Ochratoxin production and taxonomy of the yellow aspergilli (*Aspergillus* section *Circumdati*)

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Abstract: *Aspergillus* section *Circumdati* or the *Aspergillus ochraceus* group, includes species with rough walled stipes, biseriate conidial heads, yellow to ochre conidia and sclerotia that do not turn black. Several species are able to produce mycotoxins including ochratoxins, penicillic acids, and xanthomegnins. Some species also produce drug lead candidates such as the notoamides. A polyphasic approach was applied using morphological characters, extrolite data and partial calmodulin, β-tubulin and ITS sequences to examine the evolutionary relationships within this section. Based on this approach the section *Circumdati* is revised and 27 species are accepted, introducing seven new species: *A. occultus*, *A. pallidofulvus*, *A. pulvericola*, *A. salwaensis*, *A. sesamicola*, *A. subramanianii* and *A. westlandensis*. In addition we correctly apply the name *A. fresenii* (≡ *A. sulphureus* (*nom. illeg.*)). A guide for the identification of these 27 species is provided. These new species can be distinguished from others based on morphological characters, sequence data and extrolite profiles. The previously described *A. onikii* and *A. petrakii* were found to be conspecific with *A. ochraceus*, whilst *A. flocculosus* is tentatively synonymised with *A. ochraceopetaliformis*, despite extrolite differences between the two species. Based on the extrolite data, 13 species of section *Circumdati* produce large amounts of ochratoxin A: *A. affinis*, *A. cretensis*, *A. fresenii*, *A. muricatus*, *A. occultus*, *A. ochraceopetaliformis* (*A. flocculosus*), *A. ochraceus*, *A. pseudoelegans*, *A. pulvericola*, *A. roseoglobulosus*, *A. sclerotiorum*, *A. steynii* and *A. westerdijkiae*. Seven additional species produce ochratoxin A inconsistently and/or in trace amounts: *A. melleus*, *A. ostianus*, *A. persii*, *A. salwaensis*, *A. sesamicola*, *A. subramanianii* and *A. westlandensis*. The most important species regarding potential ochratoxin A contamination in agricultural products are *A. ochraceus*, *A. steynii* and *A. westerdijkiae*.

Key words: *Ascomycetes*, *Eurotiales*, Mycotoxin, Food spoilage, Indoor environment.
Taxonomic novelties: New species: *Aspergillus occultus* Visagie, Seifert, Frisvad & Samson, *A. pallidofulvus* Visagie, Varga, Frisvad & Samson, *A. pulvericola* Visagie, Seifert, Frisvad & Samson, *A. salwaensis* Visagie, Houbraken, Fotedar, Frisvad & Samson, *A. sesamicola* Visagie, Frisvad & Samson, *A. subramanianii* Visagie, Frisvad & Samson, *A. westlandensis* Visagie, Varga, Meijer & Frisvad.

Published online 12 September 2014; http://dx.doi.org/10.1016/j.simyco.2014.07.001. Hard copy: June 2014.

INTRODUCTION

Aspergillus section *Circumdati* (Gams *et al.* 1985; *Aspergillus ochraceus* species group according to Raper & Fennell 1965) includes species with biseriate conidial heads in shades of yellow to ochre.

Some species of *Aspergillus* section *Circumdati* are economically important; for example, strains of *A. ochraceus* or *A. sclerotiorum* are used for biochemical transformation of steroids, alkaloids or phenazines (Chen *et al.* 1994), while some species, e.g. *A. sclerotiorum* and *A. melleus*, are important sources of proteolytic enzymes (Luisetti *et al.* 1991) and several exometabolites (Matsukuma *et al.* 1992). Sclerotia of several species contain anti-insect compounds (Whyte *et al.* 1996, Ooike *et al.* 1997). Several species produce diverse mycotoxins harmful for animals and humans including ochratoxin A, xanthomegnin and viomellein (Lai *et al.* 1970, Ciegler 1972, Hesseltine *et al.* 1972, Durley *et al.* 1975, Varga *et al.* 1996, Frisvad *et al.* 2004, Davolos & Pietrangeli 2014).

These moulds are also known for the production of asperga-mides, notoamides, norgeamides, stephacidins, avrainvillamides, among which are several promising anti-cancer compounds (Finefield *et al.* 2011). *Aspergillus westerdijkiae*, *A. melleus*, *A. ochraceus*, *A. steynii* and *A. subramanianii* are reported to be common from a wide range of habitats such as soil, agricultural and stored foods (Kozakiewicz 1989, Frisvad *et al.* 2004, Morello *et al.* 2007, Noonim *et al.* 2008, Gil-Serna *et al.* 2009a, 2011).

Aspergillus ochraceus and *A. sclerotiorum* have also been identified as human and animal pathogens causing onychomycosis (Feuilhade de Chauvin & de Bievre 1985), allergic bronchopulmonary aspergillosis (Novey & Wells 1978, Wierzbicka *et al.* 1997), otomycosis (Harima *et al.* 2004) and antromycosis in humans (Bassiouny *et al.* 1982), and mycotic placentitis in cow (Munoz *et al.* 1989). *Aspergillus persii* was isolated from several onychomycosis cases in Italy (Zotti & Corte 2002, Zotti *et al.* 2010). Several species are involved in nail infections, including *A. sclerotiorum*, *A. ochraceus* and *A. melleus* (Machetti *et al.* 2010). Another species, *A. ochraceopetaliformis*, was

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isolated from human skin lesions (Batista & Maia da Silva 1957), and recently found to cause onychomycosis (Brasch *et al.* 2009).

An early study of the Japanese yellow Aspergilli (Nehira 1949) was followed by the taxonomic treatment of section *Circumdati* by Raper & Fennell (1965) that included nine species (*A. ochraceus*, *A. fresenii*, *A. alliaceus*, *A. sclerotiorum*, *A. auricomus*, *A. petrakii*, *A. melleus*, *A. elegans* and *A. ostianus*). Christensen & Raper (1970, 1982) described *A. robustus*, *A. campestris* and *A. bridgeri* as members of this group, while Samson (1979) added four more species: *A. dimorphicus*, *A. insulicola*, *A. lanosus* and *A. ochraceoroseus*. Later another new species, *A. persii*, was assigned to this section (Zotti & Corte 2002). In a more recent paper, seven new species were described in section *Circumdati*: *A. cretensis*, *A. flocculosus*, *A. neobridgeri*, *A. pseudoelegans*, *A. roseoglobulosus*, *A. steynii* and *A. westerdijkiae* (Frisvad *et al.* 2004). A sexually reproducing ochratoxin producing species with a *Neopetromyces* teleomorph, *A. muricatus*, was also found to belong to this section (Varga *et al.* 2000a, Frisvad & Samson 2000).

Phylogenetic analysis of sequences of parts of the ribosomal RNA gene cluster indicated that *A. campestris* and *A. lanosus* belong to *Aspergillus* sections *Candidi* and *Flavi*, respectively, while *A. dimorphicus* and *A. sepultus* are members of section *Cremei* (Peterson 1995, Varga *et al.* 2000a). Two teleomorphic species previously assigned to this section, *Petromyces alliaceus* and *P. albertensis* were found to belong to *Aspergillus* section *Flavi* (Varga *et al.* 2000b).

In this study, we examined available strains of the species proposed to belong to section *Circumdati* and provide a monographic treatment on the section. We used a polyphasic approach to species delimitation, including sequence analysis of parts of the β-tubulin and calmodulin genes and the ITS region, macro- and micro-morphological analyses and examination of the extrolite profiles of the isolates.

Table 1. Strains used for phylogenetic analyses of *Aspergillus* section *Circumdati*.

Species	Culture collection number	Source and origin	GenBank accession nr.		
			ITS	*BenA*	*CaM*
A. affinis	CBS 129190T = IBT 32310 = ATCC MYA-4773	Decomposing leave litter, Italy	GU721090	GU721092	GU721091
A. auricomus	CBS 467.65T = NRRL 391 = IBT 14581 = ATCC 16890 = IMI 172277 = LCP 89.2596 = LSHBA 41 = WB 391	Unrecorded source	EF661411	EF661320	EF661379
	CBS 613.78 = NRRL 397 = IBT 13857 = QM 9809 = Thom 5479.A42 = WB 397	Unrecorded source	EF661412	EF661321	EF661380
	CBS 638.78 = NRRL 388 = IBT 13854 = DTO 139-E9	Soil, Wyoming, USA	FJ491575	KJ775049	FJ491529
A. bridgeri	CBS 350.81T = NRRL 13000 = IBT 13380 = ATCC 44562 = IMI 259098	Soil, Wyoming, USA	EF661404	EF661335	EF661358
	NRRL 35081 = NRRL A-20312 = o-1192	Soil, Utah, USA	EF661398	EF661334	EF661366
A. cretensis	CBS 112802T = NRRL 35672 = IBT 17505	Soil, Crete, Greece	FJ491572	AY819977	FJ491534
	CBS 112805 = NRRL 35673 = IBT 23283 = IMI 001177	*Citrus* sp., Israel	FJ491584	AY819978	FJ491533
A. elegans	CBS 102.14T = CBS 543.65 = NRRL 4850 = IBT 13505 = ATCC 13829 = ATCC 16886 = IFO 4286 = IMI 133962 = QM 8912 = QM 9373 = WB 4850	Unrecorded source, USA	EF661414	EF661349	EF661390
	CBS 310.70 = CBS 614.78 = NRRL 407 = NRRL 4813 = IBT 13861 = IFO 4354 = IMI 313490 = QM 8885 = QM 9810 = Thom 5400.1 = WB 4813	Unrecorded source	EF661413	EF661348	EF661383
	CBS 615.78 = NRRL 4820 = IFO 5443 = IMI 313485 = WB 4820	Unrecorded source, Japan	FM201287	FM995522	KJ775241
A. fresenii	CBS 550.65T = NRRL 4077 = ATCC 16893 = IMI 211397 = NRRL A-5355 = NRRL A-5520 = WB 4077	Soil, Mysore, India	EF661409	EF661341	EF661382
	NRRL 35092 = NRRL A-22634	Unrecorded source, India	EF661408	EF661342	EF661367
A. insulicola	CBS 382.75T = NRRL 6138 = ATCC 26220	Soil, Venezuela	EF661430	EF661353	EF661396
A. melleus	CBS 112786 = NRRL 386 = IBT 14265	Unrecorded source	FJ491567	AY819964	KJ834523
	CBS 112788 = IBT 23399	Soil in Gauguin garden, Taboga Island, Costa Rica	FJ491578	FJ491523	FJ491543
	CBS 546.65T = NRRL 5103 = IBT 13510 = IBT 13511 = IBT 13875 = ATCC 16889 = WB 5103	Soil, India	EF661425	EF661326	EF661391
	NRRL 35105 = NRRL A-23163	Peanut field soil, Turkey	EF661426	EF661327	EF661370
A. muricatus	CBS 112808T = NRRL 35674 = IBT 19374 = IMI 36852	Grassland soil, Philippines	EF661434	EF661356	EF661377
	NRRL 35071 = NRRL A-20270	Cotton field soil, Arizona, USA	EF661433	EF661355	EF661365
A. neobridgeri	CBS 559.82T = NRRL 13078 = IBT 14026	Soil, Nebraska, USA	EF661410	EF661345	EF661359