

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

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**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  $\beta$ -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*,  $\beta$ -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 accommodate 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 °C. This species is one of the most frequently encountered mould in cereal grains and flour (Rabie *et al.* 1997; Weidenbömer *et al.* 2000; Ismail *et al.* 2004; Hocking 2003). *A. candidus* causes loss of viability and germ discolouration 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 & Schmoranzler 1970; Zaror & 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 (Saez 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 (Kamiguchi *et al.* 1998), chlorflavonin (Bird & Marshall 1969), dechlorochlorflavonin (Marchelli & Vining 1973), xanthoascins (Takahashi *et al.* 1976b), kojic acid (Kinosita & Shikata 1969, Saruno *et al.* 1979, Cole & Cox 1981), 3-nitro-propionic acid (Kinosita *et al.* 1968), and 6-sulfoaminopenicillanic 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).

**Table 1.** The *Aspergillus* section *Candidi* isolates examined in this study.

Species	Strain No.	Origin
<i>Aspergillus campestris</i>	CBS 348.81 <sup>T</sup>	Soil, North Dakota, U.S.A.
<i>Aspergillus candidus</i>	CBS 119.28	IFO 5468; <i>A. okazakii</i>
<i>Aspergillus candidus</i>	CBS 116945	Museum dust, Tiel, Netherlands
<i>Aspergillus candidus</i>	CBS 175.68	Mouse dung, Netherlands
<i>Aspergillus candidus</i>	CBS 114385	Air, Finland
<i>Aspergillus candidus</i>	CBS 120.38	No. 827/2; Unknown, J.C. Neill
<i>Aspergillus candidus</i>	CBS 225.80	Human nail, Netherlands
<i>Aspergillus candidus</i>	CBS 102.13	Japan, G. Kita
<i>Aspergillus candidus</i>	CBS 118.28	QM 9372; A. Blochwitz
<i>Aspergillus candidus</i>	CBS 566.65 <sup>T</sup>	ATCC 1002; IMI 091889; NRRL 303; unknown, J. Westerdijk
<i>Aspergillus candidus</i>	1-F9	TM 04.129 V11
<i>Aspergillus candidus</i>	13-C4	House, Utrecht, Netherlands
<i>Aspergillus candidus</i>	17-C2	House, Eindhoven, Netherlands
<i>Aspergillus candidus</i>	25-11	Indoor environment, Germany
<i>Aspergillus candidus</i>	IMI 091889	ATCC 1002, CBS 566.65
<i>Aspergillus candidus</i>	CBS 283.95	IFO 33019; JCM 10250; SRRRC 310
<i>Aspergillus taichungensis</i>	IBT 19404 <sup>T</sup>	PF1167; Soil, Taiwan
<i>Aspergillus taichungensis</i>	CBS 567.65	ATCC 16871; IMI 230752; NRRL 312; unknown, Brazil
<i>Aspergillus taichungensis</i>	CBS 112449	Indoor environment, Germany
<i>Aspergillus tritici</i>	CBS 119225	SLV 541; wheat flour, Sweden
<i>Aspergillus tritici</i>	CBS 117270	Djambee (drum), Ghana
<i>Aspergillus tritici</i>	CBS 266.81 <sup>T</sup>	Wheat grain, India
<i>Aspergillus tritici</i>	11-H7	Feed ingredient, Netherlands
<i>Aspergillus tritici</i>	SZMC 0565	Viticultural Institute, Kecskemet, Hungary
<i>Aspergillus tritici</i>	CBS 283.95	ATCC 13686=IMI 78734=NRRL 2297; P.G. Stansly, B81
<i>Aspergillus tritici</i>	SZMC 0897	Agricultural Service, Bekes county, Hungary
<i>Aspergillus implicatus</i>	CBS 484.95 <sup>T</sup>	Soil, Ivory Coast

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. tritici* (as *A. triticus*, Mehrotra & 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  $\beta$ -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 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  $\beta$ -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,  $\beta$ -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  $\beta$ -tubulin genes. During analysis of part of the  $\beta$ -tubulin gene, 496 characters were analyzed, among which 68 were found to be parsimony informative. The Neighbour-joining tree based on partial  $\beta$ -tubulin genes sequences is shown in Fig. 1. The topology of the tree is the same as the single maximum parsimony tree constructed by

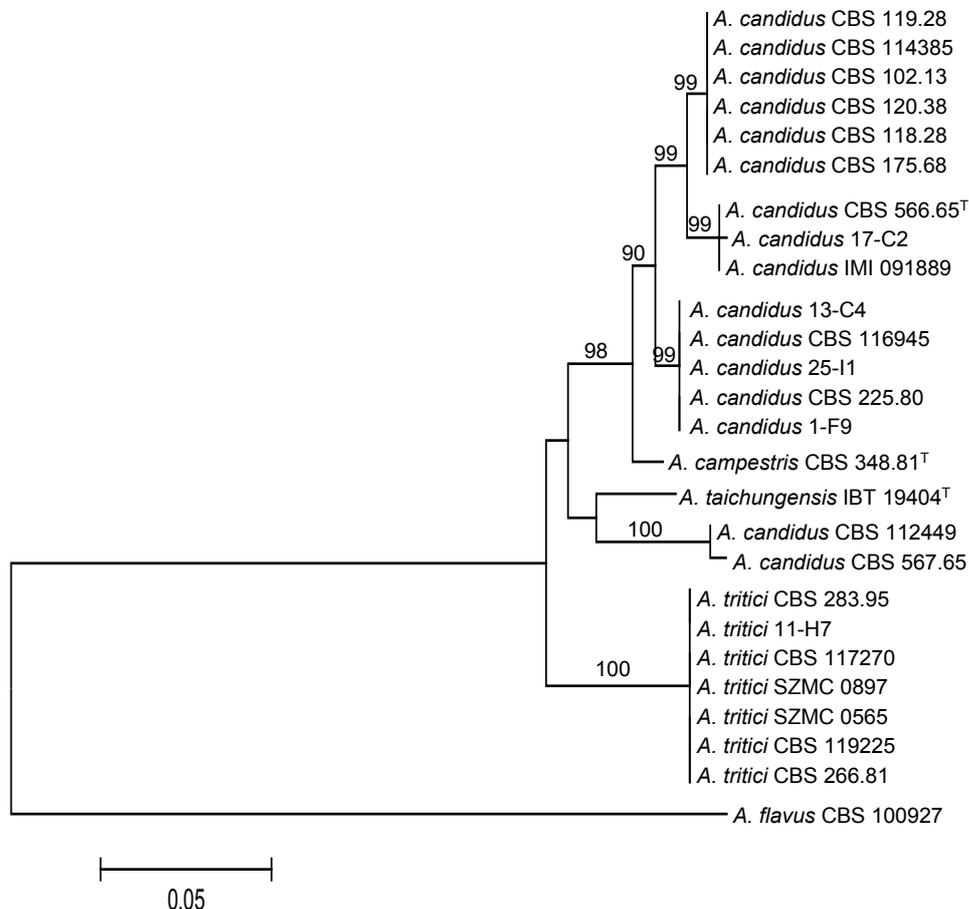


Fig. 1. Neighbour-joining tree based on  $\beta$ -tubulin sequence data of *Aspergillus* section *Candidi*. Numbers above branches are bootstrap values. Only values above 70 % are indicated.

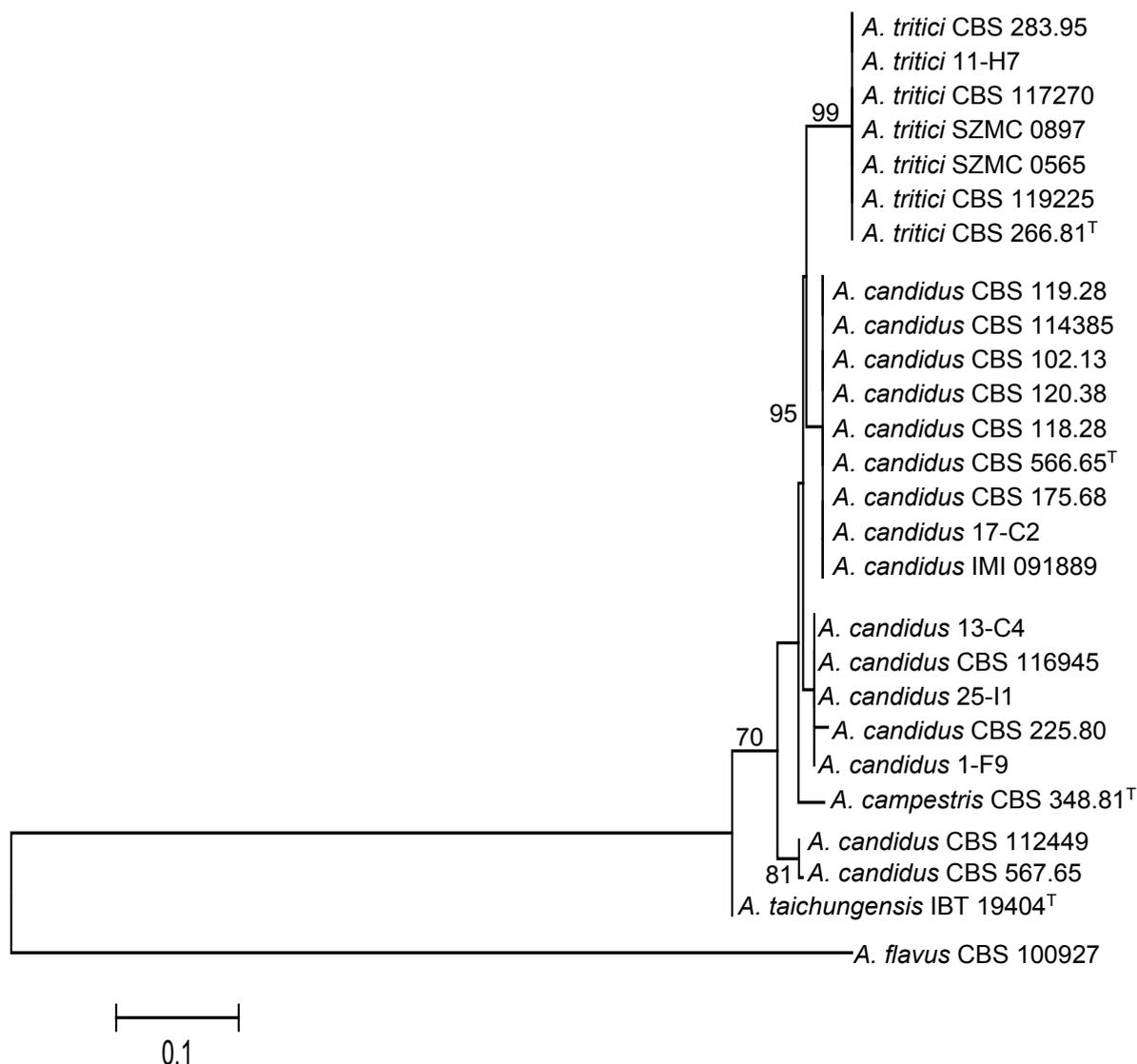


Fig. 2. Neighbour-joining tree based on calmodulin sequence data of *Aspergillus* section *Candidi*. Numbers above branches are bootstrap values. Only values above 70 % are indicated.

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 Neighbour-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 Neighbour 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  $\beta$ -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  $\beta$ -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.nbrc.nite.go.jp/jscc/idb/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

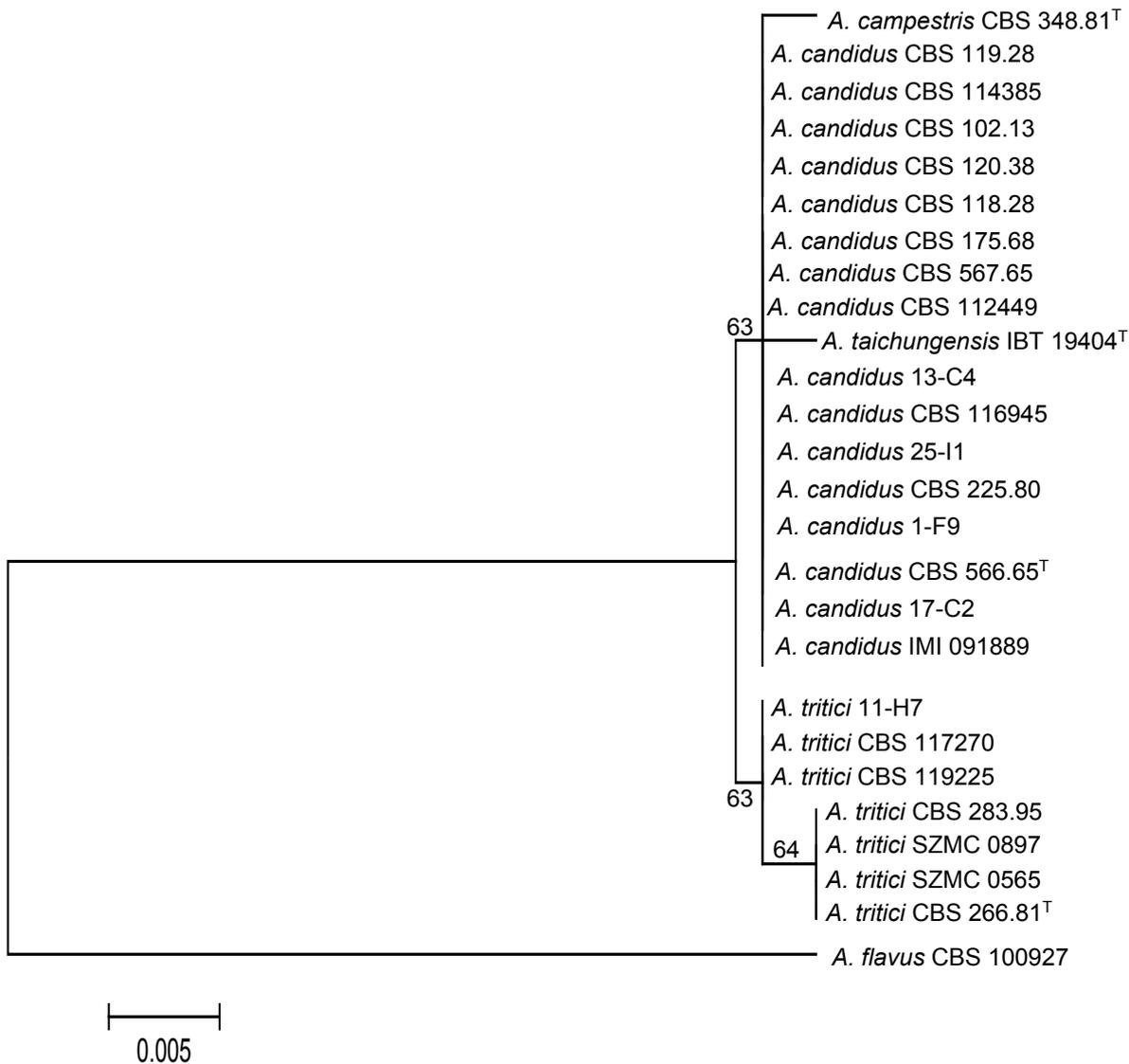


Fig. 3. Neighbour-joining tree based on ITS sequence data of *Aspergillus* section *Candidi*. Numbers above branches are bootstrap values. Only values above 70 % are indicated.

also produced chlorflavin and a chlorflavin analogue. *A. tritici* isolates differed from *A. candidus* in not producing candidusin A and chlorflavin. *A. taichungensis* produced candidusin C, terphenyllin, and 3-hydroxyterphenyllin, while the type strain of *A. campestris* also produced chlorflavin. 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, chlorflavins 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 biseriata large heads. Colonies on CYA and MEA usually slow growing, colonies white to cream coloured, reverse

usually uncoloured. Conidial heads usually biseriata, 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 or subglobose in most strains to elliptical in others, thin walled, 2.5–3.5 µm or occasionally 4 µm, smooth, colourless. Sclerotia, when produced, at first white, quickly becoming reddish purple to black, consisting of thick-walled parenchyma-like cells. *A. candidus* is unable to grow at 37 °C.

*Aspergillus taichungensis* was described by Yaguchi *et al.* (1995) from soil, Taiwan. The species is characterised by restricted growth on CZA and MEA at 25 °C, colonies white to pale yellow, velvety, reverse uncoloured. Conidial heads radiate, biseriata, conidiophores smooth, 300–450 µm long, often diminutive (90–250 µm long, biseriata), vesicles hemispherical to elongate, 5–20 µm in diam, fertile over the upper half to two-thirds, conidia hyaline, yellow in mass, globose to subglobose, microverrucose, 3–4 µm.

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

	<i>A. candidus</i>	<i>A. tritici</i>	<i>A. taichungensis</i>	<i>A. campestris</i>
<b>Morphological characteristics</b>				
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
Diminutive heads	Common	Common	Common	Common
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 37°C	-	+	+	-
Sclerotia	Purple to black	Purple to black	Dark brown	-
<b>Extrolite production</b>				
Candidusin A	+	-	-	-
Candidusin B	+	+	-	-
Candidusin C	-	-	+	+
Candidusin analogue	-	+	-	-
terphenyllin	+	+	+	+
3-hydroxyterphenyllin	+	+	+	-
chlorflavonin	+	+	-	+
chlorflavonin analogue	+	-	-	-

Dark brown sclerotia which appear on MEA after more than 25 d incubation. *A. taichungensis* is able to grow at 37 °C 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 °C, colonies velvety, sulphur yellow, reverse uncoloured. Conidial heads biserial, radiate, conidiophores usually 400–800 µm but can be up to 1300 µm long, smooth, often diminutive (up to 100 µm long, biserial), 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 °C 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 biserial, radiate, conidiophores thick-walled, septate, 130–700 µm long, often diminutive (10–75 µm, sometimes uniseriate), 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 °C.

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.



**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.

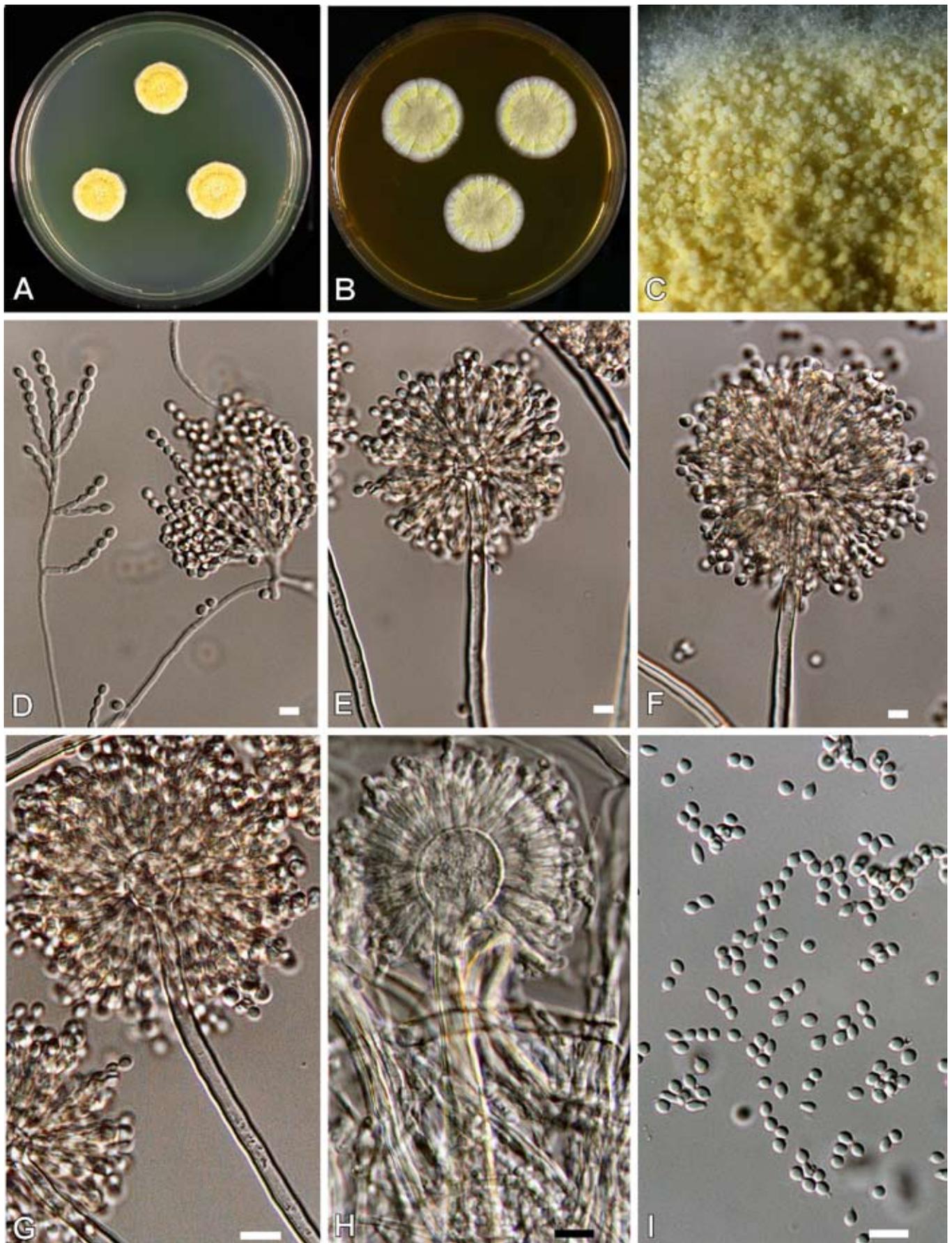


Fig. 5 *Aspergillus campestris*. A–B Colonies after 7 d at 25 °C A. CYA. B. MEA. C. Conidial heads. D–H. Conidiophores. I. Conidia. Scale bars = 10 μm.

***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  
Conidiation: abundant  
Reverse colour (CZA): uncoloured  
Colony texture: velvety  
Conidial head: radiate, splitting in age  
Stipe: 400–800(–1300) × 7–12 µm  
Vesicle diam/shape: (18–)24–36(–46) µm, globose to subglobose  
Conidium size/shape/surface texture: 3–4 × 2.3–3 µm, ellipsoidal to egg-shaped, smooth

**Cultures examined:** KACC 42091, KACC 42090 = IBT 27920, KACC 41955 = IBT 3016, UAMH 1324 (from mouse, Canada, as *A. sulphureus*), IBT 17867

**Diagnostic features:** restricted growth on all media, sulphur yellow colony colour and diminutive conidial heads

**Similar species:** -

**Ecology and habitats:** soil

**Distribution:** U.S.A., Canada

**Extrolites:** candidusin C, terphenyllins, chlorflavonin (Rahbaek *et al.* 2000), confirmed in this study

**Pathogenicity:** not reported

**Note:** Diminutive conidial heads commonly produced (100 × 10–12 µm)

***Aspergillus candidus*** Link, Mag. Ges. Naturf. Freunde Berlin 3: 16. 1809. Fig. 5.

= *Aspergillus okazakii* Okazaki (1907)

**Type:** CBS 566.65, from Westerdijk, 1909

**Other no. of the type:** ATCC 1002; IMI 091889; LSHB Ac27; NCTC 595; NRRL 303; QM 1995; WB 303

#### Description

Colony diam: CZA25: 15–30 mm; CYA25: 13–20 mm, MEA25: 8–14 mm, YES25: 19–33 mm, OA25: 9–18 mm, CYA37: 0 mm, CREA25: poor growth and no acid production  
Colony colour: white  
Conidiation: limited  
Reverse colour (CZA): uncoloured to pale yellow  
Colony texture: submerged  
Conidial head: diminutive, with few divergent spore chains  
Stipe: 500–1000 × 5–10(–20) µm, walled, smooth, occasionally septate, colourless or slightly yellowed in age  
Vesicle diam/shape: 10–40 µm, globose to subglobose  
Conidium size/shape/surface texture: 2.5–3.5(–4) µm, globose to subglobose, smooth

**Cultures examined:** CBS 119.28, CBS 116945, CBS 175.68, CBS 114385, CBS 120.38, CBS 225.80, CBS 102.13, CBS 118.28, CBS 566.65: 1-F9, 13-C4, 17-C2, 25-I1, IMI 091889, CBS 283.95, NRRL 5214

**Diagnostic features:** phialides clustered on one side of the vesicle, echinulate conidia, slow growth rate and cream-yellow reverse on CYA; unable to grow at 37 °C

**Similar species:** *A. tritici*

**Distribution:** worldwide (Bangladesh, Pakistan, Kuwait, Sri Lanka, Japan, South Africa, Somalia, Chad, Libya, Egypt, Syria, Israel, Argentina, Bahama Islands, New Guinea, Solomon Islands, China, Central America, Chile, Russia, Nepal, U.S.A., Spain, Italy, Hungary, Austria, Czechoslovakia, Germany, France, Britain, Ireland, Netherlands, Denmark)

**Ecology and habitats:** stored products, especially cereals, soil, dried fruits, dung, dried fish, indoor air

**Extrolites:** terphenyllin, 3-hydroxyterphenyllin (Rahbaek *et al.* 2000), prenylterphenyllin, 4<sup>'''</sup>-deoxyrenylterphenyllin, 4<sup>'''</sup>-deoxyisoterpenin, 4<sup>'''</sup>-deoxyterpenin (Wei *et al.* 2007), and other terphenyl-type compounds (Marchelli & Vining 1975 Kurobane *et al.* 1979; Kobayashi *et al.* 1985; Takahashi *et al.* 1976b), including candidusins (A & B) (Kobayashi *et al.* 1982) and other terpenins (Kamigauchi *et al.* 1998), chlorflavonin (Bird & Marshall 1969; Munden *et al.* 1970), dechlorochlorflavonin (Marchelli & Vining 1973), and xanthoascin (Takahashi *et al.* 1976a). The production of terphenyllin, 3-hydroxyterphenyllin, candidusin A, candidusin B, chlorflavonin and xanthoascin was confirmed by HPLC-DAD.

**Extrolites not produced by *A. candidus*:** kojic acid (Kinosita & Shikata 1969; Cole & Cox 1981), and 3-nitro-propionic acid (Kinosita *et al.* 1968) were reported from the same strain of *A. candidus* of which ATCC 44054 is representative. A re-examination of that strain showed that it was a white-spored mutant of *Aspergillus flavus*, a known producer of these two metabolites. The asterriquinone analogs, neoasterriquinone and isoasterriquinone (Alvi *et al.* 1999) have not been found in any strains of *A. candidus* by us. These asterriquinone analogues are probably produced by *A. niveus*, but this has to be confirmed by examination of isolates of the latter species. Citrinin production was observed in some studies (Timonin & Rouatt 1944; Kinosita & Shikata 1969), but the producing fungus was later identified as *A. niveus* (NRRL 1955, Raper and Fennell, 1965). The production of 6-sulfoaminopenicillanic acid by *A. candidus* (Yamashita *et al.* 1983) has not been confirmed

**Pathogenicity:** Pathogenicity of *A. candidus* is rather improbable, as this species cannot grow at 37 °C., however pathogenicity has often been reported: *A. candidus* has been claimed to be involved in a wide range of human infections including invasive aspergillosis (Rippon 1988; Ribeiro *et al.* 2005), pulmonary aspergillosis (Iwasaki *et al.* 1991), aspergilloma (Avanzini *et al.* 1991), otomycosis (Yasin *et al.* 1978; Falser 1983), brain granuloma (Linares *et al.* 1971) and onychomycosis (Kaben 1962; Fagner & Kubackova 1974; Cornere & Eastman 1975; Piraccini *et al.* 2002; Schonborn & Schmoranzner 1970; Zaror & Moreno 1980); also caused various disorders in pigs (Moreau 1979). In these cases it is more likely caused by white spored mutants of *A. flavus* or by *A. tritici*

**Note:** young heads varying in the same culture from globose masses 200 to 300 µm in diam to small heads less than 100 µm in diam; some isolates produce purple to black sclerotia

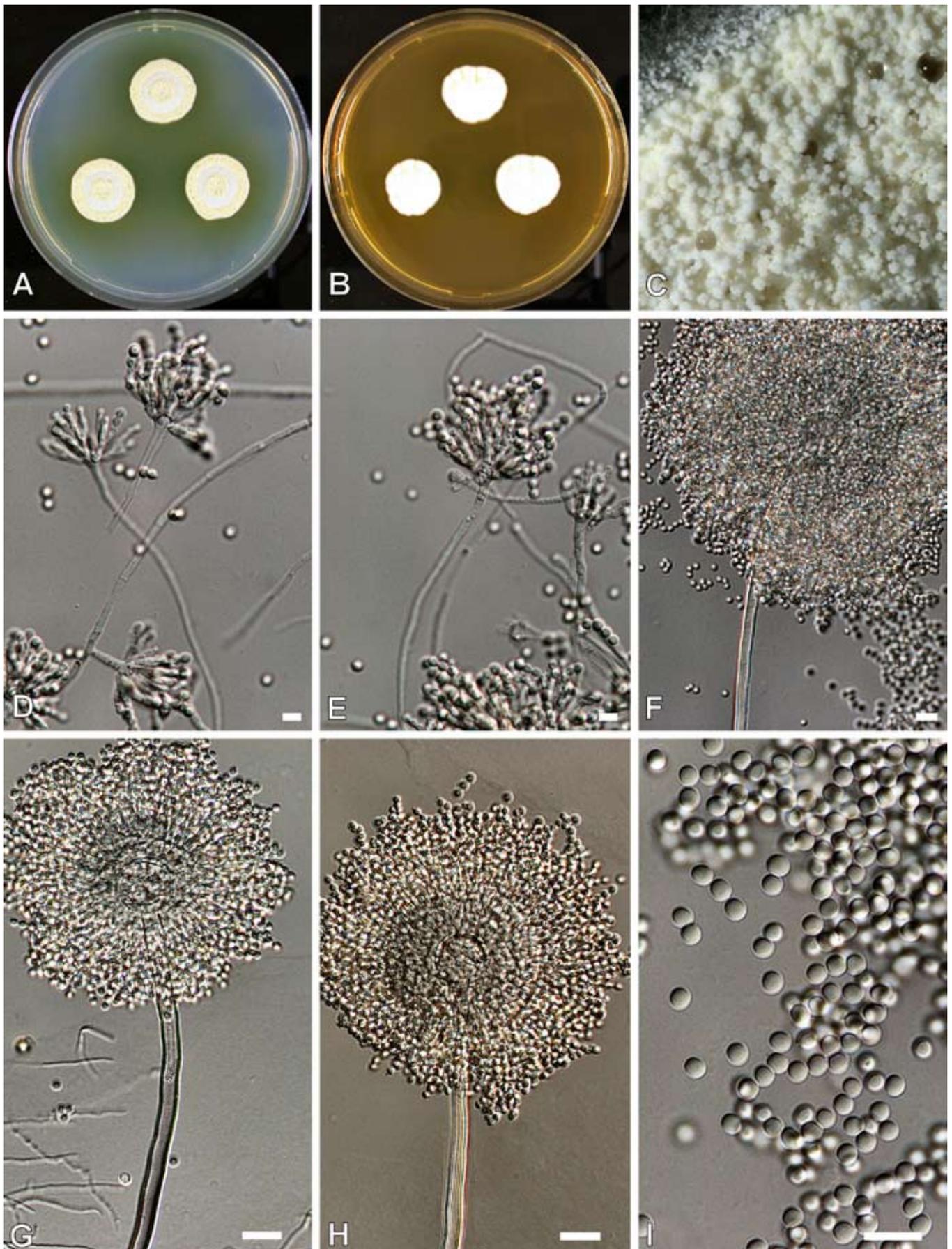
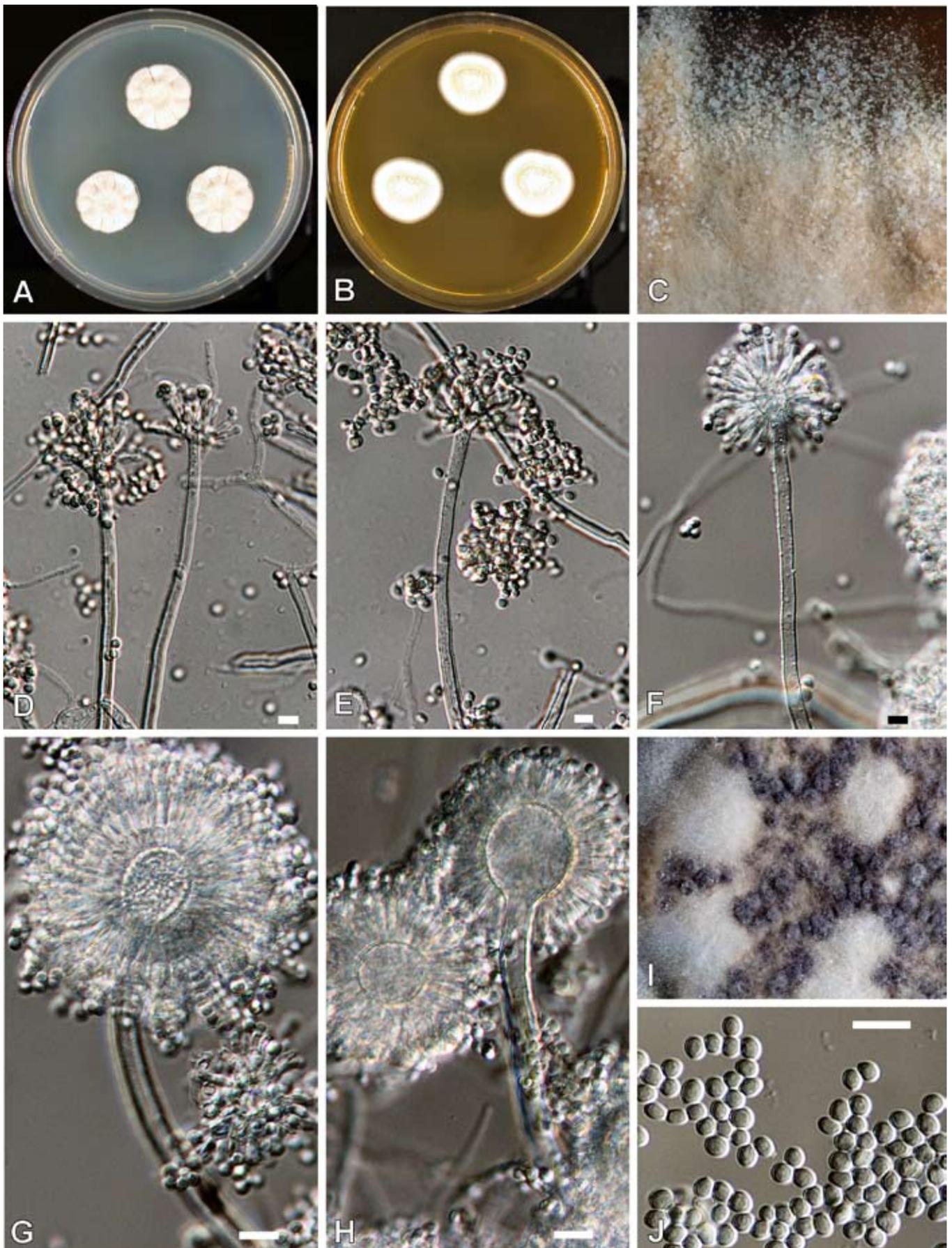


Fig. 6. *Aspergillus taichungensis*. A–B Colonies after 7 d at 25 °C. A. CYA. B. MEA. C. Conidial heads. D–H Conidiophores. I. Conidia. Scale bars = 10 μm.



**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. Sclerota. 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: floccose (MEA)

Conidial head: loose radiate

Stipe: 300–440 × 5–9 µm

Vesicle diam/shape: 5–20 µm, hemispherical to elongate

Conidium size/shape/surface texture: 3–4 µm, globose to subglobose; sometimes ovoid, 3–5 × 3–4.5 µm, microverrucose

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

**Diagnostic features:** slow growing colonies 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

**Distribution:** Taiwan, Brazil, Germany

**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

**Pathogenicity:** not reported

**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, shape: 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 283.95, 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

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