Aspergillus waksmanii sp. nov. and Aspergillus marvanovae sp. nov., two closely related species in section Fumigati

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Two new and phylogenetically closely related species in Aspergillus section Fumigati are described and illustrated. Homothallic Aspergillus waksmanii sp. nov. was isolated from New Jersey soil (USA) and is represented by the ex-type isolate NRRL 179T (=CCF 4266T=Thom 4138.HS2T=IBT 31900T). Aspergillus marvanovae sp. nov. was isolated from water with high boracic acid anions content in Dukovany nuclear power station (Czech Republic). The sexual stage of this species is unknown, but the MAT1-1 locus was successfully amplified suggesting that the species is probably heterothallic and teleomorphic but is represented by only the ex-type isolate CCM 8003T (=CCF 4037T=NRRL 62486T=IBT 31279T=IFM 60873T). Both species can be distinguished from all previously described species in section Fumigati based on morphology, maximum growth temperature, sequence data from five unlinked loci and unique secondary metabolites profiles.

INTRODUCTION

Section Fumigati is one of the most species-rich sections in the genus Aspergillus and includes species with overall significance for medicine, pharmacology, biotechnology, food and soil mycology. Many species show high levels of intraspecific variability, overlapping morphological features and siblings are often regarded as cryptic species. Numerous approaches have been proposed for the taxonomy of section Fumigati resulting in complex polyphasic species definitions (Hong et al., 2008; Samson et al., 2007).

Two undescribed taxa belonging to section Fumigati were discovered during taxonomic re-identification of culture collection isolates using molecular methods (Hubka, 2011; Peterson, 2008) and are proposed here as novel species, Aspergillus waksmanii sp. nov. and Aspergillus marvanovae sp. nov., using sequence data from five unlinked loci, macro- and micromorphology (optical and scanning electron microscopy), secondary metabolite analysis and maximum growth temperature.

METHODS

Morphology. The isolates were cultivated and their morphology was observed as described by Nováková et al. (2012). Growth at 42, 45, 47 and 50 °C was tested on malt extract agar (MEA) plates sealed with Parafilm. Scanning electron microscopy (SEM) was performed using...
RESULTS AND DISCUSSION

Phylogenetic analysis

We examined the phylogeny of *A. waksmanii* and *A. marvanovae* and other species belonging to section *Fumigati* using sequence data from *benA*, *caM*, *act1*, ITS and *RPB2* loci. The combined maximum-likelihood tree based on *benA* and *caM* loci is shown on Fig. 1. The data for ITS region, *rpb2* and *act1* loci are incomplete for section *Fumigati* and presented only on individual trees showing relationships of the closest taxa (Fig. S1).

*A. waksmanii* and *A. marvanovae* were found to form a strongly supported clade in *benA* and *caM* trees with homothallic *Neosartorya assimulata*, *Neosartorya multiplicata* and *Neosartorya tsunodae*, heterothallic *Neosartorya nishimurae* and anamorphic *Aspergillus turcosus* and *Aspergillus unilateralis*. The exact relationships of both species to other species within this clade is not fully resolved because of weakly supported deeper branching. At the *benA* and *caM* loci, *A. waksmanii* and *A. marvanovae* formed a clade and showed differences of 2% in the nucleotide sequence to each other. Other species, with the exception of *N. nishimurae*, have more than 2% bp difference at each locus. Based on the *act1* locus, *A. waksmanii* is most closely related to *N. nishimurae* and *N. assimulata* with 2% bp difference, other taxa show 4% or higher dissimilarity. The *act1* locus of *A. marvanovae* shows 2% dissimilarity from *A. turcosus*, other taxa have 4% dissimilarity. At the *RPB2* locus, sequence differences of 1–6% or higher are seen among species belonging to section *Fumigati*. *A. waksmanii* also has unique ITS sequences and can be differentiated from all other species using the barcoding ITS locus (Schoch *et al.*, 2012), in contrast to *A. marvanovae* which shares identical ITS sequence with *A. turcosus*. The level of discrimination between species from section *Fumigati* and some other sections (Jurjevic *et al.*, 2012; Novákova *et al.*, 2012; Peterson, 2012) is relatively low in the ITS region, but it is the standard and is workable.

Secondary metabolite analysis

The extrolite analysis of *A. waksmanii*, *A. marvanovae* and some phylogenetically related species showed that these species produce unique profiles of secondary metabolites with characteristic UV-spectra and retention-times (Table 1, Table S1). Some of these metabolites are common across section *Fumigati* (apolar indoloterpene-1; aszonapyrone A). On the other hand, e.g. the metabolite designated ‘SNU1’ is characteristic of and has only been detected in *A. marvanovae* and *A. turcosus* and not in other species belonging to section *Fumigati*. Among the species analysed, *A. turcosus* produces known secondary metabolites such as epiaszonalenins, gliotoxin and kotanin, whereas *N. multiplicata* produces helvolic acid (Table 1). *A. unilateralis*, *N. assimulata* and *N. multiplicata* produced aszonapyrone A, but this metabolite has also been found in other members of *Aspergillus* section *Fumigati*. *A. waksmanii* and *A. marvanovae* produced apolar indoloterpenes, but did not share other secondary metabolites. These apolar indoloterpenes, however, are also found in other *Aspergillus* section *Fumigati* species producing ascomata.

Mating locus analysis

The *MAT1-1* idiomorph, containing an alpha box domain, was partially characterized in the ex-type isolate of *A. marvanovae* CCM 8003™ using primers *alpha1* and *alpha2* (Sugui *et al.*, 2010) (accession no. HE978838). No product was observed on electrophoretograms when using primers...
Novel Aspergillus species in section Fumigati

Fig. 1. Phylogenetic tree showing relationships of A. waksmanii and A. marvanovae to other species in section Fumigati. Thick lines indicate branches that support Bayesian probabilities greater than 0.90 and a bootstrap value greater than 90%. Only bootstrap values >50% and Bayesian probabilities >0.50 are shown. The ex-type isolates are designated by a superscript T.

Accession numbers of sequences deposited in this study are in bold font. Dichotomomyces cejpii NRRL 5183’ was used as outgroup. The asterisk on N. nishimurae CBS 117265 (≡IBT 3016) indicates that this strain is not identical with isolate KACC 41955 (≡A. turcosus) as stated by Samson et al. (2008) and Hong et al. (2008). The dashed line defines taxa analysed in three single-locus trees (act1, RPB2, ITS) shown in Fig. S1.

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Heterothallism is relatively common in different clades of the Aspergillus genus. Our data indicated that isolates NRRL 179 T and CCM 8003 T are well separated from all other species in section Fumigati based on five loci examined, phenotypic analysis and secondary metabolite analysis and are proposed here as representatives of novel species.

**TAXONOMY**

Our data indicated that isolates NRRL 179 T and CCM 8003 T are well separated from all other species in section Fumigati based on five loci examined, phenotypic analysis and secondary metabolite analysis and are proposed here as representatives of novel species.

**Description of Aspergillus waksmanii Hubka, S.W. Peterson, Frisvad & M. Kolařík sp. nov. (Fig. 2)**

Aspergillus waksmanii (waks.man´i.i. N.L. masc. gen. n. waksmanii of Waksman, named to honour Dr Selman A. Waksman who isolated this fungus). Colonies on MEA attain a diameter of 48–59 mm in 7 days at 25 °C, light greenish grey (#B2BEB5) initially plane, later becoming floccose to granular due to abundant cleistothecia; conidial heads abundant; reverse vivid yellow (#F3C300). Colonies on CYA attain a diameter of 54–62 mm in 7 days at 25 °C, moderate greenish yellow (#B9B459), radially sulcate; cleistothecia sparse; conidial heads sparse; reverse vivid yellow (#F3C300). Colonies on CZA attain a diameter of 28–35 mm in 7 days at 25 °C, light yellow green (#C9DC89), plane, floccose; cleistothecia and conidial heads few in number; reverse light yellow green (#C9DC89).

Mycelium composed of hyaline, branched, septate, smooth-walled, 1.5–4.0 μm wide hyphae. Cleistothecia homothallic, white, globose to subglobose, 80–250 μm in diameter, covered by a dense felt of white hyphae; asci eight-spored, globose to subglobose 14–17(–18.5) × 11–12.5 μm; ascospores broadly lenticular, spore body 4.5–5.7 × 4–5.2 μm, with two well-separated, 1–3 μm wide and frequently irregular equatorial crests, convex surface smooth or nearly so. Conidial heads greyish green, short columnar, conidiophores hyaline, smooth-walled, usually 150–300 × 3.5–5.0 μm wide at the middle, non-septate or with occasional septum, reduced short conidiophores or solitary phialides frequently present on the aerial mycelium, vesicles clavate, subclavate, subglobose to globose, 10–22 μm diameter, phialides lageniform, born on vesicle, 5–7 × 2.5–3.5 μm, covering half to two-thirds of the vesicle; conidia green in mass, globose to subglobose, 2.5–3.5(–4.2) μm, smooth (microtuberculate in SEM). The species is able to grow at 47 °C and does not grow at 50 °C.

Holotype is PRM 860537, a dried herbarium specimen; isotype is PRM 860538.

Mycobank accession no. MB801063.

The ex-type culture is NRRL 179 T (=CCF 4266 T=Thom 4138.H52 T=IBT 31900 T), isolated from soil, New Jersey, USA, 15 August 1916, Dr S. A. Waksman.

The homothallic species closest to A. waksmanii is N. assulata with the convex surface of ascospores decorated by flaps and with less pronounced crests than A. waksmanii. The other homothallic Neosartorya species with ascospores similar to A. waksmanii (ascospore body size, smooth convex surface and distinct crests) can be distinguished by colony morphology, growth parameters and micromorphology. Colonies in shades of yellow are present in Neosartorya aurata, Neosartorya aureola and Neosartorya stramenia. Funiculose texture and slower growth parameters on MEA and CYA are present in Neosartorya galapagensis. Crests are not as wide in Neosartorya australensis and Neosartorya shendawei. Cleistothecia of...
Neosartorya papuensis are yellowish. Neosartorya glabra does not grow at 47 °C in contrast to A. waksmanii. Whitish colonies and sparse conidial heads are present in Neosartorya paulistensis on MEA. Unique secondary metabolite spectrum differentiates A. waksmanii from closely related species (Table 1), as well as from other species in section Fumigati (Samson et al., 2007).

**Description of Aspergillus marvanovae Hubka, S.W. Peterson, Frisvad & M. Kolařík sp. nov. (Fig. 3)**

Aspergillus marvanovae (mar.va.nov’ae. N.L. fem. gen. n. marvanovae of Marvanová, named to honour Dr Ludmila Marvanová who isolated this fungus).

Colonies on MEA attain a diameter of 45–50 mm in 7 days at 25 °C, greyish yellow green (#8F9779), plane, velvety to powdery; conidial heads abundant; reverse greyish moderate yellow green (#8A9A5B). Colonies on CYA attain a diameter of 45–48 mm in 7 days at 25 °C, pale orange yellow (#FAD6A5), velvety, slightly umbonate and later radially sulcate; conidial heads abundant; reverse moderate orange yellow (#E3A857). Colonies on CZA attain a diameter of 38–41 mm in 7 days at 25 °C, with nearly white, plain, submerged mycelium creating a broad zone around the borders of colonies; reverse uncoloured.

Mycelium composed of branched, septate, smooth-walled hyphae, 1.5–3.5 μm wide. Conidial heads columnar, conidiophores 50–250 × (1.8–)2.7–4.7(–5.6) μm wide in the middle, smooth-walled, frequently septate, basal area occasionally brownish-black, vesicles variable in shape, subglobose, hemispherical, pyriform, flask-shaped or subclavate (5–)7–17(–20) μm diameter, frequently set at an angle on the conidiophore with a ‘nodding’ appearance, phialides lageniform, born on vesicle, 5.1–9.5 × 2.1–3.7 μm covering half to two-thirds of the vesicle, non-septate or with occasional septum; conidia green in mass, globose to subglobose, 2.2–2.9(–3.1) μm, smooth (microtuberculate in SEM). The species is able to grow at 47 °C and does not grow at 50 °C.
Holotype is PRM 860539, a dried herbarium specimen; isotype is PRM 860540.

Mycobank accession no. MB801064.

The ex-type culture is CCM 8003T (=CCF 4037T=NRRL 62486T=IBT 31279T=IFM 60873T), isolated from water with high content of boracic acid anions, in Dukovany nuclear power station, Czech Republic, 27 May 1986, Dr L. Marvanová.

The species most closely related to *A. marvanovae* are *A. turcosus* and *A. unilateralis* (both with teleomorph unknown) and heterothallic *N. nishimurae*. In contrast to *A. marvanovae*, colonies of *A. turcosus* have similar texture and colour on CYA and MEA. Nodding or pseudo-nodding heads seen in *A. marvanovae* can also be observed in *A. unilateralis*, *A. viridinutans* and *N. udagawae*, but all these species have their maximal growth temperatures between 42–45 °C, while *A. marvanovae* grows at 47 °C. *A. unilateralis* has echinulate conidia, vesicles bearing only limited numbers of phialides, shows slower growth on all media tested and reverse on CZA is almost black after 2 weeks of cultivation (Fig. 3). Conidia of *N. nishimurae* are larger and ellipsoidal. Other species with unknown teleomorph and heterothallic species in section *Fumigati* can be distinguished by their maximum growth temperatures (Balajee *et al.*, 2007), micromorphology, and differences in colony texture and colour. *A. marvanovae* produces a spectrum of secondary metabolites which is different from closely related species (Table 1) and as well as from other members of section *Fumigati* (Samson *et al.*, 2007).

**Fig. 3.** *Aspergillus marvanovae* CCM 8003T. (a) Colonies on MEA at 25 °C after 7 days; (b) colonies on CYA at 25 °C after 7 days; (c) reverse on CZA at 25 °C after 2 weeks, *A. marvanovae* CCM 8003T on the left side and comparison with *A. unilateralis* CBS 126.86T on the right side; (d–e) conidiophores; (f) columnar conidial heads on MEA; (g) conidiophores observed by SEM; (h) conidia. Bars: (d), (e), 5 μm; (g), (h), 10 μm.

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REFERENCES


