
***Aureobasidium iranianum*, a new species on bamboo from Iran**

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An endophytic species of black yeasts, *Aureobasidium iranianum*, is described and illustrated from bamboo stem in Iran using morphological as well as sequence data from the ITS-rDNA region. The species can be distinguished from other *Aureobasidium* species by the abundant production of pigmented arthroconidia in culture and ITS barcode.

Key words – black yeast – ITS – taxonomy

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Introduction

The genus *Aureobasidium* Viala & Boyer is a yeast-like deuteromycete that accommodates species with synchronous conidium production and hyaline conidiogenous cells (Hermanides-Nijhof 1977, Zalar et al. 2008). Members of this genus produce one-celled conidia of various shapes from hyaline and terminal, lateral or intercalary conidiogenous cells (Hermanides-Nijhof 1977). Species of the genus are ubiquitous and found on different habitats such as plant materials (phyllosphere, plant debris, bark, roots, fruits, wood), water, marine sediments, marshland, soil, air, skin, nails, stone, glass and in the clinical laboratory as a contaminant or human pathogen (Hermanides-Nijhof 1977, de Hoog & Yurlova 1994, de Hoog et al. 1999, 2000, Urzi et al. 1999). Members of this genus have diverse life styles as saprophytes, plant associated endophytes or pathogens and opportunistic human pathogens (Andrews et al. 2002, Loncaric et al. 2008, Martini et al. 2009). *Aureobasidium pullulans* (De Bary) Arnaud, the

type species of the genus, is well known for its commercial significance as a producer of pullulan, a biodegradable extracellular polysaccharide (Singh et al. 2008, Zalar et al. 2008) and also has been considered as a potential biocontrol agent of post harvest plant pathogens (e.g., *Botrytis cinerea* and *Penicillium expansum*), by means of different antagonistic strategies such as competing for nutrients and space and producing numerous compounds including pectolytic enzymes, antimicrobial metabolites and high-molecular-weight polysaccharides (Castoria et al. 2001, Dugan et al. 2002, Schena et al. 1999, 2003, Seibold et al. 2004, Elmer & Reglinski 2006, Felice et al. 2008, Zalar et al. 2008, Martini et al. 2009).

During a study of microfungi associated with bamboo species in southern Iran (Bushehr Province) an endophytic black yeast fungus was isolated from apparently healthy bamboo stems. The fungus was identified as a new species of the genus *Aureobasidium* based on phenotypic features and sequence data from ITS-rDNA region.

Methods

Collection of isolates, morphological and cultural studies

Bamboo stems were sampled from southern parts of Iran, cut into small pieces, surface sterilized with 70 % ethanol, rinsed two times with sterilized distilled water, dried on sterile filter paper, and plated on acidified malt extract agar (MEA; Himedia, India). Pure cultures were established by transferring emergent hyphal tips to a fresh MEA plate. The cultures were deposited in the living Culture Collection of Tabriz University (CCTU), Tabriz, Iran. The fungus was sub-cultured on MEA, and incubated at 25°C in the dark. After 1 week of incubation, cultural and microscopic features were studied. Measurements and microphotographs were taken from slide mounts in lactic acid. Thirty measurements were made for each microscopic structure and 95th percentiles were defined for all measurements with the extremes given in parentheses.

DNA extraction, amplification and phylogenetic analysis

Fungal isolates were cultured on MEA at 25°C in dark. After 7 days, genomic DNA was extracted using the method of Moller et al. (1992). The primers ITS1 and ITS4 (White et al. 1990) were used to amplify part (ITS) of the nuclear rRNA operon spanning the 3' end of the 18S rRNA gene, the first internal transcribed spacer (ITS1), the 5.8S rRNA gene, the second ITS region and the 5' end of the 28S rRNA gene. The reaction mixture and PCR conditions were the same as Arzanlou & Khodaei (2012a, b) and Arzanlou et al. (2012). Sequences were compared with the sequences available in NCBI's GenBank nucleotide (nr) database using a megablast search.

Results

DNA Phylogeny

Based on megablast search of ITS sequence against GenBank nucleotide database, the closest hits were for *Aureobasidium pullulans* isolates with 97 % maximal identity.

Morphology

Aureobasidium iranianum Arzanlou & S. Khodaei **sp. nov.** Figs 1–8

Mycobank: MB800705

Etymology – epithet derived from the country of origin.

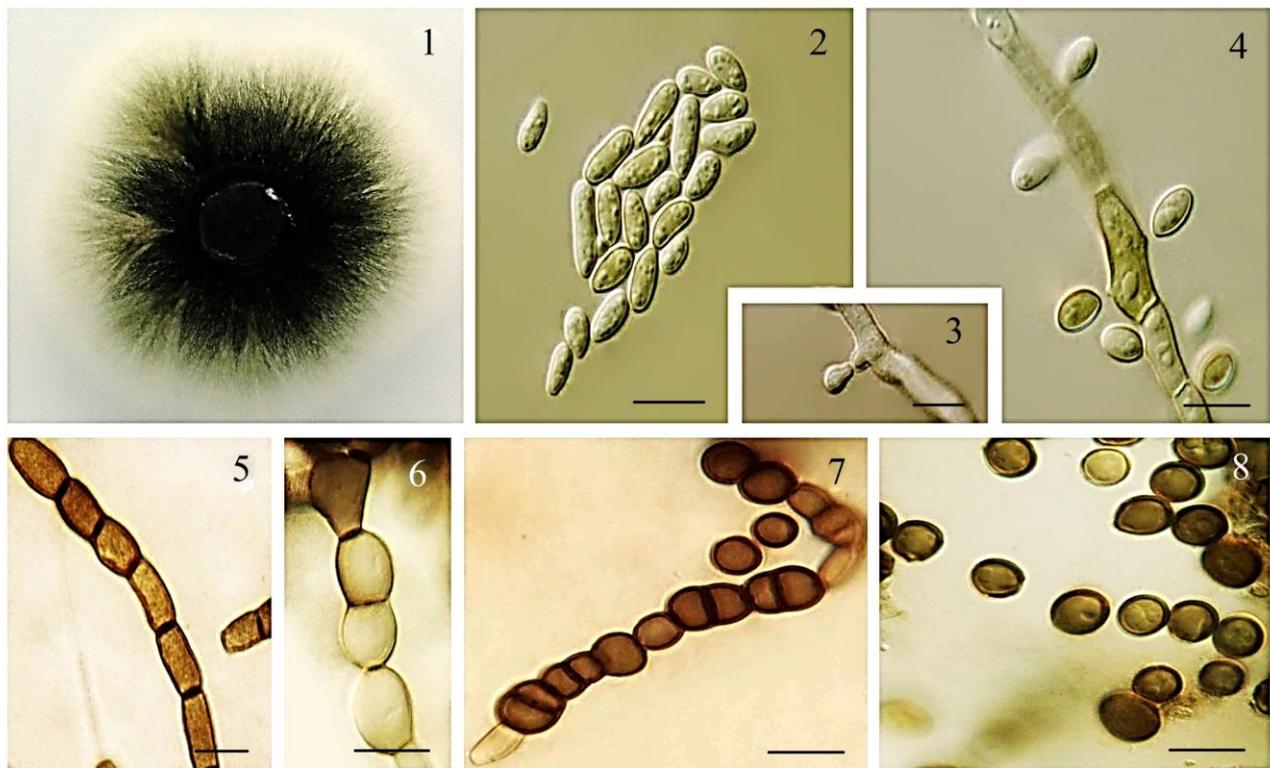
Diagnosis - distinguished from other known species of the genus *Aureobasidium* by abundant production of darkly pigmented arthroconidia and conidial dimensions.

Colonies on MEA moderately fast growing, attaining diameter of 20 mm in 7 days, spreading, smooth, flat, rapidly turning to olivaceous black, with dark green, irregular margins, covered with slimy masses of conidia (Fig 1). Mycelium immersed, no aerial mycelium. Hyphae subhyaline, smooth, thin-walled, 3-8 µm wide, septate, soon becoming brown, thick-walled. Conidiogenous cells undifferentiated, hyaline, intercalary or rarely terminal. Conidia blastic, produced simultaneously in dense clusters from small denticles, (5-)7-9(-14) × 3-6 µm, hyaline to dark brown, smooth, one-celled, thin-walled, with several guttules, variable in shape and size, ellipsoidal to spherical to ovoid, straight, rarely slightly curved, with rounded to subtruncate base and a flat basal hilum. Secondary conidia produced by terminal or sub-terminal, mono- or bipolar budding of primary conidia. Arthroconidia frequently formed, dark, 1- to 2- or rarely 4-celled, (5-)10-12(-19) × (4-)6-7 (-11) µm. Conidia and secondary conidia cicatrized at detachment. No endoconidia observed (Figs 2-8).

Material examined – IRAN, Bushehr Province, Kangan, Assaluyeh, stems of *Bambusa* sp. (Poaceae), 10 June, 2009 dried culture preserved as CCTU H- 268 **holotype** (culture **ex-type** = CCTU 268).

Discussion

The genus *Aureobasidium* belongs to the family Dothioraceae (order Dothideales, class Dothidiomycetes, Ascomycota) (Schoch et al. 2006, Zalar et al. 2008). The genus is characterized by holoblastic and synchronous conidium formation. There are several other mitosporic fungi with phylogenetic affinity to the order Dothideales viz., *Kabatiella* Bubák,



Figs 1–8 – *Aureobasidium iranianum* **1** 7-day-old colony on MEA **2** Conidia **3, 4** Conidiogenous cells and conidia **5** Dark hyphae **6, 7, 8** Arthroconidia – Bars = 10 μ m.

Hormonema Lagerb. & Melin and *Selenophoma* Maire (Yurlova et al. 1996, 1999, Zalar et al. 2008). The genus *Kabatiella* is a plant pathogen causing spot on specific plant species. Species of this genus are considered to have a narrow host range. The members of this genus are characterized by their sporodochial conidiomata on host plant leaves. However, in culture it is very difficult to distinguish *Kabatiella* from *Aureobasidium* as sporodochia are usually not formed or fall apart and micromorphology is similar to *Aureobasidium* (Zalar et al. 2008). Many *Kabatiella* species are known only from the sporodochial anamorph on the host plant and there is no living culture available for these species to determine their phylogeny. *Kabatiella* has been considered as a synonym of *Aureobasidium* by Hermanides-Nijhof (1977) who classified all *Kabatiella* species in *Aureobasidium* (Zalar et al. 2008). A phylogeny inferred from LSU sequence data of *A. pullulans* and its varieties revealed phylogenetic affinity of *Kabatiella* spp. with *A. pullulans* (Zalar et al. 2008). However, *Kabatiella* has been listed as a separate genus by Seifert et al. (2011); according to them *Kabatiella* is

morphologically distinct from *Aureobasidium*. Most of the species in the genus *Kabatiella* have sickle-shaped conidia, a feature which can be used to make a distinction between the two genera (Zalar et al. 2008). More isolates are needed to explore phylogeny of these morphologically similar genera.

Aureobasidium iranianum was identified as a member of the genus *Aureobasidium* by morphological characteristics of conidiogenous cell and conidia. Conidiogenous cells are undifferentiated from vegetative hyphae, hyaline, intercalary or rarely terminal (Yurlova et al. 1996, 1999, Zalar et al. 2008). Conidia are produced simultaneously in dense clusters from small denticles. *A. iranianum* produces 1-celled conidia which are hyaline or brown. The formation of brown conidia have been also observed in *A. pullulans* var. *melanogenum* isolates, originating either by transfer of hyaline conidia or disarticulating dark brown hyphae (arthroconidia). Formation of brown conidia occurred in *A. iranianum*; the first type of brown conidia (transfer of hyaline conidia) were always 1-celled, the second type (arthroconidia) were 1- to 2- or rarely 4-celled cells produced in abundance.

A. iranianum can be easily distinguished from *A. pullulans* var. *melanogenum* by its conidial dimensions, which in both cases (blastoconidia and arthroconidia) are larger than those of *A. pullulans* var. *melanogenum*. Besides, in *A. pullulans* var. *melanogenum* percurrent expansion of the conidiogenous cells has also been observed, which was not observed in *A. iranianum*. Percurrent expansion for the conidiogenous cells is characteristics of the genus *Hormonema* (Zalar et al. 2008).

The identity of *Aureobasidium iranianum* as new species was further confirmed by sequence data from ITS-rDNA region. ITS sequence showed 97 % similarity with *A. pullulans* isolates from GenBank. However there was no ITS sequence available for the other species of this genus in GenBank. A comparison made by Prof. Sybren de Hood at his private sequence database of black yeast fungi, housed at CBS-Fungal Biodiversity Centre in Utrecht, The Netherlands revealed that *A. iranianum* clusters outside *A. pullulans* and its varieties. The ITS sequence for CCTU 268 is available with the GenBank accession number: JX205092. The ecology of *A. iranianum* remains to be studied.

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