Polyphasic taxonomy of Aspergillus section Candidi based on molecular, morphological and physiological data

J. Varga1,3, J.C. Frisvad2 and R.A. Samson1

1CBS Fungal Biodiversity Centre, Uppsalalaan 8, NL-3594 CT Utrecht, the Netherlands; 2BioCentrum-DTU, Building 221, Technical University of Denmark, DK-2800 Kgs. Lyngby, Denmark; 3Department of Microbiology, Faculty of Science and Informatics, University of Szeged, H-6701 Szeged, P.O. Box 533, Hungary

Abstract: Aspergillus section Candidi historically included a single white-spored species, A. candidus. Later studies clarified that other species may also belong to this section. In this study, we examined isolates of species tentatively assigned to section Candidi using a polyphasic approach. The characters examined include sequence analysis of partial β-tubulin, calmodulin and ITS sequences of the isolates, morphological and physiological tests, and examination of the extrolite profiles. Our data indicate that the revised section Candidi includes 4 species: A. candidus, A. campestris, A. taichungensis and A. tritici. This is strongly supported by all the morphological characteristics that are characteristic of section Candidi: slow growing colonies with globose conidial heads having white to yellowish conidia, conidiophores smooth, small conidiophores common, metulae present and covering the entire vesicle, some large Aspergillus heads with large metulae, presence of diminutive heads in all species, conidia smooth or nearly so with a subglobose to ovoid shape, and the presence of sclerotia in three species (A. candidus, A. taichungensis and A. tritici). Aspergillus tritici has been suggested to be the synonym of A. candidus previously; however, sequence data indicate that this is a valid species and includes isolates came from soil, wheat grain, flour and drums from India, Ghana, Sweden, The Netherlands and Hungary, making it a relatively widespread species. All species produce terphenyllins and candidusins and three species (A. candidus, A. campestris and A. tritici) produce chlorflavonins. Xanthoascins have only been found in A. candidus. Each of the species in section Candidi produce several other species specific extrolites, and none of these have been found in any other Aspergillus species. A. candidus has often been listed as a human pathogenic species, but this is unlikely as this species cannot grow at 37 °C. The pathogenic species may be A. tritici or white mutants of Aspergillus flavus.

TAXONOMIC NOVELTY: revalidation of Aspergillus tritici Mehrotra & Basu.

Key words: Ascomycetes, Aspergillus section Candidi, β-tubulin, calmodulin, Eurotiales, extrolites, ITS, polyphasic taxonomy.

INTRODUCTION

Aspergillus section Candidi (Gams et al. 1995; A. candidus species group according to Raper & Fennell 1965) was established by Thom & Raper (1945) to accomodate a single white-spored species, A. candidus Link. This species frequently contaminates stored food and feeding stuff (Kozakiewicz 1989; Park et al. 2005). A. candidus is moderately xerophilic, and able to grow on stored grains with 15 % moisture content (Lacey & Magan 1991), raising the moisture level of the infested grain to 18 percent or higher, and the temperature to up to 55 ºC. This species is one of the most frequently encountered mould in cereal grains and flour (Rabie et al. 1997; Weidenbörner et al. 2000; Ismail et al. 2004; Hocking 2003). A. candidus causes loss of viability and germ discoulouration in cereals (Papavizas & Christensen 1960; Battacharya & Raha 2002; Lugauskas et al. 2006). It also occurs in soil, usually on seeds or in the rhizosphere, and also in milk (Raper & Fennell 1965; Kozakiewicz 1989; Moreau 1976).

A. candidus enzymes has also been used in the fermentation industry for the production of galacto-oligosaccharides (Zheng et al. 2006), and D-mannitol (Smiley et al. 1969), while some A. candidus metabolites including terphenyllins has antioxidant and anti-inflammatory activities (Yen et al. 2001, 2003). A. candidus is also used in the meat industry for spontaneous sausage ripening (Gracia et al. 1986; Sunesen & Stahnke 2003)

A. candidus is claimed to be involved in a wide range of human infections including invasive aspergillosis (Rippon 1988; Ribeiro et al. 2005), otomycosis (Yasin et al. 1978; Falser 1983), brain granuloma (Linares et al. 1971) and onychomycosis (Schonborn & Schmoranzer 1970; Zorar & Moreno 1980; Piraccini et al. 2002). A. candidus has also caused various disorders in pigs (Moreau 1979) and was found to be the second most prevalent Aspergillus species in a hospital surveillance project in the U.S.A. (Curtis et al. 2005). Concentration of A. candidus conidia can reach alarming levels in grain dust and was suggested to contribute to the development of the so-called organic dust toxic syndrome (Weber et al. 1993; Krysinska-Traczyk & Dutkiewicz 2000). A. candidus is able to induce both cellular and humoral response in animals (Krysinska-Traczyk & Dutkiewicz 2000). A. candidus metabolites including terphenyl compounds and terpenins exhibit immunomodulating capabilities and are highly cytotoxic (Shanan et al. 1998; Krysinka & Dutkiewicz 2000). There is some evidence that A. candidus might be toxic to chickens and rats (Marasas & Smalley 1972) and has also been isolated from birds (Saeez 1970, Sharma et al. 1971).

A. candidus has been reported to produce several secondary metabolites including candidusins (Kobayashi et al. 1982; Rahbaek et al. 2000), terpenins (Kamigawa et al. 1998), chlorflavonin (Bird & Marshall 1969), dechlorochlorflavonin (Marchelli & Vining 1973), xanthoacitin (Takahashi et al. 1976b), kojic acid (Kinosita & Shikata 1969, Saruno et al. 1979, Cole & Cox 1981), 3-nitro-propanic acid (Kinosita et al. 1968), and 6-sulfoaminopenicillic acid (Yamashita et al. 1983). A. candidus is reported to produce citrinin but the first report of citrinin production by an Aspergillus confused A. niveus with A. candidus (Timonin & Rouatt 1944; Raper & Fennell 1965). However, some later reports indicate that some isolates may produce citrinin (Kinosita & Shikata 1969; Cole & Cox 1981).
The description of *A. candidus* is admittedly broad, encompassing considerable variability among the isolates (Raper & Fennell 1965, Kozakiewicz 1989). *A. candidus* is characterised by white conidial heads, globose to subglobose vesicles, biseriate large and uniseriate small conidial heads, and smooth conidiophores and conidia (Raper & Fennell 1965, Kozakiewicz 1989). Several white-spored *Aspergillus* species described in the past have been synonymised with *A. candidus*, including *A. albus*, *A. okazakii*, or *A. dubius* (Raper & Fennell 1965). Raper & Fennell (1965) also stated that “it is possible that our current concept of *A. candidus* is too broad”. Recent studies indicated that other species including *A. campestris* (Christensen 1982; Rahbaek et al. 2000; Peterson 2000; Varga et al. 2000) and *A. taichungensis* (Yaguchi et al. 1995, Rahbaek et al. 2000) are also members of section *Candidi*. Besides, two other white-spored species, *A. triticci* (as *A. triticus*, Mehrtra & Basu 1976) and *A. implicatus* (Maggi & Persiani 1994) have also been suggested to belong to this section.

In this study, we examined available isolates of the species, proposed to belong to section *Candidi*, to clarify the taxonomic status of this section. The methods used include sequence analysis of the ITS region (including internal transcribed spacer regions 1 and 2, and the 5.8 S rRNA gene of the rRNA gene cluster), and parts of the β-tubulin and calmodulin genes, macro- and micromorphological analysis, and analysis of extrolite profiles of the isolates.

### MATERIALS AND METHODS

#### Morphological examinations

The strains examined are listed in Table 1. The strains were grown for 7 d as 3-point inoculations on Czapek agar, Czapek yeast autolysate agar (CYA), malt extract agar (MEA), and oat meal agar (OA) at 25 °C (medium compositions in Samson et al. 2004).

#### Analysis for secondary metabolites

The cultures were analysed according to the HPLC-diode array detection method of Frisvad & Thrane (1987, 1993) as modified by Smedsgaard (1997). The isolates were analyzed on CYA and YES agar using three agar plugs (Smedsgaard 1997). The secondary metabolite production was confirmed by identical UV spectra with those of standards and by comparison to retention indices and retention times in pure compound standards (Rahbaek et al. 2000).

#### 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 (Oxoid) 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 days. 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 & Donaldson 1995). Amplifications of the partial calmodulin gene were set up as described previously (Hong et al. 2005). Sequence analysis was performed with the Big Dye Terminator 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). The unique ITS, β-tubulin, and calmodulin sequences were deposited at the GenBank nucleotide sequence database under accession numbers EU076291–EU076311.

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 (Swofford 2002). 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 taxa 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 A. flavus isolate was used as outgroup in these experiments.

RESULTS AND DISCUSSION

Phylogeny

We examined the genetic relatedness of section Candidi isolates using sequence analysis of the ITS region of the ribosomal RNA gene cluster, and parts of the calmodulin and β-tubulin genes. During analysis of part of the β-tubulin gene, 496 characters were analyzed, among which 68 were found to be parsimony informative. The Neighbour-joining tree based on partial β-tubulin genes sequences is shown in Fig. 1. The topology of the tree is the same as the single maximum parsimony tree constructed by
The PAUP program (length: 240 steps, consistency index: 0.8833, retention index: 0.9263). The calmodulin data set included 532 characters, with 43 parsimony informative characters (Fig. 2). The topology of the Neighbor-joining tree was the same as that of one of the 78 maximum parsimony trees (tree length: 300, consistency index: 0.9633, retention index: 0.9396). The ITS data set included 492 characters with 5 parsimony informative characters. The Neighbor joining tree shown in Fig. 3 has the same topology as one of the more than $10^5$ maximum parsimony trees (tree length: 35, consistency index: 1.0000, retention index: 1.0000).

Phylogenetic analysis of both β-tubulin and calmodulin sequence data indicated that Aspergillus section Candidi includes 4 species, namely: A. candidus, A. campestris, A. taichungensis and A. tritici. Interestingly, the reference strain of A. candidus, CBS 283.95 was found to belong to the A. tritici species. Isolates CBS 597.65 and CBS 112449 were found to be related to the A. taichungensis type strain based on β-tubulin sequence data, and formed a distinct clade on the tree based on calmodulin sequences. Further studies are needed to clarify the taxonomic position of these isolates.

Comparison of our ITS sequence data to those available on the web site of the Japan Society for Culture Collections (http://www.ncbi.nite.go.jp/scc/ridb/search) indicated that several strains held as A. candidus represent other species. Three strains (NBRC 4389 = IFO 4389, NBRC 4037 = IFO 4037, and NBRC 4322 = IFO 4322) were found to be actually white-spored A. oryzae isolates, NBRC 5468 (= IFO 5468) and NBRC 33019 (= IFO 33019 = CBS 283.95 = SRRC 310) belong to A. tritici, while NBRC 32248 (= IFO 32248) has identical ITS sequence to A. campestris. However, further loci should also be analyzed to confirm their assignment. Other isolates including NBRC 8816, NBRC 4309, NBRC 4310 and NBRC 4311 are representatives of the A. candidus species based on their identical ITS sequences.

Aspergillus implicatus, another species previously assigned to this section (Maggi & Persiani 1994), was found to be more closely related to A. anthodesmis based on sequence data, which places this species close to Aspergillus section Sparsi (data not shown). Further studies are needed to clarify the taxonomic position of this white-spored species within the Aspergillus genus.

**Chemotaxonomy**

All strains of species in section Candidi produced terphenyllins and candidusins. Aspergillus candidus isolates produced candidusins A and B, terphenyllin, 3-hydroxyterphenyllin and some isolates
also produced chlorflavonin and a chlorflavonin analogue. *A. tritici* isolates differed from *A. candidus* in not producing candidusin A and chlorflavonin. *A. taichungensis* produced candidusin C, terphenyllin, and 3-hydroxyterphenyllin, while the type strain of *A. campestris* also produced chlorflavonin. Xanthoascin was only found in some strains of *A. candidus* and not in any other species in *Candidi*. Each species produced a large number of as yet not structure elucidated extrolites. These extrolites, including terphenyllins, candidusins, chlorflavonins and xanthoascin, have only been found in section *Candidi* and not in any other aspergilli, except for *A. ellipticus*, that produces terphenyllin and candidusin (Samson et al. 2004, 2007).

**Morphology**

*Aspergillus candidus* is a wide-spread species throughout the world. According to Raper & Fennell (1965), “a typical strain of *A. candidus* differs little from members of the *A. niger* group except for the absence of both pigmentation and roughening in the conidia”. Another interesting feature observed in *A. candidus* is the production of diminutive conidial heads which are frequently uniseriate in contrast with the biseriate large heads. Colonies on CYA and MEA usually slow growing, colonies white to cream coloured, reverse usually uncoloured. Conidial heads usually biseriate, white to cream coloured, at first globose, with spore chains later adherent in loose divergent columns, diminutive heads commonly produced, conidiophores varying with the strain from less than 500 µm to up to 1000 µm long, thick walled, smooth, occasionally septate, vesicles globose to subglobose, ranging from 40 µm or more in diam in very large heads to less than 10 µm in small heads, typically fertile over the whole surface, phialides occasionally uniseriate in small heads but typically in two series, colourless, conidia globose to subglobose, microverrucose, 3–4 µm. 

Fig. 3. Neighbour-joining tree based on ITS sequence data of *Aspergillus* section *Candidi*. Numbers above branches are bootstrap values. Only values above 70 % are indicated.
Dark brown sclerotia which appear on MEA after more than 25 d incubation. *A. taichungensis* is able to grow at 37 ºC on CYA.

*Aspergillus campestris* was described by Christensen (1982) from native prairie soil, North Dakota. The species is characterised by its restricted growth on CZA and MEA at 25 ºC, colonies velvety, sulphur yellow, reverse uncoloured. Conidial heads biseriate, radiate, conidiophores usually 400–800 µm but can be up to 1 300 µm long, septate, 130–700 µm long, biseriate, vesicles globose to slightly elongate, 25–40 µm in diam, fertile over the entire surface, conidia thin-walled, hyaline, pale yellow in mass, slightly ellipsoidal, 3–4 × 2.3–3 µm. Sclerotia not observed. *A. campestris* is unable to grow at 37 ºC on any media tested.

*Aspergillus tritici* was described as *A. triticus* by Mehrotra & Basu (1976) from wheat grains, India. Colonies are slow-growing on CZA and MEA, white to light cream coloured, reverse light brown. Conidial heads are biseriate, radiate, conidiophores thick-walled, septate, 130–700 µm long, often diminutive (10–75 µm, sometimes unsieriate), vesicles elongated, small (5–11 µm), conidia globose to subglobose, slightly roughened, 2.7–3.5 µm. At maturity conidia are embedded in a water drop giving the conidial heads a "slimy" appearance. The sclerotia are at first white, later becoming purple to black. *A. tritici* grows well at 37 ºC.

Based on a polyphasic investigation of *Aspergillus* section *Candidi*, the section includes four species: *A. candidus*, *A. campestris*, *A. taichungensis* and *A. tritici*. Phenotypic characteristics of these species are shown in Table 2. *A. campestris* was placed in section *Circumdati* because of its yellowish white conidia and it was not considered closely related to *A. candidus* by Christensen (1982). *A. taichungensis* was equivocally placed in either section *Versicolores*, *Terrei* or *Flavipedes* (Yaguchi et al. 1995). However, the phylogenetic and chemotaxonomic evidence presented here indicates that both species belong to section *Candidi*. This is strongly supported by all the morphological characteristics that are characteristic of the section *Candidi*: slow growing colonies with globose conidial heads having white to yellowish conidia, conidiophores smooth, small conidiophores common, metulae present and covering the entire vesicle, some large *Aspergillus* heads with large metulae, conidia smooth or nearly so with a subglobose to ovoid shape (albeit slightly ellipsoidal in *A. campestris*), and sclerotia present in *A. taichungensis*, *A. candidus* and *A. tritici*. Sclerotia have not been observed in *A. campestris*, but have been observed in *A. candidus* (light cream coloured turning purple to black in age). *Aspergillus tritici* has been suggested to be the synonym of *A. candidus* by Samson (1979). However, sequence data indicate that this is a valid species and includes isolates from soil, wheat grain, flour and drums from India, Ghana, Sweden, The Netherlands and Hungary, making it a relatively widespread species.

### Table 2. Phenotypic characteristics of species in *Aspergillus* section *Candidi*.

| Morphological characteristics | *A. candidus* | *A. tritici* | *A. taichungensis* | *A. campestris* |
|-----------------------------|--------------|-------------|-------------------|----------------|
| Colony colour               | White        | Light cream | Light cream       | Sulphur yellow |
| Colony reverse              | Uncoloured to yellowish | Light brown | Uncoloured         | Uncoloured     |
| Conidial heads              | Globose      | Radiate    | Radiate           | Radiate        |
| Conidiophores               | Smooth, 500–1000 µm | Septate, 130–700 µm | Smooth, 300–400 µm | Smooth, 400–800 µm |
| Vesicles                    | Globose, 40 µm | Elongated, 5–11 µm | Hemispherical, 5–20 µm | Globose, 25–40 µm |
| Conidial ornamentation      | Smooth       | Slightly roughened | Microverrucose | Smooth         |
| Conidial shape              | (Sub)globose | (Sub)globose | (Sub)globose     | Ellipsoidal    |
| Size of conidia             | 2.5–3.5 µm  | 2.7–3.5 µm   | 3–5 µm            | 3–4 × 2.3–3 µm |
| Growth at 37ºC              | -            | +           | +                 | -              |
| Sclerotia                   | Purple to black | Purple to black | Dark brown       | -              |

**Extralite production**

| Extralite               | A. candidus | A. tritici | A. taichungensis | A. campestris |
|-------------------------|-------------|------------|-----------------|--------------|
| Candidusin A            | +           | -          | -               | -            |
| Candidusin B            | +           | +          | -               | -            |
| Candidusin C            | -           | -          | +               | +            |
| Candidusin analogue     | -           | +          | -               | -            |
| terphenyllin            | +           | +          | +               | +            |
| 3-hydroxyterphenyllin   | +           | +          | +               | -            |
| chlorflavonin           | +           | +          | -               | +            |
| chlorflavonin analogue  | +           | -          | -               | -            |

*Candidusin* in *A. candidus* was placed in section *Versicolores*, *Terrei* or *Flavipedes* (Yaguchi et al. 1995). However, the phylogenetic and chemotaxonomic evidence presented here indicates that both species belong to section *Candidi*. This is strongly supported by all the morphological characteristics that are characteristic of the section *Candidi*: slow growing colonies with globose conidial heads having white to yellowish conidia, conidiophores smooth, small conidiophores common, metulae present and covering the entire vesicle, some large *Aspergillus* heads with large metulae, conidia smooth or nearly so with a subglobose to ovoid shape (albeit slightly ellipsoidal in *A. campestris*), and sclerotia present in *A. taichungensis*, *A. candidus* and *A. tritici*. Sclerotia have not been observed in *A. campestris*, but have been observed in *A. candidus* (light cream coloured turning purple to black in age). *Aspergillus tritici* has been suggested to be the synonym of *A. candidus* by Samson (1979). However, sequence data indicate that this is a valid species and includes isolates from soil, wheat grain, flour and drums from India, Ghana, Sweden, The Netherlands and Hungary, making it a relatively widespread species.
Fig. 4. Aspergillus candidus. A–B Colonies after 7 d at 25 °C. A. CYA. B. MEA. C, G. Conidial heads. D–F, H–K. Conidiophores. H. Sclerotia. L. Conidia. Scale bars = 10 µm.
Fig. 5 Aspergillus campestris. A–B Colonies after 7 d at 25 °C A. CYA. B. MEA. C. Conidial heads. D–H. Conidiophores. I. Conidia. Scale bars = 10 µm.
**Aspergillus campestris** Christensen, Mycologia 74: 212. 1982. Fig. 4.

**Type:** CBS 348.81, from soil from native prairie, North Dakota, U.S.A.

**Other no. of the type:** IBT 27921 = IBT 13382

**Description**
- Colony diam: CZA25: 10–12 mm; CYA25: 10–15 mm, MEA25: 7–10 mm, YES25: 18–24 mm, OA25: 9–12 mm, CYA37: 0 mm, CREA25: poor growth, no acid production
- Colony colour: sulphur yellow to pinard yellow
- Conidial head: diminutive, with few divergent spore chains
- Conidiation: limited
- CREA25: poor growth and no acid production
- Yes25: 19–33 mm, OA25: 9–12 mm, CYA37: 0 mm, Colony diam: CZA25: 10–12 mm; CYA25: 10–15 mm, MEA25: 7–10 mm, YES25: 18–24 mm, OA25: 9–12 mm, CYA37: 0 mm
- Colony texture: velvety
- Reverse colour (CZA): uncoloured
- Colony colur: sulphur yellow to pinard yellow
- Colony diam: CZA25: 10–12 mm; CYA25: 10–15 mm, MEA25: 7–10 mm, YES25: 18–24 mm, OA25: 9–12 mm, CYA37: 0 mm
- Conidial head: diminutive, with few divergent spore chains
- Conidiation: limited
- CREA25: poor growth and no acid production
- Yes25: 19–33 mm, OA25: 9–12 mm, CYA37: 0 mm
- Colony diam: CZA25: 10–12 mm; CYA25: 10–15 mm, MEA25: 7–10 mm, YES25: 18–24 mm, OA25: 9–12 mm, CYA37: 0 mm
- Conidial head: diminutive, with few divergent spore chains
- Conidiation: limited
- CREA25: poor growth and no acid production
- Yes25: 19–33 mm, OA25: 9–12 mm, CYA37: 0 mm
- Colony diam: CZA25: 10–12 mm; CYA25: 10–15 mm, MEA25: 7–10 mm, YES25: 18–24 mm, OA25: 9–12 mm, CYA37: 0 mm
- Conidial head: diminutive, with few divergent spore chains
- Conidiation: limited
- CREA25: poor growth and no acid production
- Yes25: 19–33 mm, OA25: 9–12 mm, CYA37: 0 mm
- Colony diam: CZA25: 10–12 mm; CYA25: 10–15 mm, MEA25: 7–10 mm, YES25: 18–24 mm, OA25: 9–12 mm, CYA37: 0 mm
- Conidial head: diminutive, with few divergent spore chains
- Conidiation: limited
- CREA25: poor growth and no acid production
- Yes25: 19–33 mm, OA25: 9–12 mm, CYA37: 0 mm
- Colony diam: CZA25: 10–12 mm; CYA25: 10–15 mm, MEA25: 7–10 mm, YES25: 18–24 mm, OA25: 9–12 mm, CYA37: 0 mm
- Conidial head: diminutive, with few divergent spore chains
- Conidiation: limited
- CREA25: poor growth and no acid production
- Yes25: 19–33 mm, OA25: 9–12 mm, CYA37: 0 mm
- Colony diam: CZA25: 10–12 mm; CYA25: 10–15 mm, MEA25: 7–10 mm, YES25: 18–24 mm, OA25: 9–12 mm, CYA37: 0 mm
- Conidial head: diminutive, with few divergent spore chains
- Conidiation: limited
- CREA25: poor growth and no acid production
- Yes25: 19–33 mm, OA25: 9–12 mm, CYA37: 0 mm
- Colony diam: CZA25: 10–12 mm; CYA25: 10–15 mm, MEA25: 7–10 mm, YES25: 18–24 mm, OA25: 9–12 mm, CYA37: 0 mm
- Conidial head: diminutive, with few divergent spore chains
- Conidiation: limited
- CREA25: poor growth and no acid production
- Yes25: 19–33 mm, OA25: 9–12 mm, CYA37: 0 mm
- Colony diam: CZA25: 10–12 mm; CYA25: 10–15 mm, MEA25: 7–10 mm, YES25: 18–24 mm, OA25: 9–12 mm, CYA37: 0 mm
- Conidial head: diminutive, with few divergent spore chains
- Conidiation: limited
- CREA25: poor growth and no acid production
- Yes25: 19–33 mm, OA25: 9–12 mm, CYA37: 0 mm
- Colony diam: CZA25: 10–12 mm; CYA25: 10–15 mm, MEA25: 7–10 mm, YES25: 18–24 mm, OA25: 9–12 mm, CYA37: 0 mm
- Conidial head: diminutive, with few divergent spore chains
- Conidiation: limited
- CREA25: poor growth and no acid production
- Yes25: 19–33 mm, OA25: 9–12 mm, CYA37: 0 mm
- Colony diam: CZA25: 10–12 mm; CYA25: 10–15 mm, MEA25: 7–10 mm, YES25: 18–24 mm, OA25: 9–12 mm, CYA37: 0 mm
- Conidial head: diminutive, with few divergent spore chains
- Conidiation: limited
- CREA25: poor growth and no acid produc
Fig. 6. *Aspergillus taichungensis*. A–B Colonies after 7 d at 25 °C. A. CYA. B. MEA. C. Conidial heads. D–H Conidiophores. I. Conidia. Scale bars = 10 µm.
Fig. 7. Aspergillus tritici. A–B Colonies after 7 d at 25 °C A. CYA, B. MEA. C. Conidial heads. D–F, G–H Conidiophores. I. Sclerotia. J. Conidia. Scale bars = 10 µm.
Aspergillus taichungensis Yaguchi, Someya & Udagawa, Mycoscience 36: 421. 1995. Fig. 6.

**Type:** PF1167, from soil, Taiwan

**Other no. of the type:** IBT 19404

**Description**

- **Colony diam:** CZA25: 12–15 mm; CYA25: 17–20 mm, MEA25: 9–13 mm in 7 d, YES25: 25–28 mm, OA25: 12–16 mm, CYA37: 7–10 mm, CREA25: poor growth, no acid production
- **Colony colour:** yellowish white to primrose
- **Conidiation:** moderate
- **Reverse colour (CZA):** colourless (CZA), light yellow to pale luteous (MEA)
- ** Colony texture:** radially furrowed
- **Conidial head:** short radiate
- **Conidium size/shape/surface texture:** 3–4 µm, globose to subglobose; sometimes ovoid, 3–5 × 3–4.5 µm, micro verrucose

**Cultures examined:** IBT 19404, CBS 567.65, CBS 112449

**Diagnostic features:** growing as a colony with globose conidial heads having white to yellowish conidia, presence of diminutive conidiophores and dark brown sclerotia

**Similar species:** A. candidus, A. tritici

**Ecology and habitats:** soil, air

**Extrolites:** candidusin C, terphenyllin, 3-hydroxyterphenyllin (Rahbaek et al. 2000, and confirmed in this study). A large number of additional extrolites, until now only found in this species, were also produced. These have not yet been structure elucidated, but had characteristic UV spectra

**Notes:** the type strain produces dark brown sclerotia 300–500 × 200–400 µm in size in 30 d (Yaguchi et al. 1995; Rahbaek et al. 2000); diminutive conidiophores present, 90–250 × 2–3 µm in size

Aspergillus tritici Mehrotra & Basu, Nova Hedwigia 27: 599, 1976. Fig. 7.

**Type:** CBS 266.81, from wheat grain, India

**Other no. of the type:** No. A x 194

**Morphological characteristics**

- **Colony diam (7 d):** CZA25: 18–23 mm; CYA25: 16–29 mm, MEA25: 11–17 mm, YES25: 18–41 mm, OA25: 13–25 mm, CYA37: 7–21 mm, CREA25: poor growth, no acid production
- **Colony colour:** white to light cream coloured
- **Conidiation:** moderate
- **Reverse colour (CZA):** light yellow to light brown with age
- **Colony texture:** radially furrowed
- **Conidial head:** short radiate
- **Stipe:** 130–700 × 4–8 µm (diminutive stipes 10–75 × 1.5–3.5 µm), septate
- **Vesicle diam:** 4.8–11 µm, small, only slightly enlarged at the end
- **Conidium size, shape, surface texture:** 2.7–3.5 µm, globose to subglobose, slightly roughened

**Cultures examined:** CBS 119225, CBS 117270, CBS 266.81, CBS 112.34, 11-H7, SZMC 0565, CBS 283.96, SZMC 0897, IBT 23116, IBT 24170

**Diagnostic features:** colonies more yellowish than those of A. candidus; able to grow at 37 °C

**Similar species:** A. candidus

**Distribution:** India, Ghana, Sweden, Hungary, Slovenia, South Africa

**Ecology and habitats:** wheat, soil

**Extrolites:** candidusin B, candidusin analogue, terphenyllin, 3-hydroxyterphenyllin, chlorflavonin (Rahbaek et al. 2000, and confirmed in this study)

**Pathogenicity:** not reported, but since this species is able to grow at 37 °C, it may have caused some of the mycoses listed under A. candidus

**Notes:** some isolates produce sclerotia purple to black in colour; in some isolates conidia are embedded in a water drop with age („slimy” appearance) and produces diminutive heads

---

**REFERENCES**

Alvi KA, Pu H, Luche M, Rice A, App H, McMahon G, Dare H, Margolis B (1999). Asteroxinones produced by Aspergillus candidus inhibit binding of the Grb-2 adapter to phosphorylated EGF receptor tyrosine kinase. *Journal of Antibiotics* 52: 215–223.

Avanzini F, Bigori A, Nicoletti G (1991). A rare case of isolated aspergilloma of the sphenoid sinus. *Acta Otorhinolaryngologica Italica* 11: 483–489. [in Italian]

Bhattacharya K, Raha S (2002). Deteriorative changes of maize, groundnut and soybean seeds by fungi in storage. *Mycopathologia* 155: 135–141.

Bird AE, Marshall AC (1969). Structure of chlorflavonin. *Journal of the Chemical Society, Chemical Communications* 1969: 2418–2420.

Christensen M (1982). The Aspergillus ochraceus group: two new species from western soils and a synoptic key. *Mycologia* 74: 210–225.

Cole RJ, Cox RH (1981). Handbook of toxic fungal metabolites. New York: Academic Press.

Cornere BM, Eastman M (1975). Oncychomycosis due to Aspergillus candidus: case report. *New Zealand Medical Journal* 82: 13–15.

Curtis L, Cali S, Conroy L, Baker K, Ou CH, Hershov R, Norlock-Cruz F, Schefl P (2005). *Aspergillus* surveillance project at a large tertiary-care hospital. *Journal of Hospital Infection* 59: 188–190.

Falsen E (1983). Pilzbesatz des Ohres. Harmloser Saprophyt oder pathognomonische Risikofaktor? *Dermatologie* 11: 483–489. [in German]

Fragner P, Kubickova V (1974). Oncychomycosis due to *Aspergillus candidus*. *Ceskoslovenská Dermatologie* 49: 322–324. [in Czech]

Frisvad JC, Thrane U (1987). Standardized high performance liquid chromatography of 182 mycotoxins and other fungal metabolites based on alkyphenone retention indices and UV-VIS spectra (diode array detection). *Journal of Chromatography A* 404: 195–214.

Frisvad JC, Thrane U (1993). Liquid chromatography of mycotoxins. In: *Betina V (ed.) Chromatography of mycotoxins: techniques and applications*. Journal of Chromatography Library 54. Amsterdam: Elsevier: 253–372.

Gane W, Christensen M, Onions AH, Pitt JI, Samson RA (1985). *Infrageneric taxa of Aspergillus*. In: *Betina V, Raper, Schemes, and other fungal metabolites based on alkyphenone retention indices and UV-VIS spectra (diode array detection). Journal of Chromatography A* 404: 195–214.

Grazia L, Romano P, Bagni A, Roggiani D, Guglielmi G (1986). The role of moulds in nasal and sphenoid sinus. *Acta Otorhinolaryngologica Italica* 11: 483–489. [in Italian]

Hillis DM, Bull JJ (1993). An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. *Systematic Biology* 42: 182–192.
Yaguchi T, Someya A, Udagawa S (1995). *Aspergillus taichungensis*, a new species from Taiwan. *Mycoscience* 36: 421–424.

Yamashita M, Hashimoto S, Ezaki M, Iwami M, Komori T, Kohsaka M, Imanaka H (1983). FR-900318, a novel penicillin with β-lactamase inhibitory activity. *Journal of Antibiotics* 36: 1774–1776.

Yasin A, Maher A, Moawad MH (1978). Otomycosis: a survey in the eastern province of Saudi Arabia. *Journal of Laryngology and Otology* 92: 869–876.

Yen GC, Chang YC, Sheu F, Chiang HC (2001). Isolation and characterization of antioxidant compounds from *Aspergillus candidus* broth filtrate. *Journal of Agricultural and Food Chemistry* 49: 1426–1431.

Yen GC, Chiang HC, Wu CH, Yeh CT (2003). The protective effects of *Aspergillus candidus* metabolites against hydrogen peroxide-induced oxidative damage to Int 407 cells. *Food Chemistry and Toxicology* 41: 1561–1567.

Zaror L, Moreno MI (1980). Onicomicosis por *Aspergillus candidus* Link. *Revista Argentina de Micologia* 3: 13–15 [in Spanish].

Zheng P, Yu H, Sun Z, Ni Y, Zhang W, Fan Y, Xu Y (2006). Production of galactooligosaccharides by immobilized recombinant beta-galactosidase from *Aspergillus candidus*. *Biotechnology Journal* 1: 1464–1470.