

## Short Communication

# Report of foliar necrosis of potato caused by *Cochliobolus lunatus* in India

Bengyella Louis<sup>1,2\*</sup>, Roy Pranab<sup>1</sup>, Sayanika Devi Waikhom<sup>3</sup> and Narayan Chandra Talukdar<sup>3</sup>

<sup>1</sup>Department of Biotechnology, The University of Burdwan, 713104 Golapbag More, West Bengal, India.

<sup>2</sup>Department of Biochemistry, University of Yaoundé I, BP812 Cameroon.

<sup>3</sup>Institute of Bioresources and Sustainable Development (IBSD), Takyelpat, 795001 Imphal, Manipur, India.

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During the winter season of 2011, *Cochliobolus lunatus* was isolated from necrotized leaves of potato in potato plantations of Burdwan District, West Bengal State, India. The isolate was identified using standard monographs and taxonomic keys and confirmed molecularly using the Ribosomal Deoxyribonucleic acid (rDNA) sequence data. The Koch's postulate was confirmed through pathogenicity test on potato cultivar 'Kufri Jyoti'. This is the first report of *C. lunatus* causing brown-to-black spots disease of potato in India.

**Key words:** Brown-to-black spots, pathogenicity, rDNA, *Solanum tuberosum* L., *Cochliobolus lunatus*.

## INTRODUCTION

During 2011 winter survey of potato farms in West Bengal State India, *Cochliobolus lunatus* Nelson and Haasis was observed on 30% of approximately 6000 plotted 3 to 4 weeks old potato (*Solanum tuberosum* L.) plants. *C. lunatus* is well known to cause leaf disease and seedling blights on diverse plants (Sivanesan, 1987), and in some cases, 100% seed loss has been observed (Roa and William, 1978). In India, the Bengal rice famine that lead to the dead of over two million people because of reduction in yield (40 to 90%) was related to *Cochliobolus* spp. disease outbreak (Scheffer, 1997). The purpose of this study was to identify the causal organism of brown-to-black leaf spots disease of potato using both morphological and molecular tools and to verify the pathogenicity of the fungus.

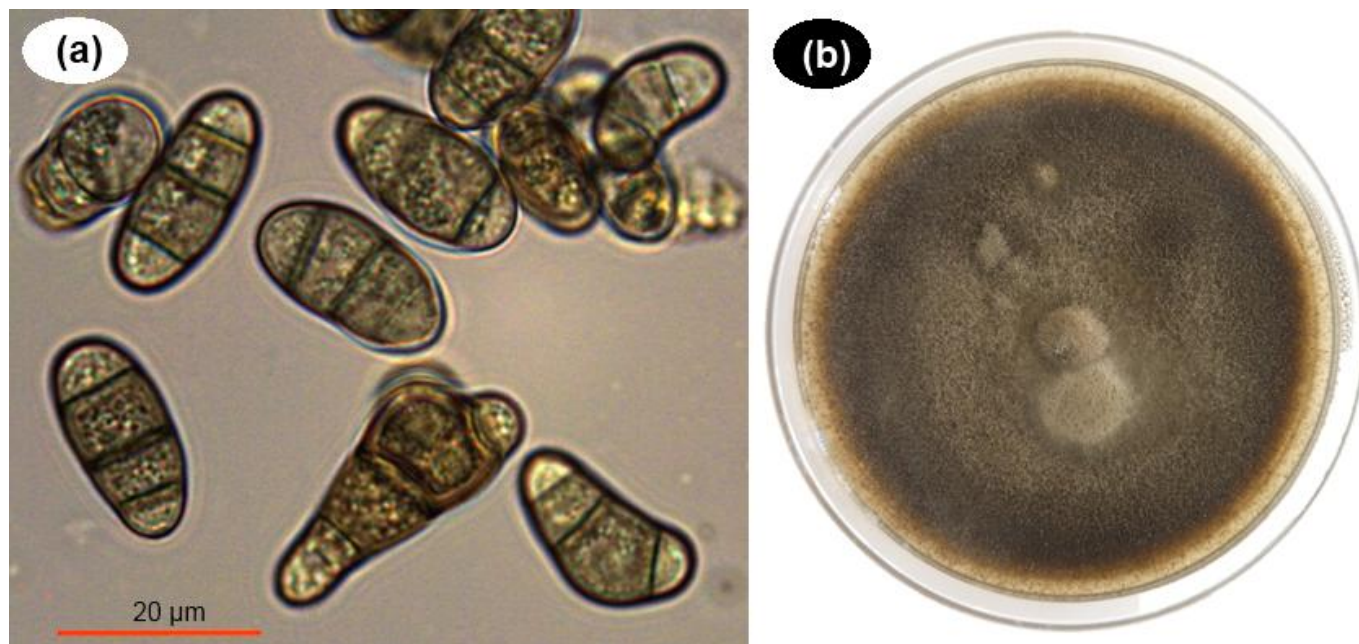
## MATERIALS AND METHODS

Infected leaves of potato were collected during November through December 2011 from Burdwan potato plantation, West Bengal, India. Isolation was accomplished by transferring excised necrotic fragments of leaves into 2% NaClO for 30 s, then wash with sterile

water with three changes and plated on potato dextrose agar (PDA). Petri dishes were sealed and incubated in continuous darkness at 25°C for two weeks. A pure culture was obtained by maintaining the fungus on PDA amended with 250 mg/L chloramphenicol and 100 mg/L ampicillin. The pathogen was identified morphologically based on standard monograph and standard taxonomic keys (Drechsler, 1934). The culture was deposited in the living Indian Type Culture Collection (ITCC), New Delhi, IARI, India. The morphological description was made based on 2 weeks old cultures on PDA exposed to 12 h photoperiod. Microscopic observations were made with Olympus BX61 microscope couple with DP7M5.0.0.5 software and an Olympus DP70 camera.

The Koch's postulates were performed in the greenhouse on 3 weeks-old sprouted potato cv. Kufri Jyoti. Leaves were inoculated with 10<sup>6</sup> spores/ml suspension prepared from a 3 weeks old culture and control plants were misted with sterile water only. The treatments were covered with polythene bags for 24 h for optimizing high humidity of about 100%. Five days after inoculation, leaves were detached and developed necrotic spots were surface sterilize and plated on PDA for re-isolation. Total genomic DNA was extracted from mycelium mat using UltraClean™ Microbial DNA isolation kits (Mo Bio Laboratories, Inc., Carlsbad, CA, USA) as described by the manufacturer. The rDNA ITS2 region was amplified using IT4 (5'-tctctccgcttattgatgc-3') and ITS3 (5'-gcatcgatgaagaacgcagc-3') primers (White et al., 1990). Sequence had been submitted into GenBank® as accession JX907828. 18 hit scores from the GenBank® with threshold identities greater than 98% was aligned in Muscle software (Edgar, 2004). The Akaike information criterion (AIC) and Bayesian information criterion were determined for selecting the best substitution model.

\*Corresponding author. E-mail: bengyellalouis@gmail.com.



**Figure 1.** (a) Conidial morphological variations of *C. lunatus* (GenBank® accession JX907828). (b) Three weeks old colony of *Cochliobolus lunatus* on PDA.

The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei, 1987) and the evolutionary distances were computed using the Jukes-Cantor method (Juke and Cantor, 1969). The tree was drawn to scale with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The rate variation among sites was modelled with a gamma (G) distribution parameter (shape parameter G= 6). A consensus tree with 1000 bootstraps test of replicates was generated. All analysis was performed in MEGA5 (Tamura et al., 2011).

## RESULTS AND DISCUSSION

The fungal colonies produced pale brown-to-dark conidia exhibiting high level of morphological variations (Figure 1a). Most prominently, fusiform and cylindrical conidia ( $26.5 \pm 3.3$  to  $12.0 \pm 1.2 \mu\text{m}$ ) with two central cells that were larger than the two terminal cells were observed in the case of four celled conidium. On PDA, *Cochliobolus lunatus* grew slowly; consistently produced greyish-white and floccose mycelia (Figure 1b). Furthermore, conidia were loosely arranged on conidiophores sparsely distributed or in closer verticils (Elis, 1971). Pure culture of this strain is available in ITCC as accession ITCC8576-11.

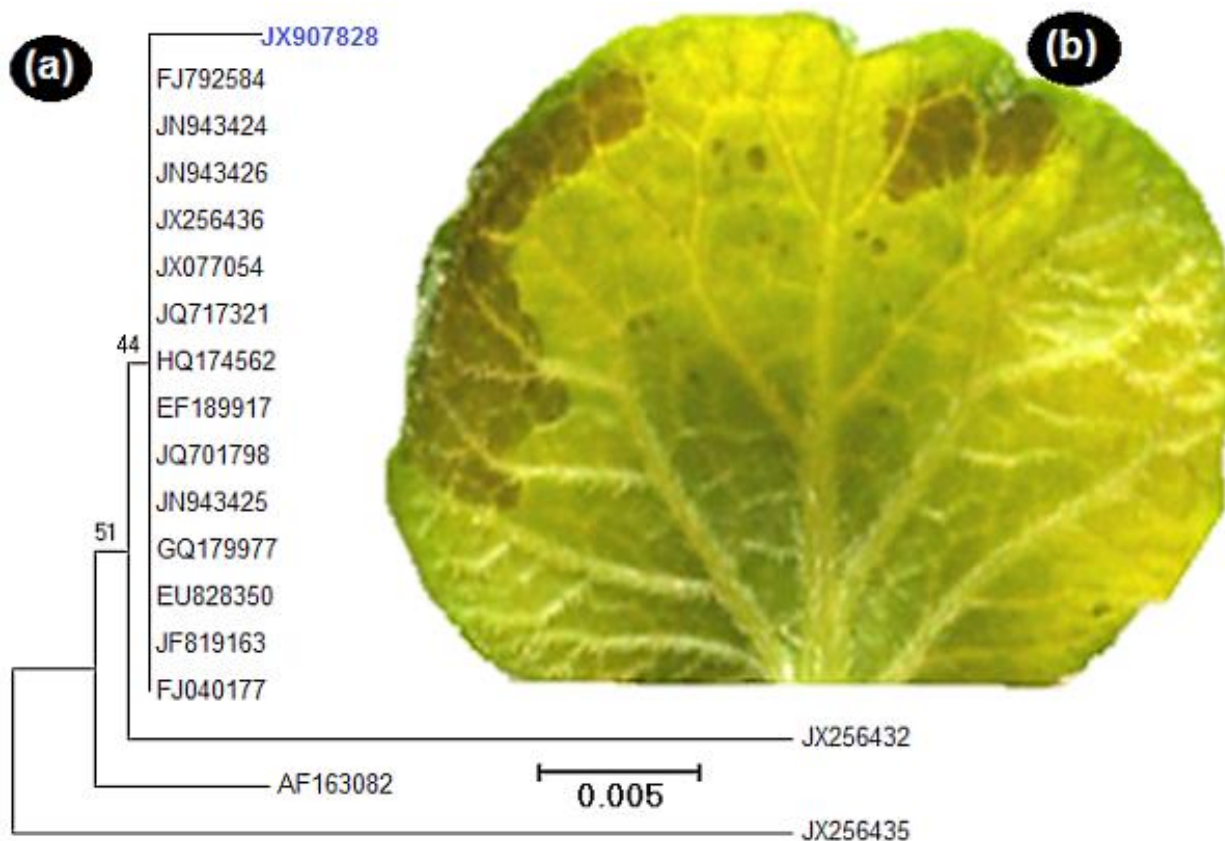
A JC + G substitution model was selected based on Akaike information criterion of 1062.249 and Bayesian information criterion of 1277.723. The optimal tree with the sum of branch length of 0.05835456 was generated and strains that clustered in the tree were supported by bootstrap values (Figure 2a). The strain causing brown-

to-black leaf spots disease of potato clustered at 44% bootstrap confidence level. Following pathogenicity test, brown-to-black circular necrotic spots (3 to 8 mm<sup>2</sup>) identical to those observed in the field developed after five days. At the advanced stage of the disease, spots coalesced into patches, and most of the parts of inoculated plants were killed within 2 weeks (Figure 2b).

Previous studies in the last 10 years in India revealed that the pathogen causes disease on *Mimusops elengi* Linn. (Selima et al., 2011), *Grewia optiva* (Cvetomir, 2011), *Amaranthus Spinosus* (Pankaj et al., 2011), strawberry (Verma and Gupta, 2010), barley (Kumar and Singh, 2002), Indian spinach (Pandey et al., 2001), groundnuts (Bansal and Mali, 1998), rice (boro seeds) (Rashid, 2001), chilli (*Capsicum anuum*) (Prabhavathy and Deddy, 1995) and rice (Lakshmanan, 1992). For any durable integrated disease control measures to be deployed, the disease causing agent must be identified and reported in time. To our knowledge, this is the first report on the occurrence of leaf spots disease of potato, as a new disease, caused by *C. lunatus*.

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**Figure 2.** The evolutionary history of *Cochliobolus lunatus* denoted by the GenBank<sup>®</sup> accessions was inferred with the Neighbor-Joining method. (a) The optimal tree with the sum of branch length = 0.05835456 is shown and the percentage of trees in which the associated strains clustered together is shown next to the branches; (b) Brown-to-black leaf spot disease of potato caused by *C. lunatus* (Genbank<sup>®</sup> accession-JX907828).

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