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Review

Aspergillus flavus: human pathogen, allergen and mycotoxin producer

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Aspergillus infections have grown in importance in the last years. However, most of the studies have focused on *Aspergillus fumigatus*, the most prevalent species in the genus. In certain locales and hospitals, *Aspergillus flavus* is more common in air than *A. fumigatus*, for unclear reasons. After *A. fumigatus*, *A. flavus* is the second leading cause of invasive aspergillosis and it is the most common cause of superficial infection. Experimental invasive infections in mice show *A. flavus* to be 100-fold more virulent than *A. fumigatus* in terms of inoculum required. Particularly common clinical syndromes associated with *A. flavus* include chronic granulomatous sinusitis, keratitis, cutaneous aspergillosis, wound infections and osteomyelitis following trauma and inoculation. Outbreaks associated with *A. flavus* appear to be associated with single or closely related strains, in contrast to those associated with *A. fumigatus*. In addition, *A. flavus* produces aflatoxins, the most toxic and potent hepatocarcinogenic natural compounds ever characterized. Accurate species identification within *Aspergillus flavus* complex remains difficult due to overlapping morphological and biochemical characteristics, and much taxonomic and population genetics work is necessary to better understand the species and related species. The *flavus* complex currently includes 23 species or varieties, including two sexual species, *Petromyces alliaceus* and *P. albertensis.* The genome of the highly related *Aspergillus oryzae* is completed and available; that of *A. flavus* in the final stages of annotation. Our understanding of *A. flavus* lags far behind that of *A. fumigatus*. Studies of the genomics, taxonomy, population genetics, pathogenicity, allergenicity and antifungal susceptibility of *A. flavus* are all required.

Introduction

The aspergilli have always been a factor in the human environment. Micheli was the first to distinguish stalks and spore heads, but it was not until the middle of the 19th century that these fungi began to be recognized as active agents in decay processes, as causes of human and animal disease and as fermenting agents capable of producing valuable metabolic products (Raper & Fennel, 1965).

First described by Link (1809), *Aspergillus flavus* is the name now used to describe a species as well as a group of closely related species. *A. flavus* is second only to *A. fumigatus* as the cause of human invasive aspergillosis. In addition, it is the main *Aspergillus* species infecting insects (Campbell, 1994), and it is also able to cause diseases in economically important crops, such as maize and peanuts, and to produce potent mycotoxins. The purpose of this review is to summarize the current knowledge about this important group of fungi.

A supplementary table showing more details of the features of members of *Aspergillus flavus* complex is available with the online version of this paper.

Ecology and geographical distribution

Like other *Aspergillus* species, *A. flavus* has a worldwide distribution. This probably results from the production of numerous airborne conidia, which easily disperse by air movements and possibly by insects. Atmosphere composition has a great impact on mould growth, with humidity being the most important variable (Gibson *et al.*, 1994). *A. flavus* grows better with water activity (a_w) between 0.86 and 0.96 (Vujanovic *et al.*, 2001). The optimum temperature for *A. flavus* to grow is 37 °C, but fungal growth can be observed at temperatures ranging from 12 to 48 °C. Such a high optimum temperature contributes to its pathogenicity in humans.

Soil

A. flavus appears to spend most of its life growing as a saprophyte in the soil, where it plays an important role as

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nutrient recycler, supported by plant and animal debris (Scheidegger & Payne, 2003). The ability of *A. flavus* to survive in harsh conditions allows it to easily out-compete other organisms for substrates in the soil or in the plant (Bhatnagar *et al.*, 2000). The fungus overwinters either as mycelium or as resistant structures known as sclerotia. The sclerotia either germinate to produce additional hyphae or they produce conidia (asexual spores), which can be further dispersed in the soil and air.

Outdoor air

A. flavus has been particularly prevalent in the air of some tropical countries (Moubasher *et al.*, 1981; Abdalla, 1988; Gupta *et al.*, 1993; Adhikari *et al.*, 2004). Climatic conditions markedly influence the prevalence of *A. flavus* in outdoor air. As an example, two Spanish studies revealed very different results. In Barcelona *A. flavus* and *A. niger* were the most frequent airborne aspergilli (Calvo *et al.*, 1980) whereas in Madrid *A. fumigatus* was the most prevalent species (54 %) (Guinea *et al.*, 2005). Comparing *Aspergillus* species in the air in London, Paris, Lyon and Marseille, Mallea *et al.* (1972) showed that *A. glaucus* and *A. versicolor* group predominated in southern France. On the other hand, *A. fumigatus* represented more than 35 % of the isolates recovered from Paris and London, whereas *A. glaucus* group never exceeded 20 % (Mallea *et al.*, 1972). In Brussels, *A. fumigatus* was the most common *Aspergillus* species whereas *A. flavus* represented only 1 % of isolates (Vanbreuseghem & Nolard, 1985).

Home and hospital air

The presence of *Aspergillus* in the air is a major risk factor for both invasive and allergic aspergillosis (Denning, 1998). Accordingly, several outbreaks of invasive aspergillosis have been associated with construction and/or renovation activities in and around hospitals (Sarubbi *et al.*, 1982; VandenBergh *et al.*, 1999), activities that markedly increase the number of spores in the air. Also, in several studies the link between infection by *A. flavus* and the contamination of the environment was clearly demonstrated by molecular typing methods (Rath & Ansorg, 1997; Diaz-Guerra *et al.*, 2000) (see below). In two studies from Iran, A. *flavus* was the most prevalent *Aspergillus* species to be recovered from the air of hospital wards and homes (Zaini & Hedayati, 1995; Hedayati *et al.*, 2005).

Water

Fungi in drinking water may alter the taste and odours of the water. Health problems are possible, including mycotoxin exposure, direct infection and allergy. More studies are needed on this subject. Surveys of fungi in drinking water have recovered many different taxa, including *A. flavus* (Gottlich *et al.*, 2002; Goncalves *et al.*, 2006) and in particular *A. fumigatus* (Warris *et al.*, 2001; Anaissie *et al.*, 2002). Contamination tends to arise from surface reservoirs and not from deep ground wells (Warris *et al.*, 2001). This variation is often attributed to factors such as raw water source (surface versus well), water temperature patterns, treatment patterns and maintenance of distribution systems. Additionally, it was reported that fungi can pass through treatment processes by means of leaks in the system, or from air in contact with water stored in distribution system reservoirs, and can even survive water disinfection with chlorine (Niemi *et al.*, 1982). Interestingly, Paterson *et al.* (1997) detected aflatoxin in water and identified *A. flavus* from a cold-water storage tank.

Genome

The recent sequencing of the *A. oryzae* genome sequence provides an excellent tool for researchers to gain insight into the basic biology of this organism (Machida *et al.*, 2005; Galagan *et al.*, 2005). The sequencing of *A. flavus* (NRRL 3357, Geiser Group 1C) is in progress, and will provide a rich source of comparative data. The primary assembly indicates that the *A. flavus* genome is 36.3 Mb in size and consists of eight chromosomes and 13 071 predicted genes. The mean gene length is 1384 bp (Yu *et al.*, 2005). *A. flavus* is genetically almost identical to *A. oryzae*. Comparative genomics will be particularly interesting as *A. flavus* is a common environmental organism whilst the sequence strain of *A. oryzae* is a 'domesticated' fungus, having been used in soy fermentation for thousands of years, and rarely causes disease.

Taxonomy

Classically, the systematics of *Aspergillus* and its associated teleomorphs have been based primarily on differences in morphological and cultural characteristics (Raper & Fennel, 1965; Samson *et al.*, 2000). *Petromyces alliaceus* and *P. albertensis* are the only two sexually reproducing species (teleomorphs) classified in *Aspergillus flavus* complex (Table 1) (Frisvad *et al.*, 2005). They were characterized by ascomata produced within closed sclerenchymatous stromata. The genus *Petromyces* belongs to the family Trichocomaceae of the order Eurotiales of Ascomycetes. Moreover, the taxonomy of the *flavus* complex group is further complicated by the existence of morphological divergence amongst isolates of the same species (Klich & Pitt, 1988).

Raper & Fennell (1965) considered the *A. flavus* group to contain nine species and two varieties, including *A. flavus*, *A. flavus* var. *columnaris*, *A. parasiticus*, *A. oryzae*, *A. oryzae* var. *effusus*, *A. zonatus*, *A. clavato-flavus*, *A. tamarii*, *A. flavo-furcatis*, *A. subolivaceus* and *A. avenaceus*. We have summarized the current species described morphologically in Table 1 (see also supplementary Table S1, available with the online version of this paper, for more data); there appear to be 23 published species or varieties. Despite the growing use of molecular genetic techniques to study the

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Table 1. Microscopic features and reported diseases of *Aspergillus flavus* complex

Fuller details are are provided in Supplementary Table S1, available with the online version of this paper.

Species	Disease	Microscopic features
A. flavus Link (1809)	The main agent of acute and chronic invasive and granulomatous Aspergillus sinusitis. Agent of otitis, keratitis, pulmonary and systemic infections in immunocompromised patients, cutaneous aspergillosis and aspergillosis in other vertebrates.	**Conidiophores** are heavy walled, uncoloured, coarsely roughened, usually less than 1 mm in length. **Vesicles** are elongate when young, later becoming subglobose or globose, varying from 10 to 65 μm in diameter. **Phialides** are uniseriate or biseriate. The primary branches are up to 10 μm in length, and the secondary up to 5 μm in length. **Conidia** are typically globose to subglobose, conspicuously echinulate, varying from 3.5 to 4.5 μm diameter.
A. oryzae (Ahlburg) Cohn (1883)	A rare agent of paranasal sinusitis, meningitis, cerebritis, pulmonary infections, ABPA, otomycosis and scleritis. There is one report of involvement in a kidney infection in an albatross.	**Conidiophores** are up to 4–5 mm in length, colourless, with walls relatively thin, definitely roughened throughout all or most of their length. **Vesicles** are sub-spherical, stigmata covering the entire surface or the upper three-fourths, up to 75 μm diameter. **Phialides** are uniseriate and biseriate, covering the entire surface or the upper three-fourths of the vesicle. **Conidia** are (sub)spherical to ovoidal, 4.5–8(–10) × 4.5–7 μm, smooth-walled to roughened, greenish to brownish.
A. subolivaceus Raper & Fennel (1965)	No documented disease.	**Conidiophores** are variable in length from 300 to 1300 μm, walls uncoloured, definitely echinulate. **Vesicles** are subglobose to somewhat elongate, variable in diameter but usually 30–55 μm × 35–60 μm. **Phialides** are biseriate, primaries 6.5–20.0 × 4.5–7.5 μm, secondaries 7.0–10.0 × 3.3–4.0 μm. **Conidia** are elliptical and delicately roughened when first formed, becoming smooth or nearly so, predominantly elliptical and mostly 4.0–4.5 × 3.0–3.5 μm.
A. thomii Smith (1951)	No documented disease.	**Conidiophores** are uncoloured, conspicuously roughened or echinulate throughout their entire length, up to 1200–1500 μm long. **Vesicles** are globose to pyriform ranging from 20–50 μm in diameter. **Phialides** are typically biseriate, primaries 6–10 × 3.3–4.0 μm, secondaries and uniseriate stigmata 6–9 × 2.2–2.8 μm. **Conidia** are elliptical and hyaline when first formed, becoming subglobose, brownish yellow and definitely roughened at maturity, varying in diameter from 3.0 to 5.5 μm.
A. terricola var. *americana* Thom & Church (1921)	No documented disease.	**Conidiophores** are 300–600 μm in length by 6–8 μm in diameter, with walls appearing granulose, uncoloured. **Vesicles** are globose to subglobose, up to 25 μm in diameter, fertile over the upper two-thirds or three-fourths. **Phialides** are usually in one series, primaries when present 7.5–9.0 × 4.5–6.0 μm, secondaries 7–10 × 3.3–4.5 μm with tips often phialiform. **Conidia** are commonly ovate to nearly globose at maturity, mostly 4.5–5.5 × 3.8–5.0 μm, rugulose, brownish yellow.
A. parasiticus Speare (1912)	No documented disease.	**Conidiophores** are variable in length, mostly 300–700 μm long, with walls colourless, smooth or nearly so in some strains, in others smooth below and definitely roughened above. **Vesicles** are 20–35 μm in diameter. **Phialides** are in one series, 79 × 3.0–4.0 μm, colourless or in pale yellow-green shades. **Conidia** are globose, coarsely echinulate, 3.5–5.5 μm in diameter, bright yellow-green.
A. sojae Sakaguchi & Yamada (1944)	No documented disease.	**Conidiophores** are short with walls smooth but sometimes roughened. **Vesicles** are subglobose to clavate, 10–30 μm. **Phialides** are definitely uniseriate. **Conidia** are globose, prominently echinulate, usually 5–6 μm.
A. toxicarius Murakami (1971)	No documented disease.	**Conidiophores** are roughened, usually less than 1 mm in length. **Vesicles** are globose to subglobose, 30–40 μm. **Phialides** are definitely biseriate. **Conidia** are globose, prominently echinulate, usually 4–5 μm.
A. caelatus Horn (1997)	No documented disease.	**Conidiophores** are hyaline, finely roughened; length variable, mostly 200–800 μm. **Vesicles** are globose or subglobose, 15–38 μm in diameter, fertile over upper three-fourths. **Phialides** are 5.0–11.0 × 3.0–6.0 μm. **Conidia** are globose and thick-walled, with spore body 5.0–6.0 μm in diameter; ornamentation coarse, consisting of tubercles and short bars <1.5 μm high.

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Table 1. cont.

Species	Disease	Microscopic features
A. pseudotamarii Ito *et al.* (2001)	No documented disease.	**Conidiophores** are hyaline, finely roughened. **Vesicles** are globose to subglobose, 26–38 μm. **Phialides** are 4.5–6.1 × 3.1–4.5 μm. **Conidia** are globose to subglobose, echinulate; variable in diameter, 3.9–9.9 μm.
A. coremiiformis Bartoli & Maggi (1978)	No documented disease.	**Conidiophores** are simple or in coremia, straight, up to 1500 μm long by 20–45 μm wide, thick-walled, walls roughened to echinulate, rarely smooth. **Vesicles** are light to deep tawny, subglobose to elongate, fertile over almost the entire surface, 50–60 × 45–60 μm. In coremia, vesicles are obclavate and spathulate, 90–150 × 85–100 μm. **Phialides** are biseriate, each series composed of closely packed sterigmata, 15–18 × 4–4.5 μm. **Conidia** are yellowish in mass, very variable in shape, from globose to oval, oblong or cylindrical, more or less encrusted, mostly 6.9–9 μm.
A. flavofurcatis Batista & da Silva Maia (1955)	No documented disease.	**Conidiophores** are erect, usually 1–2 mm in height by 11–20 μm in diameter, uncoloured, delicately granular. **Vesicles** are flask-shaped to subglobose, mostly 20–40 μm, fertile over most of their surface in large head. **Phialides** are biseriate, mostly 10–14 × 6.5–8.0 μm, conspicuously flared and thickened. **Conidia** are subglobose to globose at maturity, mostly 6.0–8.0 μm, distinctively demonstrating an olive-brown to dark brown colour change.
A. terricola var. *indica* Mehrotra & Agnihotri (1962)	No documented disease.	**Conidiophores** are usually short and branched commonly 50–150 × 6.6–10.0 μm, colourless and usually smooth. **Vesicles** are globose to subglobose, those of smaller heads 9–12 μm in diameter and fertile over the upper half, in larger heads 20–34 μm in diameter and fertile over most of the surface. **Phialides** are mostly uniseriate, 8.4–9.8 × 5.0–7.0 μm. **Conidia** are globose, varying from 4.5 to 9.8 μm in diameter, brownish yellow and conspicuously echinulate at maturity.
A. terricola Marchal (1893)	No documented disease.	**Conidiophores** vary in length but reaching 0.5–1.0 mm, uncoloured, smooth or somewhat granulose. **Vesicles** are subglobose to hemispherical, mostly 20–45 μm. **Phialides** are uniseriate or biseriate, with both conditions often observed in the same head, 7–15 × 4.5–8.0 μm. **Conidia** are subglobose to globose at maturity, extremely variable in size, 4.5–9.0 μm, brownish yellow, coarsely echinulate.
A. tamarii Kita (1913)	Implicated in a case of eyelid infection.	**Conidiophores** are usually 1–2 mm in length, hyaline, usually roughened. **Vesicles** are spherical, 10–50 μm. **Phialides** are uniseriate and biseriate, covering the entire surface of the vesicle. **Conidia** are echinulate to tuberculate, subspherical, 5–8 μm.
P. alliaceus Thom & Church (1926)	One report of a chronic otitis externa after surgery.	**Conidiophores** are smooth-walled, up to 1.2 mm long, hyaline. **Vesicles** are spherical, occasionally somewhat elongate. **Phialides** are biseriate or uniseriate, covering at least the upper half of the vesicle. **Conidia** are ovoidal to subspherical, smooth walled, yellow, 2.5–4 × 2–3.5 μm.
P. albertensis Tewari (1985)	No documented disease.	**Conidiophores** are commonly up to 3500 × 17.6 μm, somewhat sinuous, generally smooth. **Vesicles** are up to 95 μm, globose to subglobose. **Phialides** and metulae present in all heads, wedge-shaped, non-septate, 4.6–23 × 2–7 μm, phialides 5.7–9.5 × 1.7– 2.5 μm. **Conidia** are oval to subglobose, smooth 2.3–3.5 × 1.7–3.5 μm.
A. lanosus Kamal & Bhargava (1969)	No documented disease.	**Conidiophores** are commonly 1.5–3 mm, septate with smooth wall, sometimes with true branches. **Vesicles** are hemispherical to subglobose, 15–30 μm, often fertile over half to two-thirds of surface. **Phialides** are crowded, biseriate, primaries 8–12 × 3.3–4.4 μm, secondaries 6.5–8.8 × 2.2–2.3 μm. **Conidia** are smooth, globose to subglobose, 2.2–2.8 μm.
A. robustus Christensen & Raper (1978)	No documented disease.	**Conidiophores** are smooth to very slightly roughened, mostly 750–5000 × 10–21 μm. **Vesicles** are globose to somewhat elongate, thick-walled, fertile over the entire surface, mostly 40–70 μm. **Phialides** are 9.0–12.6 × 3.4–4.5 μm. **Conidia** are echinulate, thin-walled ellipsoidal, mostly 3.5–4.5 × 2.8–3.4 μm.
A. leporis States & Christensen (1966)	No documented disease.	**Conidiophores** are extremely variable, commonly 250–1100 μm, echinulate and uncoloured or pale yellow. **Vesicles** are thicked-walled, fertile over three-fourths or more of the surface, globose to subglobose, mostly 20–50 μm. **Phialides** are biseriate, metulae 6.5–16 × 4.5–5 μm. **Conidia** are globose to subglobose, smooth to delicately roughened, mostly 3–3.5 μm.
A. nomius Kurtzman *et al.* (1987) (*A. zhaoqingensis*)	No documented disease.	**Conidiophores** are uncoloured, echinulate; variable in length, mostly 300–1100 μm. **Vesicles** are globose to subglobose, 25–65 μm. **Phialides** are 3.8–6.5 × 7.6–11.3 μm. **Conidia** are globose to subglobose, echinulate; mostly 4.5–6.5 μm.

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Table 1. cont.

Species	Disease	Microscopic features
A. avenaceus Smith (1943)	No documented disease.	**Conidiophores** are commonly 1–2 mm, colourless, smooth in fluid mounts, but appearing finely roughened when examined dry. **Vesicles** are globose or slightly flattened, thick-walled, mostly 75–100 μm. **Phialides** are biseriate, primary 20–50 μm, secondary 8–13 × 3–4 μm. **Conidia** are ellipsoid, smooth, mostly 4.0–5.0 μm.
A. bombycis Peterson *et al.* (2001)	No documented disease.	**Conidiophores** are smooth-walled; 300–500 × 10–20 μm. **Vesicles** are globose, 30–50 μm, fertile over entire surface. **Phialides** are 3–4 × 8 μm, flask-shaped, metulae cylindrical 4–5 × 10–12 μm. **Conidia** are roughened, globose to subglobose, (3.5)–4–7–(8.5) μm in diameter.
A. qizutongi Li *et al.* (1998)	One report of a maxillary aspergilloma.	**Conidiophores** are 100–300(–500) μm long, straight to sinuous, roughened, septate, swollen in the upper part. **Vesicles** are flask-shaped, 9–17 μm in diameter, fertile over the upper half or three-fourths; often proliferating. **Phialides** are uniseriate, 7–12 × 2–4 μm. **Conidia** are hyaline, globose to ovoid, 5–6.5(–7) μm in diameter, smooth.
A. beijingensis Li *et al.* (1998)	One report of a maxillary aspergilloma.	**Conidiophores** are100–550 μm long, 4–5.5 μm in diameter, septate, thick-walled, smooth. **Vesicles** are clavate, sometimes flask-shaped, 7–11(–14) μm in diameter, fertile over the upper half. **Phialides** are 10–19 × 4–5 μm. **Conidia** are globose to subglobose, 3.5–6.5 × 3.5–5.5 μm, micro-verrucose.

phylogeny of these fungi (Kurtzman *et al.*, 1986; Klich & Mullaney, 1987), morphological and cultural characteristics are still routinely used for identification because of their simplicity, accessibility and feasibility.

Restriction fragment length polymorphism (RFLP) has been used to distinguish between *A. flavus* and *A. oryzae* and to infer phylogenetic relationships (Bruns *et al.*, 1991; Montiel *et al.*, 2003). Moody & Tyler (1990) demonstrated that restriction profiles of purified mitochondrial DNA can distinguish *A. flavus* Link, *A. parasiticus* Speare and *A. nomius* Kurtzman *et al.* However, for routine identification of *Aspergillus* isolates it is desirable to detect mitochondrial DNA RFLP without first separating the mitochondrial DNA from the nuclear DNA (Bruns *et al.*, 1991).

Wang *et al.* (2001) described the use of partial sequences of the mitochondrial cytochrome *b* gene (402 bp) to differentiate 77 isolates in the *Aspergillus flavus* complex into seven DNA types (D-1 to D-7). *A. sojae* were defined as D-1, *A. parasiticus* as D-2, *A. flavus* and *A. oryzae* were grouped together as D-4, *A. tamarii* was defined as D-5 and *A. nomius* as D-7. Furthermore, D-3 was found to be closely related to *A. parasiticus* (D-2), also including one strain that had been deposited as *A. flavus* var. *flavus*. DNA type D-6 included one strain that was identified as *A. flavus* and was closely related to *A. tamari*. Peterson (2000) differentiated 17 type strains in the *flavus* complex based on rDNA sequence analysis. These included *A. flavus*, *A. oryzae*, *A. parasiticus*, *A. sojae*, *A. terricola* var. *americana*, *A. subolivaceus*, *A. kambarensis*, *A. flavus* var. *columnaris*, *A. thomii*, *A. tamarii*, *A. caelatus*, *A. leporis*, *A. nomius*, *Petromyces alliaceus*, *A. avenaceus*, *A. zonatus* and *A. clavatoflavus* (Table 1). The author also indicated that *A. zonatus* and *A. clavatoflavus* were not phylogenetically part of the *flavus* complex.

Single-strand conformation polymorphism of internal transcribed spacer (ITS) regions has also been used as a genetic approach to differentiate species in the *flavus* complex (Kumeda & Asao, 1996). This complex seems to comprise distinct clades (Rigo *et al.*, 2002). The three main clades (*P. alliaceus*, *A. flavus* and *A. tamarii*) could also be distinguished based on colony colour and their ubiquinone system. Based on ITS sequences *A. robustus*, *A. caelatus*, *A. lanosus*, *A. albertensis*, *A. coremiiformis*, *A. flavofurcatis*, *A. toxicarius*, *A. terricola* var. *indica*, *A. terricola* and the species mentioned by Peterson (2000) were all located in *Aspergillus flavus* complex. In addition, *A. pseudotamarii* and *A. bombycis* were found to be closely related to *A. caelatus* and *A. nomius*, respectively (Table 1). Rigo *et al.* (2002) suggested that *A. zonatus* and *A. clavatoflavus* should be excluded from *Aspergillus flavus* complex, a suggestion previously made by Kozakiewicz (1989), based on scanning electron microscopic studies. Recently, Frisvad *et al.* (2005) found that *A. toxicarius* resembles *A. parasiticus* but differs in at least three sequence differences in the ITS regions, as compared to four strains of *A. parasiticus*. Usually, the presence of three or more sequence

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differences in ITS regions is an indication of a different species. *A. zhaoqingensis* was considered the same as *A. nomius* in this study (Frisvad *et al.* 2005).

Identification

Accurate species identification within *Aspergillus flavus* complex remains difficult due to overlapping morphological and biochemical characteristics (Table 1). In general, *A. flavus* is known as a velvety, yellow to green or brown mould with a goldish to red-brown reverse (Fig. 1). The conidiophores are variable in length, rough, pitted and spiny. They may be either uniseriate or biseriate. They cover the entire vesicle, and phialides point out in all directions (Fig. 2). Conidia are globose to subglobose, conspicuously echinulate, varying from 3.5 to 4.5 μm in diameter. Based on the characteristics of the sclerotia produced, *A. flavus* isolates can be divided into two phenotypic types. The S strain produces numerous small sclerotia (average diameter <400 μm). The L strain produces fewer, larger sclerotia (Cotty, 1989). Within the S strain, some isolates, termed SB, produce only B aflatoxins, whilst others, named SBG, produce both B and G aflatoxins (Cotty, 1989). The S strain isolates have been referred to as atypical (Nozawa *et al.*, 1989), microsclerotium producing (Saito & Tsurata, 1993) and *A. flavus* var. *parvisclerotigenu* (Geiser *et al.*, 2000). The microsclerotial strains differ from *A. flavus* and therefore it has been suggested that they represent a taxon separated from *A. flavus* (Geiser *et al.*, 2000; Frisvad *et al.* 2005). Molecular phylogenetics suggests that SB isolates are closely related to the *A. flavus* type culture and other L strain isolates (Egel *et al.*, 1994).

Molecular typing

Phenotypic methods to discriminate *A. flavus* showed only a moderate discriminatory power for distinguishing isolates (Rath, 2001). Genotypic methods that have been used for typing *A. flavus* isolates include RFLP (Moody & Tyler, 1990; James *et al.*, 2000), RAPD (Rath, 2001; Heinemann *et al.*, 2004) and microsatellite polymorphism analysis (Guarro *et al.*, 2005). Restriction endonuclease analysis of total cellular DNA has not proven to be a suitable method for discrimination of strains of *A. flavus* (Buffington *et al.*, 1994). James *et al.* (2000) evaluated a DNA fingerprinting procedure that used a repetitive DNA sequence cloned from *A. flavus* var. *flavus* to probe RFLP of genomic DNA. The discriminatory power was 0.9526. However, RFLP analysis with Southern blotting may be tedious and labour intensive. RAPD analysis is the most frequently applied method, although lack of reproducibility is a well-known limitation of this technique. Buffington *et al.* (1994) combined the products from RAPD analysis and RFLP analysis of a tester strain of *A. flavus* to produce a DNA probe for Southern blot analysis. Although a high degree of discrimination amongst strain types was achieved, the probe and target sequences remain undisclosed.

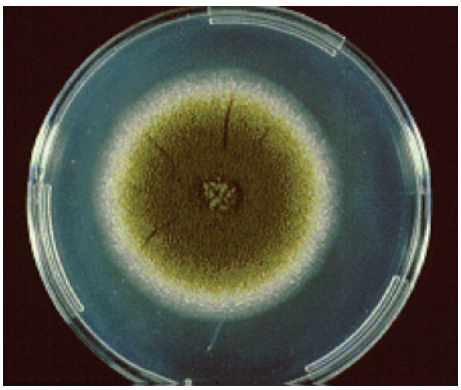

Fig. 1. Macroscopic features of *A. flavus* on Czapek's agar.

Fig. 2. Microscopic features of *A. flavus*.

Microsatellites are short tandemly repeated DNA sequences with a repetitive motif of 26 nt, forming tracts up to 100 nt long. Given the extensive polymorphism of microsatellites, they have proved to be epidemiologically useful for typing *A. fumigatus* (de Valk *et al.*, 2005). Guarro *et al.* (2005) used random amplified microsatellites (RAMS) to type isolates of *A. fumigatus* and *A. flavus* obtained from a supposed outbreak. RAMS combines microsatellite and RAPD analysis. A discriminatory power of 0.9489 was obtained with the combination of two different primers. A full understanding of population(s) of *A. flavus* and the discriminatory power of these and other typing systems awaits a full population genetics study.

Population genetics

Two papers have demonstrated that agricultural isolates of *A. flavus* can be divided into two taxonomically distinct groups (Geiser *et al.*, 1998, 2000). After analysing 314 Australian *A. flavus* isolates taken from agricultural soils,

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Geiser *et al.* (1998) found 16 different genotypes effectively forming two genetically distinct groups, namely I and II. All isolates of *A. oryzae* analysed appeared to be members of group I and almost no variation was observed amongst them. Isolates belonging to group II appeared to be more homogeneous than those in group I, implying clonal dissemination. It is unknown whether clinical isolates are members of only one or both groups. It is also unclear whether these taxonomic groupings have clinical significance in terms of mode of infection, drug resistance or virulence. The sequenced isolate NRRL 3357 aligns with group IC when the omt12 sequence is used in a phylogenetic alignment according to the parameters described by Geiser *et al.* (1998) (P. Bowyer, unpublished observations).

Although *A. flavus* is known to reproduce exclusively asexually in the laboratory, these populations are highly polymorphic in nature. In the phylogenetic study performed by Tran-Dihn *et al.* (1999) two distinct major profiles for the *A. flavus* isolates were observed by RAPD. In comparison to isolates belonging to the *A. flavus* group, RAPD profiles seemed to be considerably less variable within the groups of *A. parasiticus* isolates. Molecular typing of a larger global collection of *A. flavus* clinical isolates may contribute to a better understanding of whether there are differences in pathogenicity in the *flavus* complex. If we consider the fact that most of the outbreaks of *A. flavus* infection were caused by a single strain, it is possible that subspeciation and detailed population genetics in the *flavus* complex might be of great clinical relevance.

Outbreaks

Outbreaks of aspergillosis involving the skin, oral mucosa or subcutaneous tissues are more often associated with *A. flavus* than other species (Myoken *et al.*, 2003; James *et al.*, 2000; Heinemann *et al.*, 2004; Vandecasteele *et al.*, 2002; Allo *et al.*, 1987; Grossman *et al.*, 1985; Singer *et al.*, 1998). This is quite distinct from what is observed for outbreaks caused by *A. fumigatus*, i.e. life-threatening pulmonary or sinuses diseases in severely immunocompromised patients. In fact, clusters of invasive sinusitis or invasive pulmonary infection caused purely by *A. flavus* are fairly unusual. In a recent review that aimed to summarize the data from all nosocomial *Aspergillus* outbreaks reported to date (Vonberg & Gastmeier, 2006), 53 outbreaks were found, affecting 458 patients. Species identified most often from clinical samples were *A. fumigatus* (n=154) and *A. flavus* (n=101). Although superficial skin infections occurred in only 24 patients (5.2 % of the total), *A. flavus* was reported in almost all of these cases where *Aspergillus* species were identified to the species level.

Another important difference between outbreaks of aspergillosis caused by *A. fumigatus* and *A. flavus* is the level of genetic diversity among outbreak isolates. Molecular studies have revealed that *A. fumigatus* isolates recovered from epidemics are usually genetically distinct, meaning that every patient tends to be infected by a different strain of *A. fumigatus* (Guarro *et al.*, 2005). In contrast, most of the outbreaks caused by *A. flavus* have been associated with a single or a few different strains, indicating a point source outbreak (Myoken *et al.*, 2003; James *et al.*, 2000; Heinemann *et al.*, 2004; Vandecasteele *et al.*, 2002). There seems to be much less genetic diversity amongst clinical isolates of *A. flavus* in comparison with *A. fumigatus*.

A. flavus as a mycotoxin producer

Mycotoxins are fungal secondary metabolites that are potentially harmful to animals or humans. The word 'aflatoxin' came from '*Aspergillus flavus* toxin', since *A. flavus* and *A. parasiticus* are the predominant species responsible for aflatoxin contamination of crops prior to harvest or during storage (Yu *et al.*, 2004). The aflatoxins B1, B2, G1 and G2 are the major four toxins amongst at least 16 structurally related toxins (Goldblatt, 1969). Aflatoxin B1 is particularly important, since it is the most toxic and potent hepatocarcinogenic natural compound ever characterized (Bennett & Klich, 2003). Different *A. flavus* strains may or may not produce either aflatoxins B1 and/or B2. Other toxic compounds produced by *A. flavus* are sterigmatocystin, cyclopiazonic acid, kojic acid, β-nitropropionic acid, aspertoxin, aflatrem, gliotoxin and aspergillic acid (see http://www.aspergillus.org.uk – mycotoxin section). In addition *A. flavus* may produce some other secondary metabolites such as dihydroxyaflavinine, indole, paspalinine and versicolorin A (see http://www.aspergillus.org.uk – secondary metabolite section). *A. parasiticus* produces aflatoxin G1 and G2, in addition to B1 and B2, but not cyclopiazonic acid (Bennett & Klich, 2003; Yu, 2004). Aflatoxins are produced by some other species in *Aspergillus flavus* complex, including *A. toxicarius*, *A. nomius*, *A. bombycis* and *A. pseudotamarii*. *A. pseudotamarii* also produces cyclopiazonic acid. *A. oryzae* has long been used in the Orient to prepare various kinds of food products; it can produce cyclopiazonic acid and β-nitropropionic acid, but does not produce aflatoxin. *A. oryzae*, *A. parasiticus*, *A. sojae*, *A. nomius*, *A. bombycis*, *A. tamarii*, *A. caelatus* and *A. pseudotamarii* may produce kojic acid (Varga *et al.*, 2003). Two sexually reproducing species in the *Aspergillus flavus* complex, *P. alliaceus* and *P. albertensis*, produce a high amount of ochratoxin A (50 300 mg ml^{-1}), and are considered to be responsible for ochratoxin A contamination of figs (Bayman *et al.*, 2002).

Pathogenicity

A. flavus has been studied in animal models for over 40 years but is still rarely used in comparison to *A. fumigatus*. Early studies of invasive aspergillosis in non-immunocompromised murine models demonstrated that *A. flavus* was more virulent than almost all other *Aspergillus* species, with

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only *A. tamarii* having marginally higher virulence (Ford & Friedman, 1967). More recently, studies in both normal and immunocompromised mice have demonstrated that LD_{90} inocula for *A. flavus* are 100-fold lower than those required for *A. fumigatus* (Mosquera *et al.*, 2001; Kamai *et al.*, 2002). Following intravenous administration in non-neutropenic mice of *A. flavus* spores, the infection is rapidly concentrated in the liver and lungs within 4 h. The fungal burden in the lungs rapidly declines by 95 % over 24 h whilst the burden in the liver declines more slowly for 5 days following infection. In contrast, the burden in the kidneys and brain increases until a lethal burden develops 5–10 days post-infection (Ford & Friedman, 1967). The precise cause of death in mice with disseminated infection has not been characterized but tissue burdens immediately before death are much lower than occurs in *A. fumigatus* infections. It seems clear that aflatoxin is not a major factor in disease development, as strains which are unable to produce aflatoxin *in vitro* are similarly virulent (Richard *et al.*, 1984); additionally, infections with aflatoxin-producing strains generate infections in which aflatoxin is undetectable in tissues (Richard *et al.*, 1984).

Immunocompromised rats and rabbits have also been used as hosts of disseminated, invasive pulmonary and sinus *A. flavus* infections (Kaliamurthy *et al.*, 2003). Infection results in death between 7 and 10 days post-infection, with the highest tissue burden recovered from the lungs>liver>brain>kidneys (this is in stark contrast to the tissue burdens in mice following *A. fumigatus* infection). Rabbits have been used as a model of paranasal sinus mycoses caused by *A. flavus* following direct injection into the sinus. In these studies the rabbits were not immunocompromised but required a very high inoculum (up to 10^8 spores) to reliably establish an infection (Chakrabarti *et al.*, 1997). Domestic chickens, geese and turkey poults are all susceptible to *A. flavus* without immunosuppression. Infections occur naturally in domestic flocks and can also be established following aerosol exposure.

Human diseases

A. flavus causes a broad spectrum of disease in humans, ranging from hypersensitivity reactions to invasive infections associated with angioinvasion. After *A. fumigatus*, *A. flavus* is the second leading cause of invasive and non-invasive aspergillosis (Denning, 1998; Morgan *et al.*, 2005). The primary route of infection is inhalation of fungal spores. The bigger size of *A. flavus* spores (25 μm in diameter in comparison to 23 μm for *A. fumigatus*) favours their deposition in the upper respiratory tract. Maybe this is one of the reasons why *A. flavus* is a common aetiological agent of fungal sinusitis and cutaneous infections, but not invasive fungal pneumonia. Possibly surface characteristics of the spores other than size are also important determinants of localization (Morrow, 1980).

As mentioned before, climate and geographical factors are important determinants of the local prevalence of *A. flavus* infections. In countries like Saudi Arabia and Sudan, with semi-arid and arid dry weather conditions, *A. flavus* is the main aetiological agent of invasive aspergillosis (Khairallah *et al.*, 1992; Kameswaran *et al.*, 1992). *A. flavus* is also one of the main pathogens responsible for pulmonary aspergillosis in Africa (Mahgoub & el-Hassan, 1972). For unknown reasons, the frequency of infections caused by *A. flavus* is also elevated in some hospitals, in different locales. Even though the clinical features of aspergillosis are generally identical for all of *Aspergillus* species, some particularities regarding *A. flavus* infections are described below.

Chronic cavitary pulmonary aspergillosis (CCPA) and aspergilloma

A. fumigatus causes the vast majority of cases of CCPA and aspergilloma (Denning *et al.*, 2003). For unknown reasons, *A. flavus* has rarely been associated with CCPA (Liao *et al.*, 1988; Staib *et al.*, 1983). Approximately 10 cases have been reported so far, mostly from regions with hot and dry climate. Systemic oxalosis has mostly been associated with *A. niger* aspergillomas in diabetic patients, and it is rare with *A. flavus* (Dogan *et al.*, 2004).

Allergic bronchopulmonary aspergillosis (ABPA) and allergens

Although *A. fumigatus* is responsible for the vast majority of ABPA cases, *A. flavus* has also been implicated in some series (Khan *et al.*, 1976; Chakrabarti *et al.*, 2002), mostly in studies from India. In addition, ABPA caused by *Aspergillus flavus* complex can also occur as an occupational disease. Many reports from Japan have shown that exposure to high concentrations of *A. oryzae* spores during the production of soybean products can lead to ABPA (Akiyama *et al.*, 1987; Kurosawa *et al.*, 1990). The vast majority of patients with ABPA have asthma; however, interestingly, some of these patients did not.

Several species of *Aspergillus* have been shown to be allergenic, including *A. fumigatus*, *A. niger*, *A. flavus* and *A. oryzae*. Over 20 allergens have been characterized in *A. fumigatus*, two from *A. flavus* (Asp fl 13 and Asp fl 18) and a further four from the closely related *A. oryzae* (Asp o 13, Asp o 21, Asp o lactase and Asp o lipase) (Mari & Riccioli, 2004; http://www.allergome.org/). Recent genome sequencing projects have made it possible to survey the allergens present in *Aspergillus* species. Table 2 shows predicted *A. flavus* allergen homologues by comparison with allergens from other *Aspergillus* species. It can be seen that many allergens present in *A. fumigatus* are present at high levels of homology in *A. flavus*. Proteins with >50 % identity to allergen proteins are likely to be immunologically cross-reactive (Bowyer *et al.*, 2006). Asp o 21 and Asp o 13 allergens from the closely related *A. oryzae* are present at 98 and 100 % identity respectively and are likely to function as allergens in *A. flavus*. Additionally Asp f 1, Asp f 5, Asp f 12, Asp f 13, Asp f 18, Asp f 22 and Asp f 23 are all present

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in the *A. flavus* genome at >90 % identity and are likely to be allergenic in this species. Thus it is likely that *A. flavus* will produce many more allergenic proteins than the two currently known and may possess an allergen complement similar to that of *A. fumigatus*.

Keratitis and endophthalmitis

Fungal keratitis occurs predominantly in tropical and warm climates, and various case series have been published from Africa (Gugnani *et al.*, 1978; Cheikh-Rouhou *et al.*, 2001), the Middle East (Khairallah et al., 1992), South Asia (Wong *et al.*, 1997) and some parts of the USA (Rosa *et al.*, 1994). Amongst keratitis cases caused by *Aspergillus* spp., *A. flavus* accounted for 80 % of the total *Aspergillus* infections (Khairallah *et al.*, 1992). The major predisposing condition to *A. flavus* keratitis is trauma, generally with plant material (Gugnani *et al.*, 1978; Khairallah *et al.*, 1992; Wong *et al.*, 1997; Cheikh-Rouhou *et al.*, 2001). In some cases *A. flavus* keratitis was reported after laser and cataract surgery (Sridhar *et al.*, 2000; Mendicute *et al.*, 2000). Fungal endophthalmitis has rarely been associated with *A. flavus* (Lance *et al.*, 1988; Demicco *et al.*, 1984; Cameron *et al.*, 1991).

Cutaneous infection

Most cases of cutaneous aspergillosis are caused by *A. flavus* (van Burik *et al.*, 1998; Chakrabarti *et al.*, 1998). Skin involvement can be classified as either (i) primary, following direct inoculation of *Aspergillus* at sites of skin injury (e.g. intravenous catheter sites, traumatic inoculation, occlusive dressings, burns or surgery), or (ii) secondary, from haematogenous spread, most commonly following a pulmonary portal of entry, or from contiguous extension from a neighbouring cavity such as the maxillary sinus. The clinical presentation of cutaneous aspergillosis by *A. flavus* is characterized by the presence of violaceous macules, papules, plaques or nodules, haemorrhagic bullae, ulcerations with central necrosis with or without eschar formation, pustules or subcutaneous abscesses.

Table 2. Predicted allergens in the *A. flavus* genome

Allergen	Percentage identity of the *A. flavus* homologue
Asp f 1 Mitogillin [Afu5g02330]	97
Asp f 2 [Afu4g09580]	65
Asp f 3 PMP20 [Afu6g02280]	86
Asp f 4 [Afu2g03830]	56
Asp f 5 Metalloprotease [Afu8g07080]	95
Asp f 6 Mn SOD [Afu1g14550]	60
Asp f 7 [Afu4g06670]	48
Asp f 8 [Afu2g10100] ribosomal P2	85
Asp f 9 [Afu1g16190]	64
Asp f 10 Aspergillopepsin [Afu5g13300]	75
Asp f 11 Cyclophilin [Afu2g03720]	85
Asp f 12 HSP90 [Afu5g04170]	94
Asp f 13 [Afu2g12630] protease	94 (Asp fl 13)
Asp f 17 [Afu4g03240]	42
Asp f 18 Cell serine protease [Afu5g09210]	92 (Asp fl 18)
Asp f 22 Enolase [Afu6g06770]	93
Asp f 23 Rpl3 [Afu2g11850]	95
Asp fl 13 (previously Asp fl 1)	100
Asp n 14 β-Xylosidase	64
Asp n 25 3-Phytase B	31
Asp n Glucoamylase	68
Asp o 13 Oryzin	100
Asp o 21 α-Amylase A	98

Wound infection

A. flavus is a particularly important species in wound aspergillosis, accounting for 41 % of cases confirmed by culture (Pasqualotto & Denning, 2006). Many studies have linked the occurrence of postoperative aspergillosis with the dissemination of *Aspergillus* spores in the operating room (Pasqualotto & Denning, 2006). Diaz-Guerra *et al.* (2000) reported the simultaneous isolation of one *A. flavus* isolate from the aortic prosthesis of a heart surgery patient, and another two isolates were recovered from a dual-reservoir cooler-heater used in the operating room where this patient was operated on. Genetic typing of these isolates by RAPD revealed identical genotypes, indicating the nosocomial origin of the strain. *Aspergillus* infection should always be considered in the differential diagnosis of slowly progressive but destructive wound infections, culture-negative pleural effusion and culture-negative mediastinitis after cardiac surgery.

Endocarditis and pericarditis

A. flavus has been reported as a cause of both native and prosthetic valve endocarditis, which is occasionally a manifestation of disseminated aspergillosis (Demaria *et al.*, 2000; Rao & Saha, 2000; Irles *et al.*, 2004). Occasional cases occur in patients with no overt risk factors (Kennedy *et al.*, 1998; Khan *et al.*, 1995). In postoperative *Aspergillus* endocarditis, *A. flavus* accounts for 11.2 % of cases (Pasqualotto & Denning, 2006). A rare case of fungal endocarditis (*A. flavus*) on a permanent pacemaker has been described (Acquati *et al.*, 1987). Two reports have associated *A. flavus* with pericarditis (Cooper *et al.*, 1981).

Central nervous system infection

Case series of craniocerebral aspergillosis due to *A. flavus* in immunocompetent hosts have been reported mainly from Pakistan, India, Saudi Arabia, Sudan and other African countries (Rudwan & Sheikh, 1976; Hussain *et al.*, 1995; Panda *et al.*, 1998). Most of these cases occurred as a complication of chronic granulomatous sinusitis, described below. These reports have speculated that tropical environmental conditions (hot and dry weather), bad hygiene and poor socioeconomic status are responsible

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(Rudwan & Sheikh, 1976; Hussain *et al.*, 1995; Panda *et al.*, 1998; Alrajhi *et al.*, 2001).

Rhinosinusitis

A. flavus is more likely to be recovered from the upper respiratory tract than any other *Aspergillus* species (Chakrabarti *et al.*, 1992; Hussain *et al.*, 1995; Iwen *et al.*, 1997; Kennedy *et al.*, 1997; Panda *et al.*, 1998). Clinical presentations of *Aspergillus* rhinosinusitis include acute and chronic invasive, chronic granulomatous and non-invasive syndromes (Hope *et al.*, 2005). For an adequate diagnosis, tissue should be obtained for histopathology (fungal stains are essential), with fungal cultures of surgical specimens. Cultures of the nasal mucus are unreliable for diagnosis because the cultures reflect recent air sampling, rather than disease.

Chronic granulomatous sinusitis is a curious syndrome of chronic slowly progressive sinusitis associated with proptosis that has been also called indolent fungal sinusitis and primary paranasal granuloma. Florid granulomatous inflammation is the histological hallmark of this condition. Interestingly, almost all reports come from the Sudan (Milosev *et al.*, 1969; Gumaa *et al.*, 1992; Yagi *et al.*, 1999), Saudi Arabia (Alrajhi *et al.*, 2001) and the Indian subcontinent (Chakrabarti *et al.*, 1992; Ramani *et al.*, 1994; Panda *et al.*, 2004). There are a limited number of reports in the USA, which appear to affect almost exclusively African-Americans (Currens *et al.*, 2002). Whether this reflects climatic conditions and/or any genetic predisposition is unknown. Curiously, patients appear to be immunocompetent and are infected almost exclusively with *A. flavus* (Gumaa *et al.*, 1992; Yagi *et al.*, 1999; Alrajhi *et al.*, 2001). Bone erosion is a common finding (Yagi *et al.*, 1999) and tissue destruction occurs as a result of expansion of the mass rather than vascular invasion. Most individuals present with a unilateral proptosis (Milosev *et al.*, 1969). Frequently there is direct spread beyond the confines of the sinuses to invade the brain, cavernous sinus, orbit and great vessels (Hope *et al.*, 2005). Marked regression generally occurs following surgical procedures designed to produce adequate aeration of the sinuses. However, the recurrence rate is high (about 80 %), and some evidence suggests that the use of antifungal drugs may offer benefit (Gumaa *et al.*, 1992).

Allergic fungal sinusitis (AFS) and sinus aspergilloma

Although *A. fumigatus* seems to be the most frequent *Aspergillus* organism causing AFS, *A. flavus* is particularly frequent in some geographical areas, such as the Middle East and India (Taj-Aldeen *et al.*, 2003, 2004; Saravanan *et al.*, 2006; Thakar *et al.*, 2004). Patients with AFS may have co-existent mucosal granulomatous inflammation indicative of fungal tissue invasion (Thakar *et al.*, 2004). In these cases from India, *A. flavus* was the only pathogen identified (Thakar *et al.*, 2004). Sinus aspergilloma (fungus ball) is also usually caused by *A. fumigatus* and such infections caused by *A. flavus* are less frequent in developed countries (Milosev *et al.*, 1969; Stammberger *et al.*, 1984; Ferreiro *et al.*, 1997). Again, *A. flavus* is more commonly isolated from patients in India, Sudan and other tropical countries (Panda *et al.*, 1998; Yagi *et al.*, 1999; Chakrabarti *et al.*, 1992; Milosev *et al.*, 1969).

Osteoarticular infection

A. flavus seems to be the main aetiological agent of *Aspergillus* osteomyelitis following trauma (Fisher, 1992), a situation which resembles the elevated frequency at which *A. flavus* causes primary cutaneous aspergillosis and wound infections.

Urinary tract infection

Urinary tract aspergillosis due to *A. flavus* is rare, with few cases reported (Khan *et al.*, 1995; Perez-Arellano *et al.*, 2001; Kueter *et al.*, 2002). Usually a unilateral or bilateral fungal bezoar of the urinary pelvis is the presenting problem. Predisposing conditions include diabetes, intravenous drug addiction and schistosomiasis.

Resistance to antifungal drugs

Until recent years, the only drugs available to treat aspergillosis were amphotericin B (AmB) and itraconazole, the latter in oral and intravenous formulations. Recently voriconazole, posaconazole and caspofungin have also been approved for the treatment of aspergillosis. Although resistance to antifungal drugs is not as great a concern as resistance to antibacterial agents, there has been an increase in the number of reported cases of both primary and secondary resistance in human mycoses (Denning *et al.*, 1997). Therefore, it seems possible that resistance of the fungus to the drug or an inadequate concentration of the antifungal drug at the site of infection might contribute to the high mortality rate seen for these infections.

Amphotericin B

Although the true rate of AmB resistance is unknown, some investigators have reported isolates of *A. flavus* resistant to AmB *in vitro* (Odds *et al.*, 1998; Lass-Florl *et al.*, 1998; Seo *et al.*, 1999; Mosquera *et al.*, 2001; Gomez-Lopez *et al.*, 2003; Sutton *et al.*, 2004; Hsueh *et al.*, 2005), although this is not universally accepted. In a study from Taiwan (Hsueh *et al.*, 2005) isolates of *A. flavus* and *A. fumigatus* with reduced susceptibilities to AmB were found (MICs 2 $\mu g\ ml^{-1}$). Among the four species tested, *A. flavus* was the least susceptible to AmB; the MICs at which 50 % and 90 % of *A. flavus* isolates were inhibited were twofold greater than those for *A. fumigatus* and *A. niger*.

A preliminary report has documented a steady increase in AmB resistance *in vitro* amongst *Aspergillus* isolates recovered since 2001 (Sutton *et al.*, 2004). About 20 % of *A. fumigatus* and *A. flavus* isolates recovered in 2004 had

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minimum lethal concentrations (MLCs) of AmB $\geqslant$16 μg ml^{-1} compared to 0 % in 2001. Some investigators have hypothesized that the extensive use of AmB against fungal infections has led to the emergence of less susceptible species, such as *A. terreus* and *A. flavus* (Marr *et al.*, 2002). Recently, Lionakis *et al.* (2005) found that the proportion of *Aspergillus* spp. resistant to antifungals (especially AmB) was much higher amongst isolates recovered from cancer patients with prior exposure to AmB or triazoles.

Few data are available regarding correlations between MIC and outcome of treatment with AmB for infections caused by *Aspergillus* species. In the survey of Odds *et al.* (1998) the efficacy of AmB at 0.31 mg kg^{-1} was seen *in vivo* against *A. fumigatus* (MIC 1 μg ml^{-1}) but efficacy was not seen against *A. flavus* at the same MIC, at any dose tested. In another study (Lass-Florl *et al.*, 1998), AmB MICs of $\geqslant$2 μg ml^{-1} were associated with treatment failure amongst patients with invasive aspergillosis. Mosquera *et al.* (2001) demonstrated a lack of correlation between susceptibility to AmB *in vitro* and clinical outcome for *A. flavus* infections *in vivo* by using different susceptibility testing methods, including the Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS) M-38A method. Difficulty in treating invasive aspergillosis might relate in part to poor penetration of AmB into infected tissue (Paterson *et al.*, 2003).

Itraconazole

Itraconazole resistance in *Aspergillus* species is presumptively defined as an MIC of $\geqslant$8 μg ml^{-1} (Gomez-Lopez *et al.*, 2003). According to this criterion, Hsueh *et al.* (2005) found resistance to itraconazole in 4.2 % (4 of 96) of *Aspergillus* species, including two *A. fumigatus* and two *A. flavus* isolates. Similar rates of resistance were found amongst isolates included in previous studies (Gomez-Lopez *et al.*, 2003; Lionakis *et al.*, 2005). In the study by Hsueh *et al.* (2005), all of the *Aspergillus* isolates tested were inhibited by $\leqslant$8 μg itraconazole ml^{-1}. Recently, Lionakis *et al.* (2005) showed that 11 % of the *A. flavus* isolates in their study were itraconazole resistant based on *in vitro* susceptibility by tests performed by the CLSI method; using the E-test, only 6 % of *A. flavus* isolates could be classified as itraconazole resistant. Again, *in vitro* susceptibility test results may not reflect *in vivo* response, as demonstrated by Mosquera *et al.* (2001).

Voriconazole

Voriconazole has good *in vitro* activity against a range of *Aspergillus* species, including *A. flavus* (Pfaller *et al.* 2002; Diekema *et al.*, 2003; Lass-Florl *et al.*, 2001). Hsueh *et al.* (2005) showed that all of the *Aspergillus* isolates tested, including *A. flavus*, were inhibited by $\leqslant$1 μg voriconazole ml^{-1}. Voriconazole MICs are slightly higher than those of itraconazole for *A. flavus* (Maesaki *et al.*, 2000; Gomez-Lopez *et al.*, 2003). The precise inoculum used can alter the MIC, so higher inocula yield higher and potentially resistant end points (Mosquera *et al.*, 2001). Discordance in results with the CLSI and E-test methods with voriconazole is problematic (Lionakis *et al.*, 2005). Since validated methodology and breakpoints for voriconazole have not yet been established, the rate of resistance is not known. However, some *Aspergillus* isolates seem to show cross-resistance to itraconazole and voriconazole, as demonstrated with *A. fumigatus*, and this is strain (and presumably mechanism) dependent (Espinel-Ingroff *et al.*, 2001; Pfaller *et al.*, 2002).

Other antifungal agents

Caspofungin, anidulafungin and micafungin are members of the echinocandin group of antifungal agents that target 1,3-β-glucan synthase, disrupting hyphal growth at tips and branch points. Caspofungin and micafungin are available for the treatment of invasive aspergillosis and hold promise for treatment alone or in combination with triazoles or AmB (Marr *et al.*, 2002; Cesaro *et al.*, 2004). *A. flavus* would appear to be slightly less susceptible than *A. fumigatus* to echinocandins, based on *in vitro* parameters (Oakley *et al.*, 1998; Espinel-Ingroff, 2003) but eradication rates were 20–25 % better for *A. flavus* infection than *A. fumigatus* in two salvage studies (Maertens *et al.*, 2004; Denning *et al.*, 2006). Thus a species difference in susceptibility to echinocandins may exist, but is not obviously clinically relevant, and could reflect the difficulties in interpretation of *in vitro* results with echinocandins. No isolates of *A. flavus* have yet been described that are resistant to posaconazole.

Conclusions

A. flavus is the second most important *Aspergillus* species causing human infections. The importance of this fungus increases in regions with a dry and hot climate. In addition, many *A. flavus* isolates produce aflatoxin B1, the most toxic and potent hepatocarcinogenic natural compound ever characterized. Small studies of phylogenetic species in *A. flavus* indicate that the morphological species contains several genetically isolated species, and until a population-based discriminatory molecular typing system is applied, we will not know the full extent of diversity in *A. flavus*, *sensu lato*. Population genetics studies on isolates causing disease would be of great interest. Particularly common clinical syndromes associated with *A. flavus* include chronic granulomatous sinusitis, keratitis, cutaneous aspergillosis, wound infections and osteomyelitis following trauma and inoculation. On the other hand, *A. flavus* is rarely the aetiological agent of chronic cavitary pulmonary aspergillosis. In frank contrast to *A. fumigatus* infections, most of the investigated outbreaks caused by *A. flavus* were due to a single or a few strains, assuming that the typing systems used were sufficiently discriminatory. Finally, *A. flavus* seems to be more virulent and more resistant to antifungal drugs than most of the other *Aspergillus* species. Hopefully, recently published information about the

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Aspergillus genomes will help us to better understand the pathogenesis of these infections, as well as providing insights into toxin production and allergens.

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