First report of potato blackleg caused by biovar 3 Dickeya sp. (Pectobacterium chrysanthemi) in Greece

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Received: 20 Jun 2011. Published: 11 Nov 2011. Keywords: Dickeya solani, bacterial potato disease

In spring 2009 and 2010 significant bacterial disease symptoms were observed in potato (Solanum tuberosum cv.Spunta) crops in the Messara plain of Crete in Greece. Affected plants had blackleg and rotting symptoms on stem base, brown discolouration of vascular tissues, wilting symptoms on the foliage and soft rot in daughter tubers (Fig. 1). Incidence varied from 5%-50%. Isolations were made from soft-rotted tubers and plants with typical blackleg symptoms on crystal violet pectate (CVP) and nutrient agar glucose (NAG) media. Single bacterial colonies having characteristic pectinolytic activity (CVP) and/or "fried egg" colonies (NAG) (Fig. 2) were subcultured, purified and used for further characterisation. Twenty isolates were initially characterised as Dickeya sp. (syn. Erwinia chrysanthemi, Pectobacterium chrysanthemi). They were Gram-negative rods, facultative anaerobes, oxidase negative, sensitive to erythromycin (15 μg/disk), positive for phosphatase and indole production; grew at 37°C, negative for acid production from α-methylglucoside and trehalose; and caused soft rot in potato tuber slices (Sławiak et al., 2009). Six isolates were selected for further analysis and characterised as Dickeya sp. biovar 3. They grew on arabinose, melibiose, raffinose, mannitol and inulin but did not utilise tartrate or hydrolyse arginine under anaerobic conditions, and grew poorly at 39°C (Laurila et al., 2010). These strains were biochemically identical to the reference strain Dickeya sp. IPO2222 (kindly provided by Dr. van der Wolf) while biochemically distinct from the reference strain Dickeya dianthicola BPIC2098.

Molecular characterisation was performed on four isolates (Ds3386, Ds3378, Ds3400, Ds3405) using BOX- and ERIC-PCR fingerprinting (Fig. 3), as well as dnaX gene sequencing (Shawski et al., 2009). The obtained dnaX sequences have been deposited in GenBank (Accession Nos. JN663794 to JN663803). Results from BOX-ERIC profiling and dnaX sequencing revealed identical profiles for the isolated stains and the reference strain Dickeya sp. IPO2222; while they were clearly distinct from the related species D. dianthicola BPIC2098, P. carotovorum TEIC3036, P. atrosepticum TEIC3211 and other Erwinia spp. (Fig. 3).

Pathogenicity assays were performed on potato (cv. Spunta) either by injecting 20 μl bacterial suspension (10^7 cfu/ml) into the stem or by stabbing a tuber at the stolon end with a toothpick charged with bacterial growth. Typical disease symptoms were observed within 10 days (Fig. 2). Re-isolated strains had an identical profile with the inoculated strains and the reference strain Dickeya sp. IPO2222. This is the first report of potato blackleg caused by Dickeya sp. biovar 3 in Greece. In the last five years, Dickeya sp. has caused economic losses of up to €30M annually to the production of potato propagation material in the Netherlands (Toth et al., 2011). Further spread of the pathogen in potato production in Greece is expected to have a high economic impact.

References