; CYA37 20–35; MEA25 35–48; YES 36–41
Colony colour on CYA: blond/greyish yellow, brownish grey or greyish brown
Conidiation on CYA: abundant
Reverse colour (CYA): yellow with beige or olive brown centre
Colony texture: floccose
Conidial heads: loosely columnar
Stipe: 150–300 × 4–7 µm, smooth, brown
Vesicle diam/shape: 9–15 µm, pyriform to broadly spathulate
Conidium size/shape/surface texture: 2.7–3.5 × µm, globose, very rough ornamentation (0.5–0.8 µm high), inner and outer wall visible
Hülle cells: sparsely produced, irregularly elongated, in scattered groups
Ehrlich reaction: violet
Growth on creatine: weak to moderate growth with hyaline mycelium, no acid production

**Diagnostic features:**
- good growth at 37 °C, violet Ehrlich reaction, coarsely roughened to echinulate conidia

**Cultures examined:**
- CBS 121589, 121601–121616

**Similar species:**
- *A. pseudodeflectus*

**Distribution:**
- U.S.A., Turkey, Finland, Germany, Netherlands

**Ecology and habitats:**
- indoor air, rubber, construction material, human

**Extrilites:**
- Drimans, ophiobolins G and H, austins

**Pathogenicity:**
- pathogenic to humans (Verweij et al. 1999; Weiss & Thiemke 1983; Pavie et al. 2005; Panackal et al. 2006; Yildiran et al. 2006; Iwen et al. 1998)

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**Aspergillus granulosus** Raper & Thom, Mycologia 36: 565. 1944. Fig. 6.

**Type:** CBS 588.65, from soil, Fayetteville, Arkansas, U.S.A.

**Other no. of the type:** ATCC 16837, NRRL 279; NRRL 1726; Thom No. 4658.245

**Description**
Colony diam, 7 d, in mm: CYA 28–32; CYA37 no growth; MEA25 36–41; YES 23–30
Colony colour: almost black in center, shading through gray to white sterile floccose marginal areas
Conidiation on CYA: moderate to good
Reverse colour (CYA): yellow olive to blackish brown with age
Colony texture: floccose
Conidial head: radiate to hemispherical
Stipe: 300 × 4–8 µm, smooth, brown
Vesicle diam/shape: 11–16 µm, hemispherical to subglobose
Conidium size/shape/surface texture: 3.2–4 µm, globose, distinct roughened and inner and outer wall visible, fulgineous, the colour mostly aggregated into echinulations of the cell-wall, and even forming bars and tubercules at times
Hülle cells: variously coiled or curved, in scattered groups
Ehrlich reaction: violet
Growth on creatine: good growth with hyaline mycelium, no acid production

**Cultures examined:**
- CBS 119.58, CBS 588.65, IBT 23478

**Similar species:**
- *A. keveii*

**Distribution:**
- South Africa, Spain

**Ecology and habitats:**
- soil

**Extrilites:**
- Drimans, ophiobolins G and H, pergillin-like

**Pathogenicity:**
- caused subcutaneous infection (Gené et al. 2001)

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**Aspergillus insuetus** (Bainier) Thom & Church, Manual of the aspergilli: 153. 1929. Fig. 7. = *Sterigmatocystis insueta* Bainier (1908)

**Type:** CBS 107.25, from South Africa, Sartory

**Other no. of the type:** ATCC 1033; IFO 4128; NRRL 279; NRRL 1726; Thom No. 4658.245

**Description**
Colony diam, 7 d, in mm: CYA 28–32; CYA37 no growth; MEA25 36–41; YES 23–30
Colony colour: almost black in center, shading through gray to white sterile floccose marginal areas
Conidiation on CYA: moderate to good
Reverse colour (CYA): yellow olive to blackish brown with age
Colony texture: floccose
Conidial head: radiate to hemispherical
Stipe: 300 × 4–8 µm, smooth, brown
Vesicle diam/shape: 11–16 µm, hemispherical to subglobose
Conidium size/shape/surface texture: 3.2–4 µm, globose, distinct roughened and inner and outer wall visible, fulgineous, the colour mostly aggregated into echinulations of the cell-wall, and even forming bars and tubercules at times
Hülle cells: variously coiled or curved, in scattered groups
Ehrlich reaction: violet
Growth on creatine: good growth with hyaline mycelium, no acid production

**Cultures examined:**
- CBS 107.25, CBS 119.27, CBS 102278

**Similar species:**
- *A. keveii*

**Distribution:**
- South Africa, Spain

**Ecology and habitats:**
- soil

**Extrilites:**
- Drimans, ophiobolins G and H, pergillin-like

**Pathogenicity:**
- caused subcutaneous infection (Gené et al. 2001)
Fig. 5. Aspergillus calidoustus. A–B. Colonies at 25 °C after 7 d. A. CYA. B. MEA. C–E, G–H Conidiophores. F. Hülsie cells. I. Conidia. Scale bars = 10 µm, except F = 30 µm.
Fig. 6. Aspergillus granulosus. A–B. Colonies at 25 °C after 7 d. A. CYA. B. MEA. C–H Conidiophores. I. Conidia. Scale bars = 10 µm, except C = 30 µm.
Fig. 7. Aspergillus insuetus. A–B. Colonies at 25 °C after 7 d. A. CYA. B. MEA. C–H Conidiophores. I. Conidia. Scale bars = 10 μm, except C = 30 μm.
Aspergillus keveii sp. nov. Varga, Frisvad & Samson – MycoBank MB505570. Fig. 8.

Holotype of Aspergillus keveii, here designated as CBS 209.92* (dried culture) isolated from soil, Las Palmas, Spain.

Colonies in 7 diebus et 25 °C in agar MEA 36–41 mm, in CYA 30–39 mm, in YES 40–46 mm, in CREA 25–32 mm diam; auctus in 7 diebus et 37 °C in agar CYA nullus. Sporulatio in CYA abundans; colonia brunneognisea vel subroseobrunnea; textura coloniae floccosa; colonia reversa flavide olivaceobrunnea vel atrobrunnea. Capitula condiaia laxe columnaria; stipples 150–300 × 4–6 µm, parte laevi, brunneo; vesiculas pyrniformes, 9–13 µm in lat., biseriatae; metulae 4.7–6.7 × 2.8–3.6 µm; phialides 5.7–7 × 2–3 µm; conidia globose, 2.4–2.6 µm diam., ornamento exasperato vel echinulato. Cellulae "hülle" irregulariter elongatæ, (10–)25–40(–65) µm in long., in cumulis dispersis.

Colonies on MEA 36–41 mm, on CYA 30–39 mm, on YES 40–46 mm, on CREA 25–32 mm in diam. in 7 d at 25 °C, no growth on CYA after 7 d at 37 °C. Conidial heads abundant on CYA, colony colour brownish grey to pinkish brown, colony texture floccose, reverse yellow olive brown to dark brown. Conidial heads loosely columnar; stipes 150–300 × 4–6 µm, smooth walled, brown in colour; vesicles 9–13 µm wide, pyriform, biseriate; metulae covering the upper half to three-fourths of the vesicle, measuring 4.7–6.7 × 2.8–3.6 µm µm; phialides 5.7–7 × 2–3 µm; conidia globose 2.4–2.8 µm, coarsely roughened to echinulate. Hülle cells (10–)25–40(–65) µm in long., irregularly elongated, produced in scattered groups.

Etymology: named after Prof. Ferenc Kevei, eminent mycologist devoting his life to Aspergillus research.

Type: CBS 209.92

Ehrlich reaction: violet, with exception of CBS 113227

Growth on creatine: good growth with hyaline mycelium, no or weak acid production

Diagnostic features: no growth at 37 °C, good growth on CREA and YES, coarsely roughened to echinulate conidia; Hülle cells in scattered groups, violet Ehrlich reaction

Cultures examined: CBS 561.65, CBS 209.92 and CBS 113227

Similar species: A. insuetus

Distribution: U.S.A., Turkey, Finland, Germany, Netherlands

Ecology and habitats: indoor air, rubber, construction material, human

Extrudites: Drimans, ophioholins G and H, nidulol

Pathogenicity: not reported

Notes: CBS 113227 is deviating in having larger conidial heads and small (2.6 µm), finely roughened pinkish brown coloured conidia

Aspergillus pseudodeflectus Samson & Mouchacca, Antonie van Leeuwenhoek 41(3): 325. 1975. Fig. 9.

Type: CBS 756.74, from desert soil, Western Desert, Egypt

Other no. of the type: IMI 278381

Description

Colony diam, 7 d, in mm: CYA25 43–49; CYA37 15–20; MEA25 35–45; YES 20–30; CZA25 25-26

Colony colour: white mycelial felt intermixed with brown conidiogenous structures

Conidiation: sparse

Reverse colour (CZA): yellow

Colony texture: velvety appearance, no sporulation

Conidial head: radiate, brown

Stipe: 35–200 × 2.5–3.5 µm, rough-walled with warty protuberances, brown

Vesicle diam SHAPE: 4–12 µm, globose to clavate

Conidium size/shape/surface texture: 3.5–5 µm, globose to ellipsoidal, brown, ornamented with small warts and colour bars

Hülle cells: absent

Ehrlich reaction: none

Growth on creatine: weak to moderate growth with hyaline mycelium, no acid production

Diagnostic features: Growth at 37 °C, curved brown conidiophores and the ornamented conidia, absence of Hülle cells

Cultures examined: CBS 756.74, CBS 596.65

Similar species: A. calidoustus

Distribution: Egypt, U.S.A.

Ecology and habitats: soil

Extrudites: Drimans (Hayes et al. 1996), unknown compounds

Pathogenicity: not reported

Aspergillus punicus Kwon and Fennell, The genus Aspergillus: 547. 1965. Fig. 10.

= A. ustus var. laevis Blochwitz (1945)

Type: CBS 495.65, from soil, Zarcero, Costa Rica

Other no. of the type: ATCC 16800; IMI 126692; WB 5077

Description

Colony diam, 7 d, in mm: CYA 40–50; CYA37 no growth; MEA25 40–45; YES 48–53; CZA25 40–50 mm

Colony colour: pinkish orange near vinaceous pink, with wine red exudate droplets

Conidiation: moderate

Reverse colour (CYA): dark yellow brown or crème brown

Colony texture: floccose

Conidial head: radiate to short columnar, dull green becoming light drab with age

Stipe: 150–250(–300) × 5.5–6(–8) µm, aereally borne stipes up to 135 × 3–4 µm, straight, smooth

Vesicle diam SHAPE: 8–16 µm (subglobose), 15–18 × 13–15 µm (elliptical)

Conidium size/shape/surface texture: 2.5–3.3 µm, globose, roughened

Hülle cells: elongate, crescent shaped or irregularly twisted, often aggregated into yellowish masses

Ehrlich reaction: no reaction

Growth on creatine: moderate to good growth with bright yellow mycelium, no acid production (in some isolates weak acid production under colony)

Cultures examined: CBS 495.65, CBS 122.33, CBS 128.62, 9377, V41-02, NRRL 29173

Houbaken et al.
Fig. 8. Aspergillus kerveii. A–B. Colonies at 25 °C after 7 d. A. CYA. B. MEA. C–H Conidiophores. I. Conidia. Scale bars = 10 µm.
Fig. 9. Aspergillus pseudodeflectus. A–C. Colonies at 25 °C after 7 d. A. MEA + 40 % sucrose. B. CYA + 20 % sucrose. C. MEA. D–I. Conidiophores. H. Conidia. Scale bars = 10 µm.
Fig. 10. Aspergillus puniceus. A–B. Colonies at 25 °C after 7 d. A. CYA. B. MEA. C–H Conidiophores. I. Sclerotia. J. Conidia. Scale bars = 10 µm, except D = 30 µm.
Diagnostic features: No growth at 37 °C, good growth on creatine with brightly pigmented yellow mycelium, Hülle cells aggregated into yellowish masses.

Similar species: *A. ustus*

Distribution: Costa Rica, U.S.A., Canada, Netherlands

Ecology and habitats: soil, indoor air, human

Extrolites: ustic acids, austocystins, nidulol, versicolorins, phenylahistin, sterigmatocystin-related compound (in CBS 128.62)

Pathogenicity: isolated from mouth wash and faeces

*Aspergillus ustus* (Bainier) Thom & Church, *The aspergilli*: 152. 1924. = *Sterigmatocystis usta* Bainier (1881) = *Aspergillus humus* Abbott (1926)

Type: CBS 261.67, culture contaminant, U.S.A.

Other no. of the type: ATCC 1041; ATCC 16818; IMI 211805; NRRL 275; QM 7477; WB 275; Thom 3556

Description

Colony diam, 7 d, in mm: CYA 36–43; CYA 37 no growth; MEA 25–39; YES 37–46

Colony colour: greyish brown to dark brown

Conidiation on CYA: moderate

Reverse colour (CZA): yellow-olive edge with olive brown centre

Colony texture: floccose, plane, sulcate or umbonate

Conidial head: radiate to hemispherical

Stipe: 400 × 3–6 µm, aerially borne stipes up to 125 × 2–5 µm, smooth, brownish

Vesicle diam/shape: 7–15 µm, hemispherical to subglobose

Conidium size/shape/surface texture: 3.2–4.5 µm, globose, roughened, greenish to dark yellow brown

Hülle cells: irregularly ovoid or elongate, usually scattered

Ehrlich reaction: no reaction

Growth on creatine: good growth with faint yellow mycelium, no acid production

Cultures examined: CBS 116057, CBS 114901, CBS 261.67, CBS 133.55, CBS 239.90, CBS 113233, NRRL 285, NRRL 1609, NRRL 29172

Diagnostic features: No growth at 37 °C; good growth on creatine with faint yellow pigmented mycelium; Hülle cells typically scattered or form irregular masses and not associated with pigmented mycelium

Similar species: *A. puniceus*

Distribution: Costa Rica

Ecology and habitats: soil

Extrolites: Found in this study: Sterigmatocystin, versicolorins, Mer-NF8054X. Literature data: emethallicins A–F (Kawahara et al. 1989, 1990a), 5'-hydroxyaveranthin (Yabe et al. 1991), emetherone (Kawahara et al. 1988), emesterones A & B (Hosoe et al. 1998), 5'-hydroxyaveranthin (Yabe et al. 1991), Mer-NP8054X (Mizuno et al. 1995).

Pathogenicity: not reported
Fig 11. Aspergillus ustus. A–B. Colonies at 25 °C after 7 d. A. CYA. B. MEA. C–E. G–H Conidiophores. F. Hüle cells. I. Conidia. Scale bars = 10 µm, except F = 30µm.
Fig. 12. Emericella heterothallica. A–C. Colonies at 25 °C after 7 d. A. CYA. B. MEA. C. Crossing of mating strains. D–E. Conidiophores. F–H. Conidia. Scale bars = 10 µm.
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