

NEW DISEASE REPORT

First report of *Pantoea ananatis* causing leaf blight disease of pomegranate in India

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Email: anbarasibt08@gmail.com**KEYWORDS**Bacterial blight, Phytopathogen, *Punica granatum*

Pomegranate (*Punica granatum*) is an economically important fruit crop and India ranks first for its cultivation globally (Chathalingath & Gunasekar, 2023). During December 2022, pomegranate in Tamil Nadu, India was found with leaf abnormalities. Diseased leaves showed brown necrotic spots surrounded by yellowish margins and the edges of the leaves were wrinkled (Figure 1). Diseased leaves were collected and surface sterilised with 0.2% sodium hypochlorite, and then

the lesion-bearing areas were cut and ground with sterile water. The suspension was spread onto nutrient glucose agar and incubated at $28\pm 2^{\circ}\text{C}$ for five days (Doddaraju et al., 2019). All the colonies on the medium showed similar morphological features and a single colony was selected and assigned the name PBL5. The isolate was Gram-negative and yellow-pigmented, circular with a glistening morphology, positive for starch hydrolysis, catalase, oxidase and methyl red but negative for

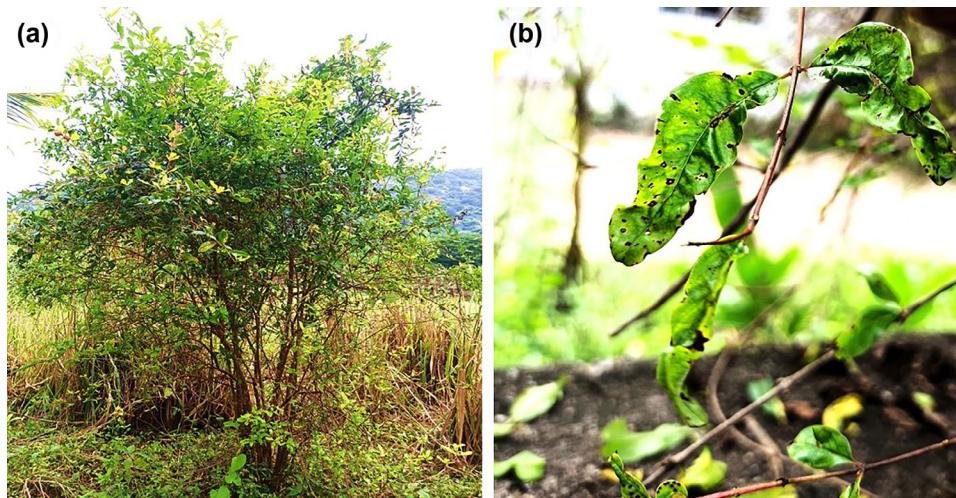


FIGURE 1 (a) Blight-infected pomegranate tree and (b) diseased leaves of pomegranate showing dark necrotic patches with yellowish margins and the leaves were curled at the outer margins

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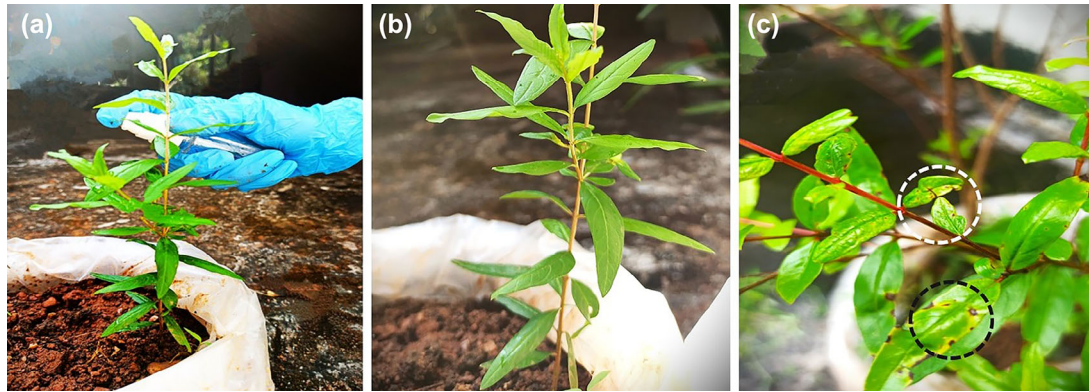


FIGURE 2 (a) Pathogenicity test of *Pantoea ananatis* strain PBL5 on pomegranate seedlings; (b) leaves sprayed with sterile distilled water showed no symptoms while (c) those sprayed with isolate PBL5 showed necrotic spots with leaf curling, six days after inoculation

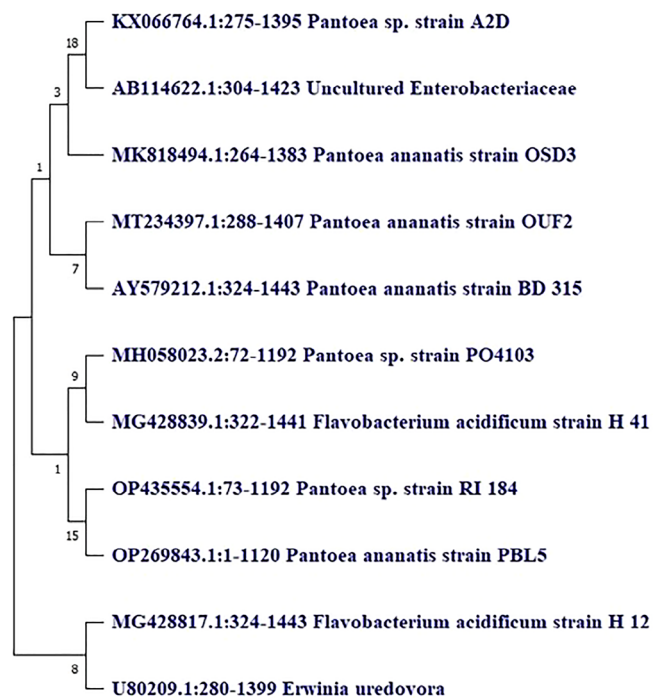


FIGURE 3 Phylogenetic tree analysis of *Pantoea ananatis* with closely associated taxa. The phylogram was generated based on the 16S rRNA gene sequences with 1000 bootstrap resampling by the neighbour-joining method using MEGA 11. Both *Erwinia uredovora* and *Flavobacterium acidificum* H12 were used as outgroups

citrate utilisation, Voges-Proskauer and casein hydrolysis. The isolate was incapable of producing indole and urease.

The pathogenicity of the isolate was tested by the foliar spray method on seedlings. Pathogenicity was confirmed after observation of symptoms, resembling those seen in the field, within a week of inoculation (Figure 2). The pathogen was successfully isolated from the inoculated leaves and identified by PCR amplification with universal 16S rRNA primers (27F and 1492R) followed by Sanger sequencing (Chathalingath et al., 2023). An amplicon of 1400 bp was sequenced (GenBank Accession no. OP269843.1) and BLAST analysis of this

sequence showed 99.91% of identity with the 16S ribosomal RNA gene of *P. ananatis* strain OSD3 (MK818494.1). A phylogenetic study revealed that PBL5 had affinity with *Flavobacterium acidificum*, *Pantoea* sp., an unnamed species of *Enterobacteriaceae* and *Erwinia uredovora* (Figure 3).

Pantoea ananatis has already been identified in many food crops. For instance, leaf blights caused by *P. ananatis* have been reported in rice in India (Mondal et al., 2011) and in strawberries in Canada (Bajpai et al., 2020). Liao et al. (2016) also found a *P. ananatis*-induced soft rot bacterial disease in peach fruit in China. To the best of our knowledge, this is the first evidence of *P. ananatis* as the cause of bacterial blight of pomegranate in India and worldwide. Since *P. ananatis* causes a range of diseases in economically important food crops, a detailed study is required to understand the mode of transmission since this is still unknown.

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