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Bacterial speck caused by *Pseudomonas syringae* pv. *tomato* race 0: first report in Nepal

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Pseudomonas syringae pv. *tomato* causes bacterial speck of tomato worldwide. During the spring of 2007, small necrotic flecks surrounded by chlorotic haloes 1·5–3·0 mm in diameter were observed on leaves of tomato plants (*Solanum lycopersicum*) of a local cultivar Baglung Local (BL), in an experimental farm in Kirtipur, Kathmandu district, central region, Nepal.

Bacteria were isolated from the diseased tissues on Nutrient agar supplemented with 5% sucrose, and incubated at 26 ± 1°C. Isolates were positive for levan production, tobacco hypersensitivity, fluorescent pigment production, and negative for arginine dihydrolase, oxidase activities and ice-nucleation activity. Pathogenicity was confirmed on cv. BL in greenhouse tests by spraying 20 healthy potted plants with a bacterial suspension (10^8 *cfu* mL^{-1}) *and 20 plants with sterile distilled water. Control plants remained healthy, and all inoculated plants showed symptoms similar to those observed in the field within one week after inoculation. Bacteria typical of the inoculated strain were re-isolated from the necrotic lesions.*

The race of the pathogen was determined by pathogenicity tests, using the same bacterial concentration (10^8 *cfu* mL^{-1}), *on cv. Rimone (Pto/Pto* gene) and cv. Riogrande (bearing *pto/pto* gene), which are respectively resistant and susceptible towards *P. syringae* pv. *tomato* race 0 (Bogatzevska *et al*., 1989). The Nepalese isolate (PST5N07) caused bacterial speck symptoms on cv. Riogrande but not on cv. Rimone, indicating that it belongs to race 0. Molecular identification was achieved by sequencing the 16S rDNA region (GenBank Accession No. FJ590508). The sequence shared 99.9% identity with the analogous sequence of *P. syringae* pv. *tomato* type strain DC3000 (AE016853). Pathogen identification was further refined by using two pathovar-specific primers (Zaccardelli *et al*., 2005) which amplified a 532 bp fragment from $hrpZ_{Pst}$.

This disease is of regulatory importance since Nepal shares its boundaries with Tibet and with Uttar Pradesh, Bihar, West Bengal and Sikkim States of India where this disease has not been reported, and similarly in the neighbouring countries Bhutan and Bangladesh. It has been reported in southwest India (Patel & Patel, 1991) and in northwest and northeast China. Contaminated seeds and/or transplants may have been the source of introduction of the pathogen to this region of Nepal.

Acknowledgements

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