First report of potato blackleg caused by *Pectobacterium carotovorum* subsp. *brasiliensis* in the Netherlands

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Potato blackleg and stem rot are caused by various soft rot coliforms belonging to the genera *Pectobacterium* and *Dickeya* (van der Wolf & De Boer, 2007). In Europe, *D. solani*, *D. diantichica*, *P. atrosepticum* and *P. wasabiae* are the main causative agents of the diseases. Recently, in the Netherlands, blackleg diseased plants were found in seed lots produced in different parts of the country, which were positive in a PCR assay for *P. carotovorum* subsp. *brasiliensis* (Pcb; Duarte et al., 2004) indicating that Pcb had caused the disease. It was estimated that in 2012, Pcb was responsible for approximately 10 percent of blackleg incidence.

Double-layer crystal violet pectate (DL-CVP) medium (Hélias et al., 2012) was used to isolate pectinolytic bacteria from the stems of blackleg affected plants. Eleven isolates derived from ten seed lots, positive in the Pcb-specific PCR (Duarte et al., 2004), were characterised with multilocus sequence analysis (MLSA) using sequences of 23acn, gpaA, icda, mdh, mlio and proA (Panda et al., 2012) (GenBank Accession Nos. KJ376801-KJ376810, KJ396350-KJ396399, KJ608062-KJ608071). The phylogenetic analysis was done by constructing a maximum likelihood tree using sequences of reference strains *P. atrosepticum*, *P. wasabiae*, *P. carotovorum*, *P. odoriferum*, *P. betavasculorum* and *Dickeya paradisiaca*, derived from GenBank, for comparison (Fig. 1). The MLSA confirmed the identity of the Pcb strains. The Dutch strains clustered together in a homogeneous branch, which also showed that these strains were highly similar. Strains were motile, able to reduce sucrose and clustered together in a homogeneous branch, which also showed that these strains were highly similar. Motile strains were able to reduce sucrose and to grow at 39°C, resistant to erythromycin, and unable to ferment lactose according to the procedures described by Hayman et al. (1998). Two Pcb isolates were tested for virulence in a field experiment in the Netherlands in 2013. Two months after planting vacuum-infiltrated seed tubers, a high percentage of the plants (more than 30%) showed typical symptoms, whereas water-treated plants remained disease-free (Fig. 2). The pathogen was re-isolated from the diseased plants and identified as Pcb using MLSA. This is the first time Pcb is described as a potato blackleg-causing agent in the Netherlands.

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Figure 1


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