



First report of blackleg and soft rot of potato caused by *Pectobacterium carotovorum* subsp. *brasiliensis* in New Zealand

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Blackleg and stem rot of potato occur sporadically in New Zealand, causing economic damage under optimal temperature and humidity conditions for disease development. Both *Pectobacterium atrosepticum* (*Pba*) and *P. carotovorum* subsp. *carotovorum* (*Pcc*) have previously been isolated from potato tubers with soft rot symptoms in New Zealand (Crowhurst & Wright, 1998) whereas only *Pba* has been shown to cause blackleg. A collection of 89 enterobacteria were recently isolated from potato tubers from commercial crops in the Auckland, Waikato, Manawatu-Wanganui and Canterbury regions of New Zealand. The majority were initially assigned as *Pcc* by their growth at 37°C, carbon utilisation profiles and restriction fragment length polymorphisms (Pitman *et al.*, 2008). These isolates were mostly unable to cause blackleg symptoms, although several were shown to be highly aggressive upon stem infection. Further characterisation of these aggressive isolates using PCR assays to distinguish *Pcc* from *P. carotovorum* subsp. *brasiliensis* (*Pbr*) was carried out using primers EXPCCF and EXPCCR (Kang *et al.*, 2003) and Br1f and L1r (Duarte *et al.*, 2004), respectively. From 18 isolates, occurring throughout the four major growing localities (Fig. 1), a 322-bp fragment specific to *Pbr* was amplified using Br1f and L1r (Fig. 2), whereas no PCR product (550-bp) was obtained typical of *Pcc*. The 16S rRNA gene was amplified (Wang & Wang, 1996) from one of the putative *Pbr* isolates, NZEC1 (deposited in the International Collection of Microbes from Plants as ICMP 19477). BLAST analysis of the 16S rRNA DNA sequence (GenBank Accession No. JQ771053) showed 100% identity to the 16S rRNA of *Pbr*.

Multi-locus sequence analysis was performed using concatenated DNA sequences of *acnA*, *gapA*, *icdA*, *mdh*, *mtlD*, *pgi* and *proA* from NZEC1 (JQ820114-JQ820120) and 46 related taxa (Ma *et al.*, 2007). The resulting majority rule consensus tree constructed using MrBayes v. 3.0b4 (Fig. 3) clustered NZEC1 with *Pbr*, readily distinguishing it from the closely related subspecies *Pcc*. Pathogenicity assays were conducted using potato plants (cv. 'Ilam Hardy') by injecting 10 µl of a bacterial suspension (10 cfu/ml) into either stems or tubers. Each time, severe blackleg (Fig. 4) or tuber soft rot (Fig. 5) were observed on plants inoculated with *Pbr* whereas *Pcc* strains were unable to cause blackleg. The results were consistent with observations throughout the world that suggest *Pcc* is largely unable to cause this stem disease.

To our knowledge, this is the first report of *Pbr* infecting potato in New Zealand. The prevalence of *Pbr* isolates in Canterbury is of particular note

due to the high proportion of seed tuber production that takes place in this region. As *Pcc* is usually unable to cause blackleg and *Pba* is only rarely isolated from tubers in New Zealand (Pitman *et al.*, 2008), *Pbr* is likely to be an important component of the blackleg syndrome in New Zealand. Consequently, this pathogen is expected to contribute to economic losses resulting from both blackleg and tuber soft rot. *Pbr* also causes severe economic impacts both in Brazil and South Africa.

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Figure 1



Figure 2

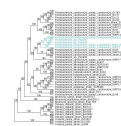


Figure 3



Figure 4



Figure 5

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