



First report of *Pantoea ananatis* causing fruitlet blight of Nagpur mandarin (*Citrus reticulata*) in India

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Since April 2016, new symptoms have been observed on fruitlets of Nagpur mandarin orange (*Citrus reticulata*) in commercial groves in the Nagpur and Amravati districts of the Vidarbha region of Maharashtra state in India. The disease was observed in crops which had blossomed in January-February. Affected tissue first had an irregular water-soaked appearance that quickly turned dark brown or black and became sunken (Figs. 1 - 2). Over time, the blackened fruitlet became dry, wrinkled and often dropped off. Symptoms did not advance into the shoots. Nearly 30% of the trees were affected with over 20% of fruitlets (12-20 mm in diameter) on those trees blighted.

Diseased tissues were surface-sterilised with 1% NaOCl, macerated in sterile water, streaked onto nutrient agar (HiMedia Laboratories, Mumbai, India) plates and incubated at 28°C for 48 hr. Isolated bacterial colonies were shiny, yellow, convex, and circular with entire margins (Fig. 3). Following sub-culture from single colonies, eight representative isolates (CITNM01 - CITNM08) were subjected to biochemical tests. They were Gram-negative, facultatively anaerobic, catalase and gelatinase positive, hydrolysed starch, produced acid from D-glucose, D-mannitol, D-melibiose, L-arabinose and inositol, and negative for nitrate reduction and hydrogen sulphide production, urease and arginine dihydrolase. DNA of all isolates was extracted using an AxyPrep Bacterial Genomic DNA Miniprep Kit (Axygen Biosciences, USA). BLASTn analysis of the PCR-amplified c.1500 bp 16S rDNA gene sequence (GenBank Accession No. MN108150) showed 99% identity to *Pantoea ananatis* strain PA2512 (KT741001). Three housekeeping genes, *gyrB*, *atpD* and *rpoB*, were also amplified and sequenced using degenerate primers (Brady *et al.*, 2008). All sequences of the eight strains were identical for each of these genes. BLASTn analysis of the sequences of the *gyrB*, *atpD* and *rpoB* genes of the representative strain CITNM02 (MN393080, MN397139 and MN397140, respectively) showed 99% identity with *P. ananatis* strain LMG 20103 (CP001875), strain BCC367 (LT708142) and strain FANS-17-1 (MH880819), respectively.

To fulfil Koch's postulates, a bacterial suspension (10^8 CFU/ml) of two strains, CITNM01 and CITNM02 and sterile distilled water (control) were sprayed until run-off onto five Nagpur mandarin fruitlets each. Fruits were allowed to dry and placed in clear polythene bags and maintained at 28°C for five days. Symptoms similar to those of natural infections were observed on inoculated fruits but not on the negative controls (Fig. 4). Bacteria were isolated from diseased tissues and identified as *P. ananatis*

by *gyrB* sequencing.

P. ananatis is pathogenic on several economically important horticultural crops including pineapple (Serrano, 1928), netted melon (Kido *et al.*, 2008), peach (Liao *et al.*, 2016) and onion (Carr *et al.*, 2010), and also rice (Azizi *et al.*, 2019). To the best of our knowledge, this is the first report of *P. ananatis* causing fruitlet blight of Nagpur mandarin in India. This disease may threaten the Nagpur mandarin production system if effective management strategies are not implemented.

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Figure 1



Figure 2



Figure 3



Figure 4

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