Aspergillus terreus Thom a new pathogen that causes foliar blight of potato

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Foliar blight of potato caused by *Aspergillus terreus* is reported for the first time. The blight is characterized by a brown leaf apex amounting to 35–65% of the total leaf surface. The pathogen was identified on the basis of morphological characters and ribosomal DNA sequence data. The fungus produced effuse white colonies, branched hyphae, broom-like conidiophores, and globose accessory conidia measuring 1.5–2.3 µm in diameter. Koch's postulates were confirmed by performing pathogenicity test on healthy potato plants.

Key words – Accessory conidia – Conidiophore – pathogenicity test – rDNA – *Solanum tuberosum* L

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Introduction

Aspergillus terreus Thom is omnipresent in the environment. So far, the pathogen commonly colonizes grassland soils, compost heaps, stored corn, barley and peanuts (Kozakiewicz 1989). Nevertheless, of recent *A. terreus* has largely been used as a biocontrol agent in agricultural science. Here, we report a pathogenic strain causing foliar blight of potato (*Solanum tuberosum* L.) for the first time. The purpose of this study was to characterize this pathogenic strain of *A. terreus* based on phenotypic and ribosomal DNA data, and verify its pathogenicity on potato.

Methods

Isolate and morphology

A leaf of potato showing foliar blight was collected from a potato farm in Burdwan District, West Bengal, India (23°14´N, 87°51´E) during January 2011. Diseased leaf fragments were surface disinfected in 0.1% sodium hypochlorite (5 min), 70% ethanol (2 min) and rinsed in sterile water with three changes. The leaf pieces were plated on potato dextrose agar (PDA) medium (HiMedia®). Cultures were maintained at 25 ± 1°C under 12 hours photoperiod. A pure culture was obtained by maintaining the fungus on PDA amended with 350 mg/L chloramphenicol. A 12 days-old culture was used for phenotypic characterization as follows. Conidia were harvested. Two to three drops of 0.01 %w/v Rose Bengal disodium salt (Fisher®) dissolved in 0.1 M NaOH solution was applied for staining, and observed under a bright-field Olympus BX61 microscope coupled with

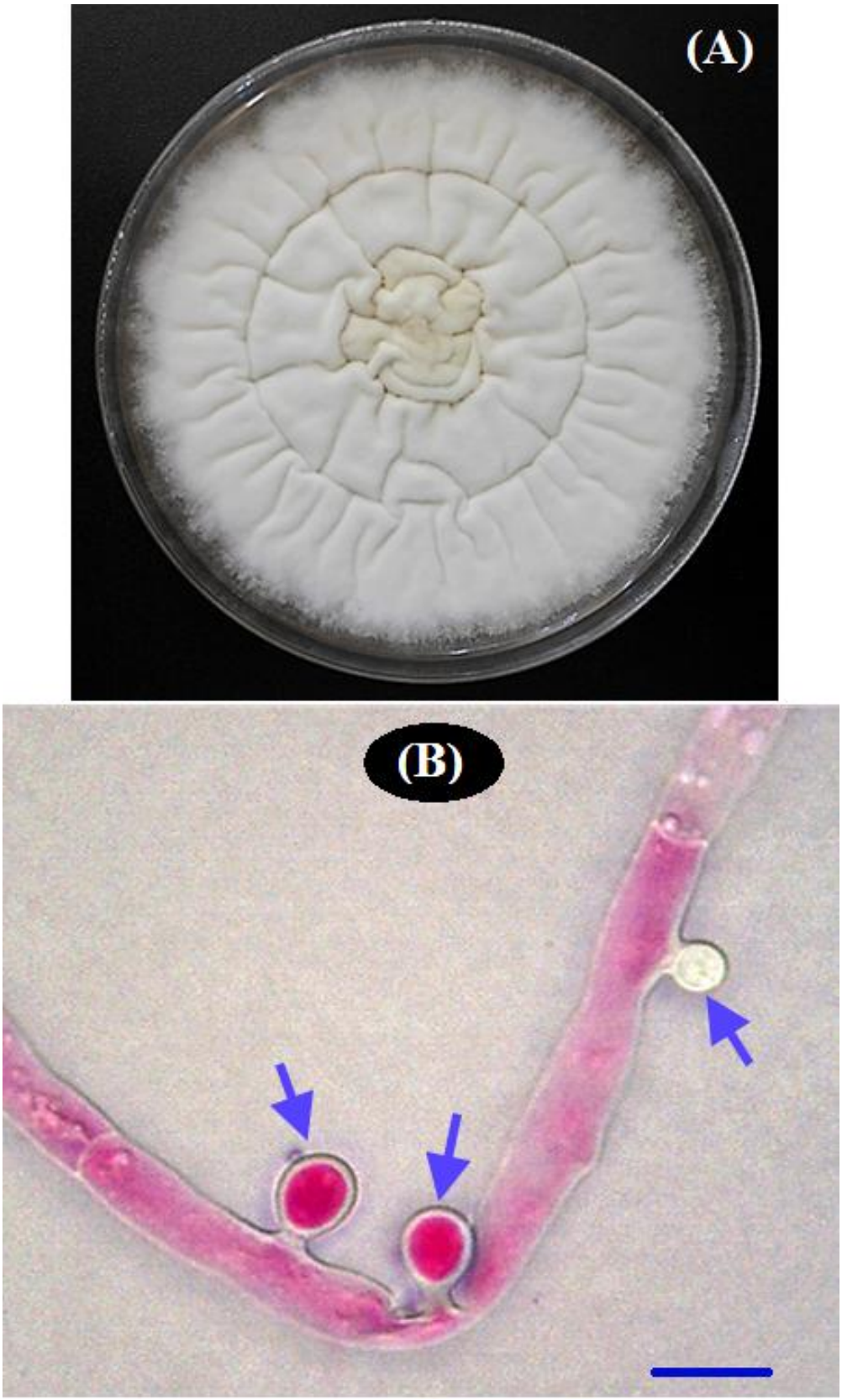

Fig. 1 – **A–B** *A. terreus*. **A** 12–days–old culture on PDA. **B** Details of hyphae and the accessory conidia indicated by an arrow. Scale bar=10 µm.

DP7M5.0.0.5 software and Olympus DP70 camera. The characterized pathotype has been deposited in the living Indian Type Culture Collection (ITCC), IARI, New Delhi-India.

Fig. 2 – Broom-like conidiophore of *Aspergillus terreus.* Scale bar=30 µm.

DNA Phylogeny

The fungus was grown in potato dextrose broth (HiMedia®). The genomic DNA was isolated using UltraClean™ Microbial DNA isolation kits (Mo Bio Laboratories, Carlsbad, CA, USA) as described by the manufacturer. Partial 5.8S rRNA gene, complete internal transcribed spacer two and partial 28S rRNA gene were amplified using IT4 (5'-tcctccgcttattgatatgc-3') and ITS3 (5'-gcatcgatgaagaacgcagc-3') primers (White et al. 1990). The PCR product was sequenced and has been submitted to GenBank®.

Pathogenicity test

To verify the ability of the pathotype to cause foliar blight, a pathogenicity test was performed on 3 weeks–old disease–free potato plants (cv. Kufri Jyoti) grown in 7 L capacity pots under greenhouse conditions. A suspension of 10^6 conidia/ml was sprayed on leaves using a hand-sprayer compressor till run-off. Control plants were sprayed with sterilized water only. Plants were covered with polyethylene bags to create a relative humidity of about 100%, and incubated for 48 hours at 20 ± 2ºC. Following removal of plastic bags,

the plants were observed every 24 hours for the development of symptoms.

Results

Isolate and morphology

Aspergillus terreus grew well on PDA producing an effuse whitish colony with an average diameter of 70 mm after 12 days of incubation (Fig. 1A). The fungus produced septated and branched hyphae. The hyphae bored irregular distributed globose accessory conidia with an average diameter of 1.5-2.3 µm (Fig. 1B). Furthermore, the archetypal conidiophores is long, smooth-columnar with colorless tip and bears sub-spherical vesicles; giving an overall broom-like structure (Fig. 2). The accession number of the living culture is ITCCI-8575.11 and it is available on demand.

DNA Phylogeny

The sequence has been submitted in the GenBank nucleotide database as accession JX155853. A blast search of our strain showed 99.82% sequence similarity to *A. terreus*, GenBank type isolate AB644383.

Pathogenicity test

Severe disease occurred 4 days after inoculation. Blighted leaves generally turned yellowish at the advance stage of the disease (Fig. 3). Disease often spread along the midrib. Whitish mycelial patches were often seen on the blighted area (Fig. 4). At the advance stages of the infection, leaves curled and the apex dried-off. The affected area amounted to 35–60% of the total leaf surface. Following pathogenicity test, the pathogen was re-isolated confirming Koch's postulates.

Discussion

The cosmopolitan nature of *Aspergillus terreus*, and its interaction with animals has been the centre of focus. In agriculture, *A. terreus* is widely used as an antagonist for controlling other pathogenic fungi (Itamar et al. 2006). Notwithstanding this beneficial role, *A. terreus* has been reported to cause diseases in wheat and ryegrass (Dewan & Sivasithamparam 1988) and huge postharvest loses in many cash crops (Kozakiewicz 1989). With this result, it is understandable that *A. terreus* may switch roles either as a pathogenic agent or an antagonist biocontrol agent for some phytopathogens, depending on the host plant association. Until now, there exists no report on potato/*A. terreus* interaction. Nevertheless, this is the first report that *A. terreus* causes foliar blight of potato in India.

Fig. 3 – Leaf blight of potato (cv. Kufri Jyoti) caused by *Aspergillus terreus.*

Fig. 4 – Development of whitish mycelial patches on infected leaf following pathogenicity test.

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