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New record of *Scedosporium dehoogii* from India

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Abstract — Three strains of *Scedosporium* were isolated from Pimpri (Pune), Maharashtra, India, during a survey of soil fungi in industrial areas. Morphological and sequence (ITS1-5.8S-ITS2 rDNA) analyses confirm them to be *Scedosporium dehoogii* (reported here as a new record for India) and *S. apiospermum*.

Key words — disease, *Microascaceae*, phylogeny, scedosporiosis, taxonomy

Introduction

Scedosporium Sacc. ex Castell. & Chalm. (teleomorph *Pseudallescheria* Negroni & I. Fisch.; *Microascaceae*) are cosmopolitan fungi found in soil, sewage, and water, some of which can cause infection in humans. Five medically important *Scedosporium* species — *S. apiospermum* Sacc. ex Castell. & Chalm., *S. aurantiacum* Gilgado et al., *S. boydii* (Shear) Gilgado et al., *S. dehoogii*, and *S. prolificans* (Hennebert & B.G. Desai) E. Guého & de Hoog (Gilgado et al. 2005, 2008) — cause various diseases (broadly termed as scedosporiosis; Guarro et al. 2006, Cortez et al. 2008) that mostly infect immunocompromised patients, causing mycetoma, septic arthritis, osteomyelitis, meningitis, brain abscesses, etc. (Cooley et al. 2007). In India, there have been several reports of *Scedosporium* infection (Lingappa & Lingappa 1962, Acharya et al. 2006, Gopinath et al. 2010, Nath et al. 2010, Mathew et al. 2012). During the present study we isolated one *Scedosporium apiospermum* strain and two *S. dehoogii* strains. Our survey of the literature and fungal checklists (Bilgrami et al. 1991, Jamaluddin et al. 2004) indicates that *S. dehoogii* is a new record for India.

Materials & methods

The fungi were isolated from soil collected from industrial region of Pimpri (Pune), Maharashtra, India, on 23 December 2011. Isolated strains were cultured on five media

obtained from Hi-Media (India) — 2% malt extract agar (MEA), potato dextrose agar (PDA), Czapek Dox agar (CDA), potato carrot agar (PCA), oatmeal agar (OA) — at 10–40±0.2°C in the dark. Morphological characteristics were observed on PDA at 25±0.2°C (7 d). Cultures have been deposited at the Microbial Culture Collection (MCC), National Centre for Cell Science, Pune, India as *S. apiospermum* MCC 1042 and *S. dehoogii* MCC 1043 and MCC 1044, and a specimen of MCC 1044 has been deposited in the Ajrekar Mycological Herbarium (AMH), Agharkar Research Institute, Pune, India. Microscopic characters were studied in lactophenol stained with cotton blue (Hi-Media, India) using Nikon YS100 microscope (Nikon, Japan). Microphotographs were taken on Olympus BX53 (Olympus Corporation, Japan) fitted with ProgRes C5 camera (Jenoptik, USA). Samples were coated with platinum on Jeol minor coater (Jeol-JFC 1600) for examination on a Jeol-JSM 6360A scanning electron microscope at 10 kV. Fungal DNA was isolated from pure 7 d old cultures with beads and lysis buffer in 2 ml Eppendorf tubes according to Sharma & Gräser (2011). Total DNA was checked on gel and NanoDrop spectrophotometer (NanoDrop, USA). PCR was conducted with a Gene Amplifier PCR System 9700 (Perkin Elmer, USA) using ITS1 and ITS4 primers for ITS1-5.8S-ITS2 region (White et al. 1990). A 25 µl mixture containing 10 U Taq polymerase buffer (New England Biolabs), 2 mM dNTPs, 10 pM primers, 1 unit Taq polymerase (New England Biolabs), and 10 ng DNA was amplified following a protocol of initial denaturation at 94°C for 2 min, 35 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min and extension at 72°C for 1 min, final extension at 72°C for 10 min. The PCR product was checked on 1.0% agarose gel and purified by PEG-NaCl (Polyethylene glycol-NaCl). Sequencing was done on ABI 3730xl Automated Sequencer using 'ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit' (Perkin Elmer Applied Biosystems Division, Foster City, CA). An NCBI Blastn search for ITS region was conducted for sequence similarity (Altschul et al. 1990). Sequences of MCC 1042, MCC 1043 and MCC 1044 were generated in lab and other sequences retrieved from GenBank and deposited in European Molecular Biology Laboratory (EMBL) database. The sequences were aligned and edited in Clustal W of Molecular Evolutionary Genetics Analysis (MEGA 5.0) computer program (Higgins et al. 1994, Tamura et al. 2011). Phylogenetic analyses were done by Kimura 2-parameter model using Neighbour Joining (NJ) statistical method. Gaps and missing data was removed by complete deletion option of the software. The bootstrap consensus tree was inferred from 1000 replicates and bootstrap values ≤50% were removed from the tree (Saitou & Nei 1987; Tamura et al. 2011).

Taxonomy

Scedosporium dehoogii Gilgado, Cano, Gené & Guarro, J. Clin. Microbiol. 46(2): 768. 2008. Plate 1

The PDA colonies, which reached 32 mm diam. after 7 d at 25±0.2°C, were radially zonate, dense, cottony to lanose, grayish-white to white and with a whitish lobate or irregular and fimbriate margin; the reverse was yellowish fading towards margin and with radial zones also visible. Other strain was effused, white to pale gray, fast growing, 75 mm after 7 d at 25±0.2°C on PDA

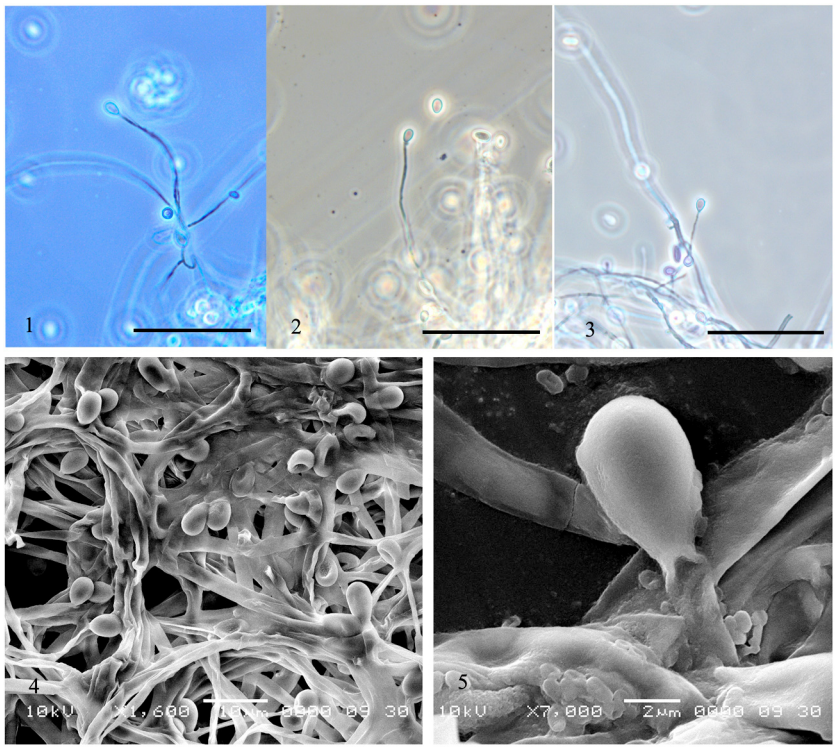

Plate. 1. *Scedosporium dehoogii* MCC 1044. Fig. 1–3. Conidiogenous cells with conidia. Fig. 4. SEM of mycelial mat with conidia. Fig. 5. SEM of sessile conidia. Scale bars: 1–3 = 50 µm; 4 = 10 µm; 5 = 2 µm.

with colorless reverse. Solitary conidiophores were subhyaline smooth-walled, 10–54 × 1–1.4 µm, and produced pale brown, obovoid or ellipsoidal, 5.9–6.2 × 3.6–4.4 µm conidia. Sessile conidia subhyaline, thick-walled, mostly obovate, (6.9–)7.3(–7.8) × (3.5–)3.8(–4.0) µm. Hyphae hyaline to subhyaline, 1.2–2.5 µm. A teleomorph was not observed even after 60 d of incubation.

Distribution & habitat: Pimpri (Pune), Maharashtra, India (soil from industrial area). (First described from garden soil in Barcelona, Spain.)

Material examined: **INDIA**, **Maharashtra State**, Pimpri (Pune), 18°37(07.04(N 73°48(13.43(E, soil, 23.XII.2011, Rohit Sharma, culture S9-H (AMH-9561; MCC 1044; GenBank HF570054); culture S9-D (MCC 1043; GenBank HF570053).

Results & discussion

Both *Scedosporium dehoogii* strains had similar optimum and maximum growth temperatures, but the MCC 1043 PDA colonies grew around two times faster than those of MCC 1044 (Plate 2). *Scedosporium apiospermum* MCC

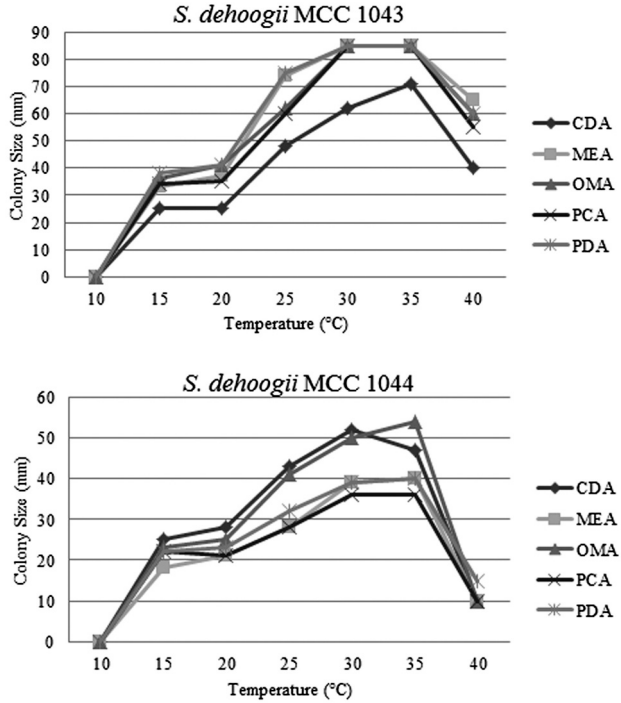

Plate. 2. Temperature-growth relationship of *Scedosporium dehoogii* MCC 1043 and MCC 1044 on Malt Extract Agar (MEA), Czapek Dox Agar (CDA), Oatmeal Agar (OA), Potato Dextrose Agar (PDA), Potato Carrot Agar (PCA) (mm diam. of 7-d old colonies).

1042 had an optimum temperature of 35±0.2°C on OMA (15±0.2°C minimum and 40±0.2°C maximum). The sessile conidia of *S. dehoogii* MCC 1044 are narrower than in the ex-type culture CBS 117406 (5–6 µm; Gilgado et al. 2008). ITS sequences of 62 strains retrieved from GenBank were aligned with the sequences from our *S. apiospermum* and *S. dehoogii* strains. Of the 545 bp used to construct the phylogeny, 434 were conserved, 107 variable, 76 parsimony-informative, and 31 singletons. The NJ-phylogenetic analysis clustered MCC 1043 and MCC 1044 in the *S. dehoogii* species group, which forms a separate branch clearly distinguishing it from other *Scedosporium* species (Plate 3).

Scedosporium apiospermum and *S. prolificans* have been reported several times from India and are associated with various types of infections in both immunocompromised and immunocompetent patients (Gopinath et al. 2010, Nguyen & Raychaudhuri 2011, Patil et al. 2011, Mathew et al. 2012, Kaur et al. 2013, Matlani et al. 2013); however, *S. dehoogii* is reported for the first time from

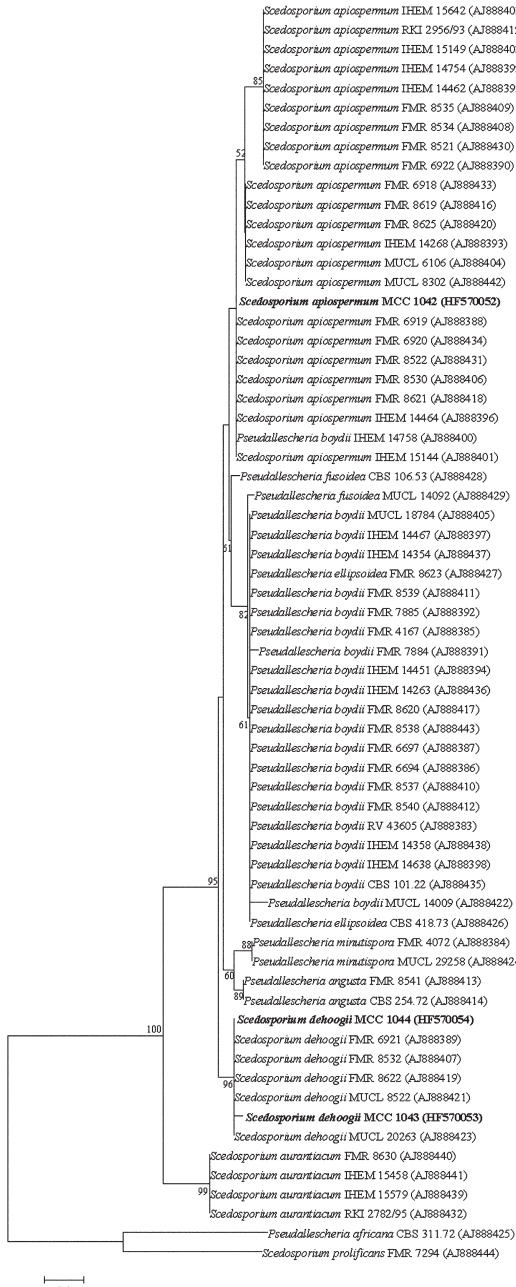

Plate. 3. Neighbor-joining tree derived from the ITS region rDNA sequences of *Scedosporium* spp. Bootstrap values were calculated from 1000 replications. Bootstrap below 50% were deleted.

India and outside Europe. Isolation of these strains from a single soil sample is an important factor as there are growing numbers of immunocompromised patients in Pune, India due to immunopulmonary infections (Salvi & Barnes 2009), AIDS, H1N1, and other viral infections caused by compromised hygiene in many hospitals. Although Gilgado et al. (2009) has shown that *S. dehoogii* is pathogenic to immunocompetent mice, detailed surveys of different natural and human dominated habitats are required to confirm distribution of *Scedosporium* in India as noted by Kaltseis et al. (2009) for Austria and the Netherlands. Research on secondary fungal infections of immunocompromised patients may also determine whether the victims are actually succumbing to secondary infection caused by *Scedosporium* or similar fungi.

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