



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

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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 discoloration 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 (Ślawiak *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 (Ślawiak *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 (10⁷ 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.

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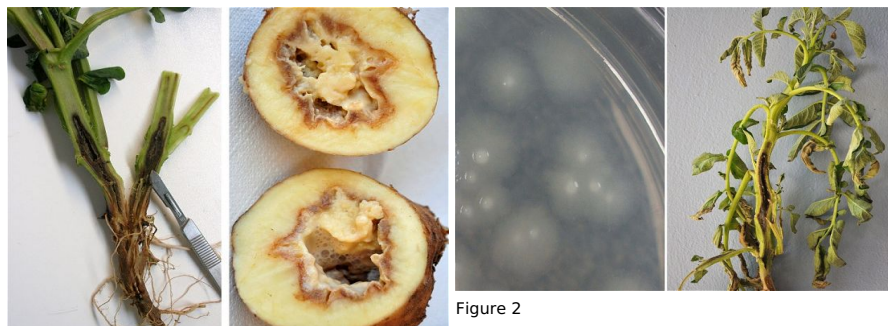


Figure 1

Figure 2

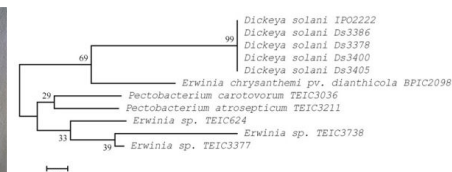


Figure 3

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