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A new record of *Gliocephalotrichum simplex* from India

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Abstract — During a survey of interesting and rare fungi infecting economically important plants in the forests of the Western Ghats in India, an uncommon fungal species was isolated from fruits of *Terminalia chebula*. The fungus has distinctive morphological features such as a whorl of sterile arms subtending penicillate branches bearing yellowish masses of elongated to ellipsoidal conidia. Based on morphological characters and a comparison of sequences of the internal transcribed spacer region of rDNA (ITS1-5.8S-ITS2), the fungus was determined to be *Gliocephalotrichum simplex,* a species not previously known from India.

Key words — anamorph, fungal diversity, *Hypocreales,* ITS sequence

Introduction

India is a tropical country that harbors considerable fungal biodiversity (Bilgrami et al. 1991, Jamaluddin et al. 2004). As part of our ongoing effort to discover and preserve fungi, we are making regular surveys and isolating rare and unusual fungi. During 2008–09 partially degraded fruits of *Terminalia chebula* were collected from the forest floor in Western Ghats in Maharashtra state, India. From these fruits a fungus was isolated and identified as *Gliocephalotrichum simplex*. This fungus has never been recorded from India. The present communication describes this fungus from India on artificial media isolated from fruits of *T. chebula*.

Materials and methods

Isolation, pure culture and microscopic examination

Samples of *Terminalia* fruits were collected in separate paper bags and transported to the laboratory. The fruit samples were surface-sterilized by dipping in 70% ethanol for 10 min, then rinsed in distilled water and incubated in a moist chamber at 25 ± 2°C. A yellowish to grayish fungal growth appeared on the fruit surface after 4 days. Direct

streak and serial dilution plate methods were used to isolate the fungus as a pure culture. Potato dextrose agar (potatoes, peeled, sliced 200 g, dextrose 20.0 g, agar 20 g, water 1L) and V8 (HIMEDIA) were used as the isolation medium. The isolation plates were sealed with parafilm (M250-HIMEDIA) and incubated at ambient lab temperature (25°C).

A Nikon trinocular stereozoom microscope (Model SMZ- 1500 with Digi CAM) was used for direct observation of the fungal growth pattern on the fruit surface. For microscopic details and photomicrographs, an Olympus CX-41 microscope was used. Specimens were mounted in lactophenol-cotton blue and distilled water for microscopic studies. Measurements of fungal structures were made with an ocular micrometer.

The specimen is deposited in Ajrekar Mycological Herbarium (AMH, according to Holmgren et al. 1990) and a pure culture is deposited in the National Fungal Culture Collection of India (NFCCI-WDCM 932), MACS' Agharkar Research Institute, Pune, India.

DNA isolation

The fungal strain was maintained on PDA slants. DNA was extracted from cultures grown on PDA plates for two weeks at 28°C by first homogenizing the mycelium in FastPrep®24 tissue homogenizer (MP Biomedicals GmbH, Germany) and then using the CTAB method of Graeser et al. (1999).

PCR amplification

For ITS-PCR the universal primers ITS4 (5' TCC TCC GCT TAT TGA TAT GC3') and ITS5 (5' GGA AGT AAA AGT CGTAAC AAG G 3') amplifying a DNA fragment of about 700 bp of the rDNA gene were used (White et al. 1990). The PCR mixture contained reaction buffer (10 mM TrisHCl pH 8.0, 50 mM KCl, 1.5 mM $MgCl_2$), 200 µM of each deoxynucleoside triphosphates (Genei, Bangalore, India), 50 pM each of primers, 1U of Taq polymerase (Genei, Bangalore, India), and 25 ng of template DNA. Samples were overlaid with sterile mineral oil and amplified through 30 cycles in a thermocycler (Eppendorf MastercyclerAG, Hamberg, Germany) as follows: initial denaturation for 5 min at 95°C, denaturation for 1 min at 95°C, annealing for 1 min at 56°C, and extension for 1 min at 72°C. This was followed by a final extension step for 10 min at 72°C. The resulting PCR product was checked on 1.2% agarose gel (Sigma).

Sequencing

PCR products were cleaned with Axygen PCR cleanup kit (Axygen Scientific Inc, CA, USA) and sequenced using primers ITS4 and ITS5 (White et al. 1990) on an automated DNA Sequencer ABI 3130 (Applied Biosystems, USA).

Sequence Alignment & Phylogenetic tree

rDNA sequences (ITS1-5.8S-ITS2) of the Indian isolate of *G. simplex* were manually aligned with those of known *G. simplex* sequences and the other six species of *Gliocephalotrichum* in the NCBI database (Table 1) using text editor option of the software MEGA for similarity. The manually edited sequence of NFCCI1496 was deposited in the EMBL nucleotide sequence database (FN550111) and was also subjected to a BLAST search. The neighbor-joining tree was derived from analyses of ITS1-5.8S- ITS2 sequences using Mega4.0 software.

Taxonomic description

Gliocephalotrichum simplex (J.A. Mey.) B.J. Wiley & E.G. Simmons, Mycologia 63(3): 578, 1971. Figs 1–8

Habitat: On rotting fruit of *Terminalia chebula* Retz. (*Combretaceae*).

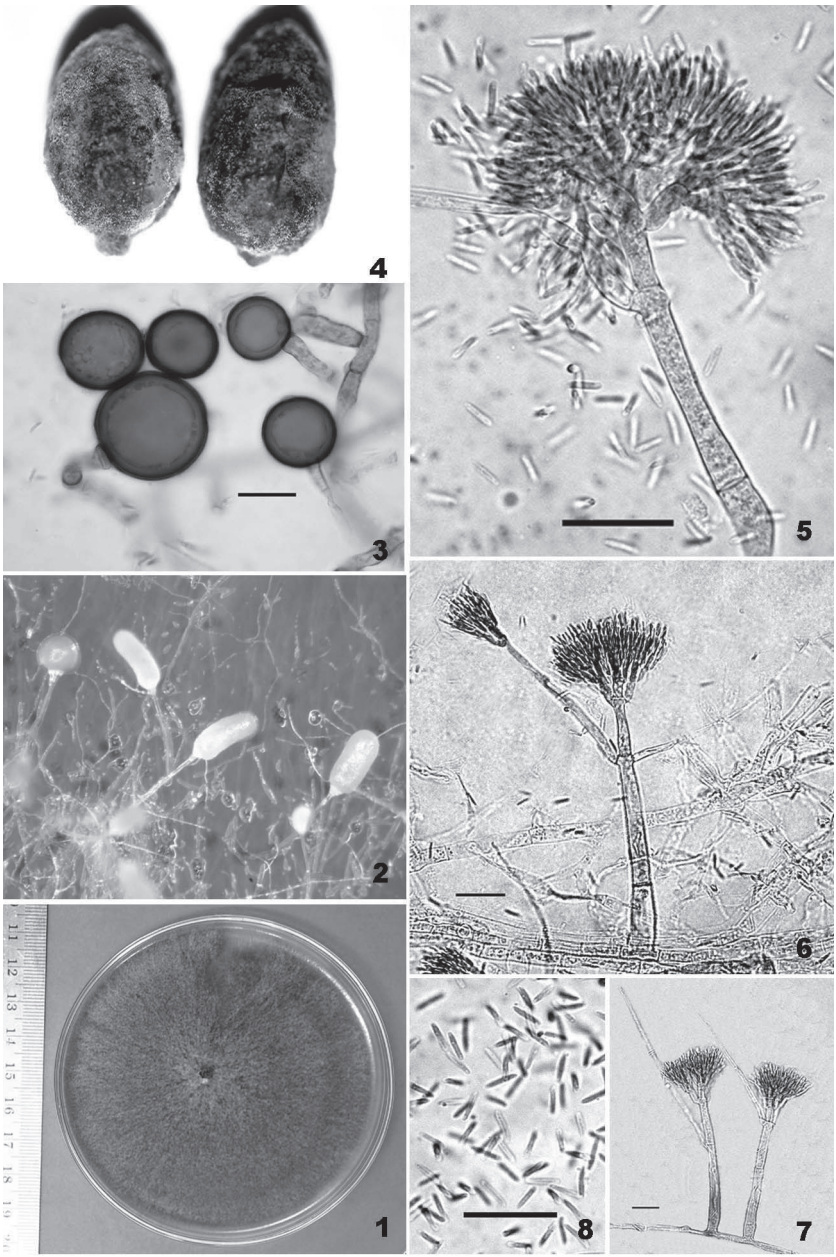
Teleomorph: Unknown.

Anamorph: Optimum temperature for growth 25–28°C. Colony radius after 3 d on PDA (80 mm), CMA (75 mm) and V8 (70 mm). Colonies on PDA off-white in centre, floccose cottony, buff, golden brown, sporulating, margin irregular. Reverse buff. Appearance in nature: Substrate brown to blackish, covered with grayish-white colonies that later turn yellowish and spread over entire outer surface. Hyphae branched, septate, hyaline, smooth, 7.5–10.5 µm wide. Chlamydospores one-celled, terminal to intercalary or lateral, subglobose to mostly globose, thick-walled, golden brown with short stalk, 20–35 × 20–32 µm diam. Sterile hairs 1–2, originating from branching point of conidiophores or beneath septum subtending penicillus, hairs hyaline 3–8 septate, 125–412 µm long, base broad, tip narrow. Conidiophores erect, simple to branched, arising directly from submerged mycelium, hyaline to subhyaline, 80–162.5 × 7.5–10 µm, broad at base gradually narrower towards apex, 2–6-septate, at apex bearing a compact penicillus, with slimy head. Penicillus of successive branches, primary branches 7–10 × 4–6 µm, secondary branches 6–8 × 4–5 µm, tertiary branches 5–7 × 4–5 µm, quaternary branches 5–6 × 2–4 µm. Conidia cylindrical to ellipsoidal, smooth, hyaline, 7.5–9(–10) × 1–1.5 µm.

Specimen examined: India, Mahabaleshwar (17°55'15"N 73°′39'21"E), Maharashtra, on degraded fruits of *Terminalia chebula* (*Combretaceae*), Oct. 2008, *L. S. Yadav*, AMH 9279, Culture No. NFCCI1496

Notes—The genus *Gliocephalotrichum* J.J. Ellis & Hesselt., typified by *G. bulbilium* J.J. Ellis & Hesselt., is mainly characterized by the origin of the sterile arms and the conidia along with the morphology and dimension of chlamydospores (Ellis & Hesseltine 1962, Decock et al. 2006). This genus has been expanded to include six additional species: *G. bacillisporum* Decock & Huret, *G. cylindrosporum* B.J. Wiley & E.G. Simmons, *G. longibrachium* Decock & Charue, *G. microchlamydosporum* (J.A. Mey.) B.J. Wiley & E.G. Simmons, *G. ohiense* L.H. Huang & J.A. Schmitt, and *G. simplex* (Ellis & Hesseltine 1962, Wiley & Simmons 1971, Huang & Schmitt 1973, Decock et al. 2006).

Sequencing of rDNA (ITS1, ITS2 and 5.8S) shows that our isolate is *Gliocephalotrichum simplex*, a species not previously recorded from India. The present strain NFCCI 1496 is part of the clade formed by other strains of *G. simplex* (Fig. 9), however, it differs slightly from its closest strain MUCL46551 from Singapore by three nucleotide positions, i.e. two transition of C→T at 202 and 316 bases along with an insertion of an A at position 8 (Fig. 10).


4
3
5
2
6
1
8
7

Initially, morphological differences viz. number and size of sterile arms and branched conidiophores produced on different media showed slight variation in morphological features from *G. simplex* (Wiley & Simmons 1971), but rDNA sequence comparisons showed that our isolate is indeed *G. simplex*. The setae of our isolate originate directly below the penicillus unlike the descriptions of this species. *Gliocephalotrichum simplex* is distinguished by the presence of 1–3 sterile hairs originating from 10–15 µm below the penicillus and cylindrical conidia measuring 7.5–9(–10) × 1–1.5 µm (n = 100 spores) accommodate this isolate in *G. simplex* (Wiley & Simmons 1971).

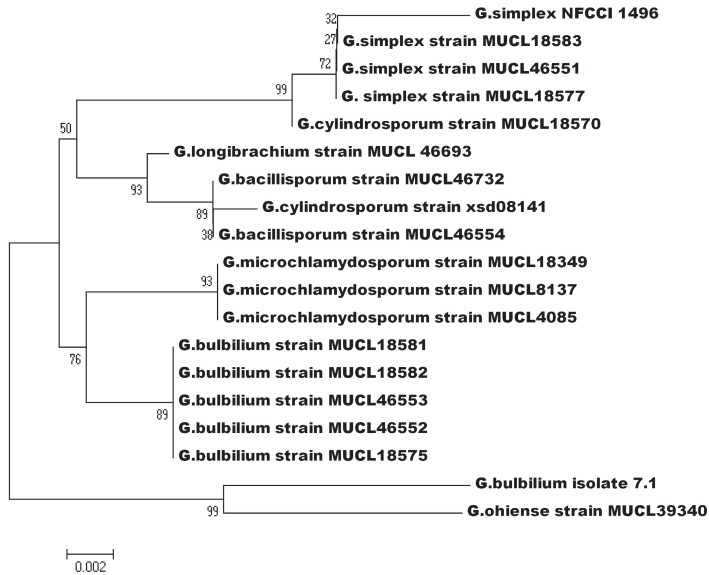


Fig. 9. Neighbour joining tree based on ITS1-5.8S-ITS2 sequences showing the relationships among 21 *Gliocephalotrichum* strains representing 7 species.

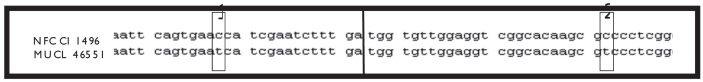


Fig. 10. Inversion (C to T) noted at two locations in *Gliocephalotrichum simplex* MUCL46551 from Singapore in comparison to NFCCI-1496 from India: position 1-base 202 (part of 5.8S gene); position 2 - base 316 (part of ITS2 region of rDNA).

Figs. 1–8 (left). *Gliocephalotrichum simplex*. 1. Colony on PDA after 5 days. 2. Stereoscopic view of yellowish conidial heads. 3. Thick walled, golden brown, globose chlamydospores. 4. Infected fruits of *Terminalia chebula* 5. Mature conidiophores with penicilli. 6. Conidiophores with fertile stipe extensions. 7. Conidiophores with sterile arms (setae). 8. Conidia. Scale bars = 20 µm.

Table 1. Comparison of the rDNA sequences (ITS1-5.8S-ITS2) among isolates of *Gliocephalotrichum*.

Species	Strain Accession §	Similarity *	Genebank/ EMBL Acc.
G. simplex	NFCCI 1496	-	FN550111
G. simplex	MUCL 46551	99%	DQ366704
G. cylindrosporum	MUCL 18570	98%	DQ366705
G. bacillisporum	MUCL 46554	97%	DQ374408
G. longibrachium	MUCL 46695	97%	DQ278422
G. bulbilium	MUCL 18582	96%	DQ381952
G. microchlamydosporum	MUCL 18349	96%	DQ366701

§ NFCCI: National Fungal Culture Collection of India, Pune, India;
MUCL: Mycotheque de l'Universite Catholique de Louvian, Louvain-la-Neuve, Belgium.
* with NFCCI 1496

Source: NCBI (http://www.ncbi.nlm.nih.gov/)

There is no previous record of *G. simplex* from India (Bilgrami et al. 1991, Jamaluddin et al. 2004). Earlier records of *G. simplex* from various parts of the world are mainly from soil and debris (Watanabe & Nakamura 2005), although it has been reported on fruit of rambutan (Nishijima et al. 2002). The isolate from India is reported for the first time from fruits of *Terminalia chebula,* a plant that has been used as a traditional medicine. Therefore, the present fungus is documented here as new record from India.

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