Polyphasic taxonomy of Aspergillus section Usti

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Abstract: Aspergillus 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 ustic 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. puniceus, A. granulosus, A. pseudodeflectus, A. calidoustus, A. insuetus and A. keveii together with Emericella heterothallica.

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. 2006). 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 1995), while A. deflectus has been reported to produce ustic 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. puniceus, A. granulosus, A. pseudodeflectus, A. calidoustus, A. insuetus and A. keveii together with Emericella heterothallica.

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

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 alkyphenone retention indices and diode array UV-VIS detection as described by Frisvad & Thrane (1987), with minor modifications as described by Smedsgard (1997). Standards of ochratoxin A and B, aflavamine, asperazine, austamide, austidol, 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 (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.

<table>
<thead>
<tr>
<th>Species</th>
<th>Strain No.</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. calidoustus</td>
<td>CBS 112452</td>
<td>Indoor air, Germany</td>
</tr>
<tr>
<td>A. calidoustus</td>
<td>CBS 113228</td>
<td>ATCC 38849; IBT 13091</td>
</tr>
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<td>A. calidoustus</td>
<td>CBS 114380</td>
<td>Wooden construction material, Finland</td>
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<td>A. calidoustus</td>
<td>CBS 121601</td>
<td>Bronchial secretion, proven invasive aspergillosis, Nijmegen, The Netherlands</td>
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<td>A. calidoustus</td>
<td>CBS 121602</td>
<td>Bronchial secretion, proven invasive aspergillosis, Nijmegen, The Netherlands</td>
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<td>A. calidoustus</td>
<td>CBS 121589</td>
<td>Autopsy lung tissue sample, proven invasive aspergillosis, Nijmegen, The Netherlands</td>
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<td>A. calidoustus</td>
<td>CBS 121603</td>
<td>Elevator shaft in hospital, Nijmegen, The Netherlands</td>
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<td>A. calidoustus</td>
<td>CBS 121604</td>
<td>Patient room, Nijmegen, The Netherlands</td>
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<tr>
<td>A. calidoustus</td>
<td>CBS 121605</td>
<td>Laboratory, Nijmegen, The Netherlands</td>
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<tr>
<td>A. calidoustus</td>
<td>CBS 121606</td>
<td>Sputum, Nijmegen, The Netherlands</td>
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<td>A. calidoustus</td>
<td>CBS 121607</td>
<td>Feces, Nijmegen, The Netherlands</td>
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<td>A. calidoustus</td>
<td>CBS 121608</td>
<td>Bronchoalveolar lavage, Nijmegen, The Netherlands</td>
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<td>A. calidoustus</td>
<td>7843</td>
<td>Pasteur Institute, Paris, France</td>
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<td>A. calidoustus</td>
<td>8523</td>
<td>Oslo, Norway</td>
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<td>A. calidoustus</td>
<td>9331</td>
<td>Mouth wash, Nijmegen, The Netherlands</td>
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<td>A. calidoustus</td>
<td>9420</td>
<td>Bronchial secretion, Nijmegen, The Netherlands</td>
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<td>A. calidoustus</td>
<td>9692</td>
<td>Hospital ward, Nijmegen, The Netherlands</td>
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<td>A. calidoustus</td>
<td>V02-46</td>
<td>Tongue swab, Nijmegen, The Netherlands</td>
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<td>A. calidoustus</td>
<td>V07-21</td>
<td>Bronchial secretion, Nijmegen, The Netherlands</td>
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<td>A. calidoustus</td>
<td>V17-43</td>
<td>Bronchial secretion, Nijmegen, The Netherlands</td>
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<td>A. calidoustus</td>
<td>V22-60</td>
<td>Skin biopsy, Nijmegen, The Netherlands</td>
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<td>Post-cataract surgery endophthalmitis, Turkey</td>
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<td>A. calidoustus</td>
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<td>Post-cataract surgery endophthalmitis, Turkey</td>
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<tr>
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<td>CBS 121610</td>
<td>Post-cataract surgery endophthalmitis, Turkey</td>
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<td>A. calidoustus</td>
<td>351</td>
<td>Osteorickets</td>
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<td>A. calidoustus</td>
<td>482</td>
<td>Post-cataract surgery endophthalmitis</td>
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<td>CBS 121611</td>
<td>Patient 4, Washington, U.S.A.</td>
</tr>
<tr>
<td>A. calidoustus</td>
<td>CBS 121616</td>
<td>Environmental, Washington, U.S.A.</td>
</tr>
<tr>
<td>A. calidoustus</td>
<td>FH 165</td>
<td>Patient 5b, Washington, U.S.A.</td>
</tr>
<tr>
<td>A. calidoustus</td>
<td>CBS 121614</td>
<td>Patient 5a, Washington, U.S.A.</td>
</tr>
<tr>
<td>A. calidoustus</td>
<td>CBS 121615</td>
<td>Patient 6, Washington, U.S.A.</td>
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<td>A. calidoustus</td>
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<td>Patient 2, Washington, U.S.A.</td>
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<td>A. calidoustus</td>
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<td>Patient 1, Washington, U.S.A.</td>
</tr>
<tr>
<td>A. calidoustus</td>
<td>FH 91</td>
<td>Patient 1a, Washington, U.S.A.</td>
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<tr>
<td>A. calidoustus</td>
<td>NRRL 26162</td>
<td>Culture contaminant, Peoria, U.S.A.</td>
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<td>A. calidoustus</td>
<td>NRRL 281</td>
<td>Thom 5634</td>
</tr>
<tr>
<td>A. calidoustus</td>
<td>NRRL 277</td>
<td>Thom 5698.754, Green rubber</td>
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<tr>
<td>A. granulosus</td>
<td>CBS 588.65</td>
<td>Soil, Fayetteville, Arkansas, U.S.A.</td>
</tr>
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<td>A. granulosus</td>
<td>CBS 119.58</td>
<td>Soil, Texas, U.S.A.</td>
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<td>A. granulosus</td>
<td>IBT 23478 = WB 1932 = IMI 017278iii = CBS 588.65</td>
<td>Soil, Fayetteville, Arkansas, U.S.A.</td>
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<tr>
<td>A. insuetus</td>
<td>CBS 107.25</td>
<td>South Africa</td>
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<td>A. insuetus</td>
<td>CBS 119.27</td>
<td>Unknown</td>
</tr>
<tr>
<td>A. insuetus</td>
<td>CBS 102278</td>
<td>Subcutaneous infection left forearm and hand of 77-year-old woman</td>
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<tr>
<td>A. keveii</td>
<td>CBS 209.92</td>
<td>Soil, La Palma, Spain</td>
</tr>
<tr>
<td>A. keveii</td>
<td>CBS 561.65</td>
<td>Soil, Panama</td>
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<td>A. keveii</td>
<td>IBT 10524 = CBS 113227 = NRRL 1254</td>
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Table 1. (Continued).

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

†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 (Amersham Pharmacia Biotech, Piscataway, NJ) equilibrated in double-distilled water and analyzed on the ABI PRISM 310 Genetic Analyzer (Applied Biosystems).

Data analysis. The sequence data was optimised using the software package Seqman from DNAStar Inc. Sequence alignments were performed by using CLUSTAL-X (Thompson et al. 1997) and improved manually. The neighbour-joining (NJ) method was used for the phylogenetic analysis. For NJ analysis, the data were first analysed using the Tamura–Nei parameter distance calculation model with gamma-distributed substitution rates (Tamura & Nei 1993), which were then used to construct the NJ tree with MEGA v. 3.1 (Kumar et al. 2004). To determine the support for each clade, a bootstrap analysis was performed with 1000 replications.

For parsimony analysis, the PAUP v. 4.0 software was used (Swoford 2000). Alignment gaps were treated as a fifth character state and all characters were unordered and of equal weight. Maximum parsimony analysis was performed for all data sets using the heuristic search option with 100 random tax additions and tree bisection and reconstruction (TBR) as the branch-swapping algorithm. Branches of zero length were collapsed and all multiple, equally parsimonious trees were saved. The robustness of the trees obtained was evaluated by 1000 bootstrap replications (Hillis & Bull 1993). An Aspergillus versicolor isolate was used as outgroup in these experiments. Unique sequences of the ITS, actin, calmodulin and β-tubulin gene sequences have been deposited in the GenBank under accession numbers EU076344–EU76377.

RESULTS

Phylogenetic analyses

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 10^4 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 (>10^4 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. insueutus 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.
A. granulosus

E. heterothallica

A. ustus

A. puniceus

A. keveii

A. insuetus

A. calidoustus, A. pseudodeflectus

Fig. 4. Neighbour-joining tree based on ITS sequence data of Aspergillus section Ustí. Numbers above branches are bootstrap values. Only values above 70% are indicated.


**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. pseudodefectus* 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. puniceus*, *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. puniceus* 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 (Vleggaar et al. 1974), austostymins (Steyn & Vleggaar 1974; Kfir et al. 1986), brevianamide A (Steyn 1973), sterigmatocystin (Rabie et al. 1977), austalides (de Jesus et al. 1987), austamidine (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), dimrins (Hayes et al. 1996), diacetoxycirpenol (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. puniceus* produced ustic acids in common. *A. ustus* and *A. puniceus* also produced austocystins and versicolorins. In the second chemical group, *A. pseudodefectus* produced dimrins (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), emeheterone (Kawahara et al. 1988), emestroterones 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 austodiol 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 the subgenus *Nidulantes*, nidulol and versicolorins are also produced by members of sections *Versicoloare* 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*, *Versicoloare* 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, drimans, Mer-NF8054X, austalides, deoxybrevianamides and austamide and thus this section is chemically unique. We have not examined the species for production of emethallicins, emestroterones 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. puniceus*, *A. conjunctus* and *A. deflectus*. Later, Kozakiewicz (1989) revised the taxonomy of the group, and included *A. ustus*,

### Table 2. Overview of morphological criteria to differentiate between the members of Aspergillus section *Usti*.

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

* All have violet reaction, except CBS 113227
** Colour according Methuen handbook of colours
Table 3. Extrolites produced by species assigned to Aspergillus section Usti.

<table>
<thead>
<tr>
<th>Species</th>
<th>Extrolites produced</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Chemical group I</strong></td>
<td></td>
</tr>
<tr>
<td>A. ustus</td>
<td>Ustic acids, austocystins (and versicolorins), australides, a compound related to sterigmatocystin, nidulol</td>
</tr>
<tr>
<td>A. granulosus</td>
<td>Ustic acids, a compound resembling sterigmatocystin, nidulol, drimans</td>
</tr>
<tr>
<td>A. puniceus</td>
<td>Ustic acids, austocystins (and versicolorins), phenylahistin, a compound related to sterigmatocystin, nidulol</td>
</tr>
<tr>
<td><strong>Chemical group II</strong></td>
<td></td>
</tr>
<tr>
<td>A. pseudodeflectus</td>
<td>Drimans, unknown compounds</td>
</tr>
<tr>
<td>A. calidoustus</td>
<td>Drimans, ophiobolins G and H, austins</td>
</tr>
<tr>
<td>A. insuetus</td>
<td>Drimans, ophiobolins G and H, pergillin-like</td>
</tr>
<tr>
<td>A. keveii</td>
<td>Drimans, ophiobolins G and H, nidulol</td>
</tr>
<tr>
<td><strong>Chemical group III</strong></td>
<td></td>
</tr>
<tr>
<td>E. heterothallica</td>
<td>Emethallicins A, B, C, D, E &amp; F, emeheterone, emesterones A &amp; B, 5&quot;-hydroxyaveranthin, Mer-NF8054X, sterigmatocystin, versicolorins</td>
</tr>
</tbody>
</table>

Aspergillus ustus (Bainier) Thom & Church was redescribed by Thom & Church (1926) based on Sterigmatocystis ustis Bainier. In this manual, A. insuetus (Bainier) Thom & Church was also accepted based on S. insuetas 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. puniceus, 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 x 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 119.58, CBS 588.65, IBT 23478

Similar species:

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/shapes: 11–16 µm, hemispherical to subglobose
Conidium size/shape/surface texture: 3.2–4 µm, globose, distinct roughened and inner and outer wall visible, fuligeous, 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

Diagnostic features: no growth at 37 °C, violet Ehrlich reaction, restricted growth on YES, coarsely roughened to echinulate conidia

Ecology and habitats: soil (?), human

Extrolites: 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. Hüle 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 CZA nullus. Sporulatio in CYA abundans; colonia brunneoargentea vel subroseobrunnea; textura coloniae floccosa; colonia reversa flaviovalveobrunnea vel atroseobrunnea. Capitula conidia laxe columnaria; stipples 150–300 × 4–6 µm, partete laevi, bruneo; vesiculae pyriformes, 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 elongatae, (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; after 7 d at 37 °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

**Extrolites:** Drimans, ophiobolins 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

**Extrolites:** 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, aerially 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
Fig. 8. Aspergillus kervei. A–B. Colonies at 25 °C after 7 d. A. CYA. B. MEA. C–H Conidiophores. I. 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.
**Aspergillus ustus** (Bainier) Thom & Church, *The aspergilli*: 152. 1924.

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

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

*Description*

Colony diam, 7 d, in mm: CYA 36–43; CYA37 5–8; MEA25 40–42; YES25 38–42

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, echinulate, yellow green

Hülle cells: irregularly ovoid or elongate, usually scattered

Ehrlich reaction: no reaction

Pathogenicity: isolated from biopsy of man with brain tumour (CBS 239.90). However, this isolate does not grow at 37 °C on normal agar media and might therefore be a culture contamination.


*Type*: CBS 489.65, from soil, Costa Rica

*Other no. of the type*: ATCC 16824; IHEM 2064; IMI 139278; RV 34434; WB 5097; IBT 22604

*Description*

Colony diam, 7 d, in mm: CYA25 35–39; CYA37 5–8; MEA25 40–42; YES25 38–42

Colony colour: cream to yellow to orange

Conidiation: limited

Reverse colour (CYA): yellow to orange to pink becoming dark reddish brown

Colony texture: floccose

Conidial head: hemispherical to short columnar

Stipe: 185–410 × 5–11 µm, generally sinuous, brownish with age, smooth

Vesicle diam/shape: 13–20 µm

Conidium size/shape/surface texture: 2.5–4 µm, globose, echinulate, yellow green

Hülle cells: 600–700(–1000) µm, pyriform to oval to elongate to twisted, in globose to subglobose masses

Cleistothecia: produced in a heterothallic manner, 270–510 µm, cinnamon to dark purple, surrounded by Hülle cells

Ascospores: 4–4.5 × 3.5–4 µm, lenticular, orange brown in colour, with two pleated equatorial crests (1.5–2 µm), with convex smooth Ehrlich reaction: none

Pathogenicity: isolated from biopsy of man with brain tumour (CBS 239.90). However, this isolate does not grow at 37 °C on normal agar media and might therefore be a culture contamination.
ACKNOWLEDGEMENTS

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REFERENCES


