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Final Report

Review Bacterial Blackleg Disease and R&D Gaps with a Focus on the Potato Industry

Project leader:

Dr Len Tesoriero

Delivery partner:

Crop Doc Consulting Pty Ltd

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Level 8
1 Chifley Square
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Telephone: (02) 8295 2300

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Content

Summary

Soft rot bacterial species *Dickeya* and *Pectobacterium* are listed globally in the top 10 of important bacterial plant pathogens based on their economic impact. They are comprised of a genetically diverse group of plant pathogens affecting a wide range of plant species. Postharvest losses are greatest in poorer countries that lack refrigerated cool chain practices. In Australia crop losses from potato blackleg disease are generally considered to be low. However, there are cases of greater losses occurring when wet and windy weather conditions prevail, or of soft rots following planting seed tubers into warmer soils. Across other horticultural commodities, there are crops that are affected by certain of these same potato pathogens, while others have their own species or subspecies affecting them. This diverse genetics of these bacterial species or subspecies causing blackleg and soft rot diseases makes it harder to easily distinguish important ones. There are several documented cases overseas of biosecurity breaches that suggest there are significant threats for Australian horticulture amongst this group of bacteria. Accurately estimating biosecurity risks and implementing appropriate mitigation or management strategies for these bacteria will require two simultaneous undertakings: a formal study to update our knowledge of which bacteria are currently present in Australia; and access to accurate diagnostic tests which include the capability to distinguish important subspecies of these bacterial pathogens. There will be a need for sufficient validation of these tests to estimate their reliability, sensitivity and selectively. In particular there will be a need to ensure no interference from related environmental species in test results. Output data from these studies will inform authorities of any need to review potato seed production guidelines and any considerations for national and regional quarantine and biosecurity. There may be economic implications flowing from any survey results and reporting of bacterial taxa not previously recorded in Australia. In particular export and interstate trade in seed potatoes may be adversely affected. Similarly, introduction of testing for imported products such as ornamental bulbs and tubers will increase costs to the cut-flower and nursery industries. These considerations need to be weighed carefully against the potential benefits that comes with a greater understanding of the causes of blackleg and soft rots in Australia, and the preparedness to deal with any future incursions.

Keywords

Potato, Blackleg, Soft Rot, *Dickeya*, *Pectobacterium*

Introduction

Bacterial soft rot diseases affect a wide range of plant species. They are caused by a range of both gram positive and gram negative bacterial genera (Charkowski, 2018). The major causal agents of potato soft rot and blackleg disease relevant to this review are species of *Dickeya* and *Pectobacterium* (once referred to as certain species and subspecies of the genus *Erwinia*). Soft rot diseases caused by members of these bacterial genera have been described on many important agricultural crops and ornamental plants (Charkowski *et al*. 2012; Joko *et al*. 2014). *Dickeya* and *Pectobacterium* species are listed in the top 10 most important bacterial plant pathogens based on their economic impact (Mansfield *et al*. 2012). These pathogens can spread asymptomatically during plant propagation as well as being carried on machinery, in water and with handling. Disease symptoms can express at planting, throughout the growing season, during transit or in storage.

Blackleg of potatoes develops at the stem base following rotting of the mother tuber. Contaminated seed has long been known to be an important source of bacterial pathogens causing blackleg and soft rot diseases (Pérombelon 1974; Nielsen 1978). Blackleg can also be caused by bacterial infection through wounded stems. The wet, black and slimy rot symptoms on potato stems and tuber soft rot caused by *Dickeya* and *Pectobacterium* bacterial species are largely indistinguishable. Tuber soft rots result from bacterial infections through lenticels, wounds or via stolons.

Soft rots causing postharvest spoilage occurs on many commodities particularly where there are failures in cool chain practices and where there is sub-optimal ventilation. Besides potatoes, the most commonly affected vegetables in storage are reported to be carrots, onions, lettuce and brassicas (Ma *et al*. 2007; Rimmer *et al*. 2007).

Figure 1.Bacterial soft rot of potato (left) and Chinese cabbage (right)

In Australia soft rot and blackleg diseases of potatoes are widespread and in certain cases they cause significant losses when favourable environmental conditions prevail. One example is where potato seed is sown into hot and wet soils (Cother and Gilbert 1990; Persley *et al*. 2010). Cother and Gilbert (1990) also detected soft rot bacteria (*E. chrysanthemi*) in water of natural ecosystems several hundred kilometres upstream from where river water was used to irrigate potatoes. Presumably these would now be classified as *Dickeya* species. Blackleg and soft rot pathogens of potatoes are not well characterised in Australia and are commonly referred to under their former names as

Erwinia species (*E. carotovora* subsp. *carotovora*; *E. carotovora* subsp. *atroseptica* and *E. chrysanthemi*) (Persley *et al*. 2010). There are four records of *P*. *carotovorum* subsp. *brasiliense* recorded in Queensland from potatoes, lettuce and capsicum (Australian Plant Diseases Database, http://collections.ala.org.au). Three Tasmanian potato isolates from the 1970s that had been lodged in the Scottish (SCRI) culture collection were recently confirmed as *P. carotovorum* subsp. *carotovorum* (Potrykus *et al*. 2014). *Dickeya zeae* has been recorded on pineapples in Queensland (A. Manners pers. comm.). Clearly there is a need to re-examine Australian historical isolates and survey for fresh isolates associated with blackleg and soft rots.

The recent detection of *Dickeya dianthicola* on potatoes in Western Australia (Wright *et al*. 2018) and the involvement of dahlia tubers as a potential source (Anon 2017) highlight biosecurity threats where there can be spread of these pathogens between different commodities and industry sectors. The emergence of *D. solani* as a pathogen of potatoes in Europe was thought to have been through a shift in hosts from monocot bulbs which are sometimes rotation crops (Parkinson *et al*. 2015). Moreover, international spread of *D. solani* was confirmed by Chen *et al*. (2015) who recorded this bacterium on hyacinth bulbs in China that had been imported from the Netherlands. In Europe the spread of blackleg caused by *Dickeya solani* in the early 2000s was thought to have been largely due to the movement of potato seed tubers (Toth *et al* 2011). Similarly the spread of *D. dianthicola* across the East Coast of North America in 2014 was thought to have been assisted by movement of potato seed tubers (Jiang *et al*. 2016) but it had likely been present for several years before detection. Cross-infection between ornamental hosts and potato was first suggested for *D. dianthicola* and *D. solani* by Parkinson *et al.* (2009). A similar scenario has been reported for bacterial heart rot of pineapples in Hawaii where *Dickeya* species appear to have been moved into their production region with planting material from Central America and the Philippines (Sueno *et al*. 2014). These examples provide us with further evidence that members of these bacterial genera pose biosecurity threats which are real and present across horticultural industries and are commonly associated with global and domestic trade of propagative material.

Bacterial Nomenclature and Taxonomy

Dickeya and *Pectobacterium* genera are pectolytic gram negative enterobacteria. They are facultative anaerobes with peritrichous flagella that were previously included in the genus *Erwinia*. This group has undergone several revisions over the past 20 years particularly since the development of phylogenomic classification systems. Most recently Adeolu *et al*. (2016) used genome analyses to divide the family *Enterobacteriaceae* into seven newly proposed families together with the establishment of the new order *Enterobacterales*. Several earlier taxonomic revisions have occurred at the genus, species and sub-species levels (Hauben *et al*. 1998; Gardan *et al*. 2003; Samson *et al.* 2005; Nykyri *et al*. 2012; Khayi *et al*. 2016). Most notably, Hauben *et al*. 1998 reclassified *Erwinia carotovora* into multiple species of the genus *Pectobacterium* while Samson *et al.* (2005) reclassified *Pectobacterium chrysanthemi* into several species of the genus *Dickeya*. A series of other authors (de Boer, 2012; Nabhan *et al*. 2012; Slawiak *et al*. 2013; Waleron *et al*. 2013) determined that potato isolates that had been described as *P. carotovorum* subsp. *carotovorum* were more similar to *P. wasabiae*. Khayi *et al*. (2016) then used a range of genetic analyses to transfer potato isolates of *P. wasabiae* to a new species *P. parmentieri.* This has helped to distinguish these potato isolates from those originally described by Goto & Matsumoto (1987) affecting Japanese horseradish (wasabi). Re-examination of isolates collected over the past 40 years has demonstrated that *P. parmentieri* has a wide geography occurring across North and South America, Europe, Asia and New Zealand (Khayi 2015). It has not been recorded in Australia but given this wide distribution it deserves further study.

Unfortunately with all these taxonomic changes and different criteria used to separate taxa many of the GenBank accessions for members of these genera are misnamed (Zhang *et al*. 2016) which can lead to further confusion. Not all the proposed name changes have been ratified either, particularly recent elevation of certain subspecies of *Pectobacterium* to species level. A check of Australian culture collections and records of blackleg and soft rot pathogens for this report suggests that many need to be reviewed in the light of these recent taxonomic reclassifications.

Recently named new *Dickeya* and *Pectobacterium* species and key historical name changes are listed in Table 1.

Table 1 Name changes and newly described species of *Dickeya* and *Pectobacterium*

Current name	Old name	Disease/ Hosts	Reference
'Candidatus Pectobacterium maceratum'	Newly named (but not ratified)	Soft rot of potatoes and cabbage	Shirshikov *et al*. 2018
Pectobacterium polaris	Newly named	Soft rot of potatoes	Dees *et al*. 2017
Pectobacterium parmentieri	*P. wasabiae* (potato isolates)	Soft rot of potatoes	Khayi *et al*. 2016
Dickeya fangzhongdai	Newly named	Bleeding canker of pear trees	Tian *et al*. 2016

Current name	Old name	Disease/ Hosts	Reference
Dickeya aquatica	Newly named	Water – no known hosts	Parkinson *et al*. 2014
Pectobacterium aroidearum	*P. carotovorum* cluster PcV	*Zantedeschia aethopica*; Soft rot of potato	Nabhan *et al.* 2013
Dickeya solani	Newly named	Soft rot of potatoes	van der Wolf *et al.* 2014
Pectobacterium wasabiae	*P. carotovorum* subsp. *carotovorum* (strain SCC3193)	Soft rot of potatoes	Nykyri *et al.* 2012
D. dadantii subsp. dieffenbachiae	*D. dieffenbachiae*	Soft rot of *Philodendron*	Brady *et al.* 2012
Dickeya spp.: *D. chrysanthemi*; *D. dadantii*, *D. dianthicola*; *D.dieffenbachiae*, *D. paradisiaca* & *D. zeae*	*Pectobacterium chrysanthemi*	Soft rot &/or blackleg of potato	Samson *et al.* 2005
P. carotovorum subsp. *brasiliense*	Newly named	Blackleg of potatoes	Duarte *et al*. 2004
Pectobacterium atrosepticum	*P. carotovorum* subsp. *atrosepticum*	Soft rot &/or blackleg of potato	Gardan *et al*. 2003
Pectobacterium wasabiae	*P. carotovorum* subsp. *wasabiae*	Soft rot of potatoes	Gardan *et al*. 2003
Pectobacterium carotovorum	*P. carotovorum* subsp. *carotovorum*	Soft rot of potatoes	Gardan *et al*. 2003
Pectobacterium chrysanthemi	*Erwinia chrysanthemi*	Soft rot of potatoes	Hauben *et al*. 1998
P. carotovorum subsp. *wasabiae*	*Erwinia carotovora* subsp. *wasabiae*	Soft rot of potatoes	Hauben *et al*. 1998
P. carotovorum subsp. *carotovorum*	*Erwinia carotovora* subsp. *carotovora*	Soft rot of potatoes	Hauben *et al*. 1998
Erwinia carotovora subsp. *odoriferum*	Newly named	Chicory: Soft rot	Gallois *et al*. 1992
Erwinia	Newly	Soft rot of	Goto &

Current name	Old name	Disease/ Hosts	Reference
carotovora subsp. *wasabiae*	named	wasabi rhizomes	Matsumoto 1987

Host Range

There is a high level of genetic and phenotypic variation in most *Dickeya* and *Pectobacterium* genera. Surveys are constantly uncovering new species and new hosts. In 2007 Ma *et al*. listed *Pectobacterium* species on 16 dicotyledonous and 11 monocotyledon plant families. Four new plant families have been added to this host list in the past decade (Charkowski 2018). In this period, more than 20 new plant host species have been recorded for *Dickeya* and *Pectobacterium* species. Key records are summarised in Table 2. These are in addition to taxa and host records that had undergone taxonomic revision and listed in Table 1. *P. carotovorum* is generally considered to have the widest host range of all soft rot bacteria (Pérombelon 2002) but more recent records suggest other species and subspecies are more widespread than previously thought.

Table 2 Recently published new host records of *Dickeya* and *Pectobacterium* species

Published name	Disease/Host	Country	Reference
***Pectobacterium* species**			
P. atrosepticum	Soft rot of calla lily	Serbia	Popovic *et al*. 2017
	Stalk & head rot of sunflowers	Turkey	Bastas *et al*. 2009
	Eggplant	Italy	Catara *et al*. 2001
P. betavasculorum	Beet	USA & Mexico,	Gardan *et al*. 2003
P. carotovorum subsp. *actinidiae*	Canker of kiwifruit	China	Wu *et al*. 2017
P. carotovorum subsp. *brasiliense*	Soft rot of cucumber	China	Meng *et al*. 2017
	Soft rot of bell pepper	Venezuela	Gillis *et al*. 2017
	Soft rot of kale	Brazil	Queiroz *et al*. 2017
	Leaf blight of tobacco	China	Wang *et al*. 2017
	Stem rot of tomatoes	USA	Rosskopf & Hong 2016
	Rot of sugar beet	North America	Secor *et al*. 2016

Published name	Disease/Host	Country	Reference
	Pith soft rot of artichoke	Italy	Cariddi & Bubici 2016
	Soft rot of paprika	Korea	Choi & Kim 2013
P. carotovorum subsp. *carotovorum*	Soft rot of sweet potato	China	Gao *et al*. 2016
P. carotovorum subsp. *odoriferum*	Soft rot of cabbage & Chinese cabbage	Poland	Oskiera *et al*. 2017
	Soft rot of sweet potato	China	Gao *et al*. 2016
	Pith soft rot of cauliflower	Italy	Cariddi & Bubici 2016
	Soft rot of potatoes & stored vegetables (Chicory, leek, celery, parsley, carrot, onion)	France, Poland & Switzerland	Walderon *et al*. 2014
***Dickeya* species**			
Dickeya spp.	Phalaenopsis orchid	Slovenia	Alič *et al*. 2017
D. chrysanthemi	Chrysanthemum	Hungary	Vegh *et al*. 2014
	Orchid (*Vanda* sp.)	USA	Cating *et al*. 2008
D. dadantii	Sheath rot of banana	China	Liu *et al*. 2016
	Soft rot of vanilla	China	Gao *et al*. 2016
D. dadantii subsp. dieffenbachiae	Soft rot of pak choi	Malaysia	Golkhandan *et al*. 2016
D. dianthicola	Potato	Europe	Toth *et al*. 2011
D. dieffenbachiae	Soft rot of *Philodendron*	China	Lin *et al*. 2012
D. fangzhongdai	Bleeding canker of Pear (*Pyrus* sp.)	China	Tian *et al*. 2016
D. paradisiana	Banana (*Musa* sp.)		Samson *et al*. 2005
	Soft rot of	China	Chen *et*

Published name	Disease/Host	Country	Reference
D. solani	Hyacinth bulbs	(imported from Netherlands)	*al*. 2015
	Blackleg & soft rot of Potato	Europe	Slawiak *et al*. 2009
	Bacterial heart rot of Pineapple	Philippines, Malaysia Hawaii	Sueno *et al*. 2014
D. zeae	Soft rot of banana	China	Zhang *et al*. 2014
	Heart rot of pineapple	Australia	A Manners (pers. comm.)

Pathogenicity and Epidemiology

Typical of necrotrophic pathogens *Dickeya* and *Pectobacterium* soft rot bacteria are characterised by the co-ordinated production and release of plant cell wall degrading enzymes (PCWDEs) to obtain nutrients from dead cells. These are pectinases, cellulases, hemicellulases and proteinases that are secreted through Type I and II secretion systems (Davidsson *et al*. 2013; Charkowski *et al*. 2012). The general action of these enzymes is to macerate plant tissue which also partly explains their broader host ranges. In addition, these pathogens secrete proteins that promote cell death such as necrosis-inducing protein and effector protein DspE (Mattinen *et al*. 2004; Kim *et al*. 2011). Although production of these enzymes and toxins are a hallmark of necrotrophic pathogens in the symptomatic phase of infection, it has been recognised that early stages of infection are latent and more consistent with biotrophic pathogens. After entering plants through wounds or natural openings such as lenticels, stomata or hydathodes these bacteria persist in intercellular spaces and vascular tissue until environmental conditions become conducive for disease development. In particular, field infection is promoted by high soil moisture and low oxygen concentrations which lower plant resistance and favour bacterial growth. When their cell density reaches a threshold, a quorum-sensing mechanism switches on secretion of PCWDEs and necrotrophy (Liu *et al*. 2008). Other virulence determinants which sense physiological or environmental cues such as plant derived organic acids and pectin derivatives are controlled by other mechanisms (Charkowski *et al*. 2012).

Horizontal gene transfer has been indicated as a mechanism for the acquisition of determinants in *Pectobacterium* and *Dickeya* which benefit the bacterium at the early stages of infection (Bell *et al*. 2004; Nykyri *et al*. 2012). One example is the gene cluster which encodes a Type IV secretion system that enhances virulence of *P. atrosepticum* isolates by facilitating translocation of DNA and/or proteins across bacterial or eukaryote cell walls (Bell *et al*. 2004). Other horizontally acquired gene clusters include Type VI secretion systems (T6SS) which at least in the case of *P. atrosepticum* were shown to contribute to virulence (Liu *et al*. 2008). This system is also present in *Dickeya* species and

is thought to function as an injectisome mechanism for trans-membrane protein translocation and which is shared with bacteriophages (Sarris *et al*. 2012). Although their functionality is not fully understood at the present time these horizontally acquired gene clusters are known to differ between *Pectobacterium* species and isolates, and may contribute to differences in host specificity and aggressiveness. There is also evidence that T6SS have a broader biological function such as mediating cooperative or competitive interactions between bacteria, biofilm formation, or to promote commensal or mutualistic relationships between bacteria and eukaryotes (Sarris *et al*. 2012).

Pathogenesis of pectinolytic bacteria is influenced and modulated by temperature, humidity and oxygen availability (Pérombelon 2002). In particular, rising temperature and greater rainfall can affect their spread, survival and disease development. Hugovieux-Cotte-Pattat *et al*. (1996) determined that temperature is one of the most important external factors influencing the survival of *D. dadantii* in the environment. Furthermore it also increases the growth rate and metabolic activity and subsequently its ability to cause disease. Golanowska *et al.* (2017) hypothesised that the apparent rapid spread of *D. solani*. *P. wasabiae* and *P. carotovorum* subsp. *brasiliense* in Europe was connected with an increase in spring and summer temperatures arising from global warming and more intense international seed potato exchange.

P. atrosepticum and *P. carotovorum* subsp. *brasiliense* cause potato blackleg but *P. carotovorum* subsp. *carotovorum* does not. Similarly, *P. parmentieri* is often found associated with other bacteria causing blackleg but it does not appear to be the primary cause of this disease. However, it is a major cause of decay of storage tubers in the Northern Hemisphere (Charkowski 2018). *Pectobacterium* species tend be quicker to rot potato tubers than *Dickeya* species. *P. carotovorum* subsp. *brasiliense* appears to be the most aggressive blackleg pathogen across several different geographical areas (van der Wolf *et al*. 2014).

The genus *Dickeya* is a very diverse group of which many species cause diseases in tropical, sub-tropical and cool temperate regions. There are currently eight recognised species: *D. aquatica*, *D. chrysanthemi*, *D. dadantii*, *D. dianthicola*, *D. fangzhongdai*, *D. paradisiaca*, *D. solani* and *D. zeae*. In Europe *D. dianthicola* isolates from potato are adapted to cooler regions. *D. solani* strains causing blackleg and soft rot of potato have been isolated from across Europe, Scandinavia and Israel. In the past decade it has become a more significant potato pathogen than *D. dianthicola* in these countries because it is able to induce diseases at lower inoculum levels and produces a greater range of plant cell wall degrading enzymes. Of note is that it also possesses T5SS/T6SS secretion proteins (Toth *et al*., 2011; Pedron *et al*., 2014). *D. solani* spreads systemically through potato plants via vascular tissue to progeny tubers (Czajkowski *et al.* 2010) Another unique feature of *D. solani* is that it does not share the high genetic variation exhibited by other species; it is essentially a clonal population that perhaps reflects a single genetic shift allowing a monocot pathogen to attack potatoes.

Although *Dickeya* and *Pectobacterium* soft rot bacteria have been studied for decades very little is known about their survival strategies between growing seasons. They are not thought to overwinter in soils. Depending on environmental conditions they are only thought to persist in soils for weeks or months (Czajkowski *et al*. 2011).

An interesting genome feature of some *Dickeya* and *Pectobacterium* isolates is the presence of an *evf* gene, which encodes a virulence factor for their persistence in the gut of Drosophila flies (Basset *et al*. 2003). This allows these flies to be vectors for soft rot

bacteria. Shirshikov *et al*. (2018) suggested that acquisition of this gene by a *P. carotovorum* subsp. *odoriferum* isolate contributed to its divergence into their newly named species *Pectobacterium maceratum*.

Recently described species *P. carotovorum* subsp. *actinidiae* (Wu *et al*. 2017) and *D. fangzhongdai* (Tian *et al*. 2016) cause canker diseases on woody hosts rather than soft rots. These and other species are potential biosecurity threats in Australian horticulture.

Detection and differentiation of *Dickeya* and *Pectobacterium* species

There has been a continuous development of new detection methods for pathogenic *Dickeya* and *Pectobacterium* taxa. Most recently, several different DNA amplification and sequencing techniques have been developed for their improved detection as well as for taxonomic studies. Following are two examples:

1. Cigna *et al*. (2017) described a PCR-sequencing assay to characterise 22 isolates *Dickeya* and *Pectobacterium* taxa causing blackleg and soft rot diseases of potatoes in France. They identified 'signature nucleotides' to create a molecular barcode for the housekeeping gene *gapA*, which distinguished their taxa to species and sub-species levels.
2. Golanowska *et al.* (2017) compared *D. solani* isolates from different geographic locations (Poland, Finland & Israel). Despite the fact that they had identical electrophoretic profiles when compared by restriction fragment length polymorphism-Pulse Field Gel Electrophoresis and repetitive sequence-based polymerase chain reactions (rep-PCR: REP, BOX and ERIC repetitive sequences) Polish isolates differed from the others when their phenotypes were compared by assessing their activities of pectinolytic, cellulolytic and proteolytic enzymes and their capacity to macerate potato tuber tissue. Polish strains had higher activities of these enzymes at all temperatures examined which correlated with their more aggressive pathogenicity when tested by potato tuber maceration tests. This result is significant since it suggests that these detection methods for *D. solani* do not imply knowledge of their aggressiveness.

Most detection and isolate characterisation studies deploy several different methods based on genetic and biological properties. Commonly used methods (and their abbreviations) are summarised in Table 3.

Table 3 Methods used for isolation, detection and differentiation of Dickeya and Pectobacterium spp.

Method	Key reference	Comment
Immunomagnetic separation	Van der Wolf (1996a,b)	Used prior to plating to media; sensitivity <100 Pba cells/L tuber peel extract
Crystal Violet Pectate agar medium (CVP)	Cuppels & Kelman (1974)	CVP medium is semi-selective; characteristic cavities on bacterial colonies
Miller-Schroth's medium	Pierce (1992)	Pink-red-orange cavities; suppressed cavities on saprophytic isolates
Biochemical characters	Hyman *et al.* (1998) Schaad *et al.*	Labourious procedures; lacks specificity because of variations occur within taxa; key tests used to confirm

Method	Key reference	Comment
	(2001)	isolates (along with Gram morphology test)
Fatty acid methyl ester (FAME) analysis	Van Der Wolf (2014)	Can distinguish *Dickeya* spp. from *Pectobacterium* spp.; but cannot differentiate *Dickeya* to species; does not distinguish Pc from Pw
Serology	De Boer & McNaughton (1987)	Generally lack specificity; prone to false reactions; Serogroups for lipopolysaccharides antibodies studied in Europe
*rec*A sequence phylogeny	Stead *et al*. 2010	
*dna*X; *icd*A & *mdh* sequence phylogenies	Dees *et al*. 2017	Named new species *P. polaris* which was separated from *P. carotovorum* subsp. *odoriferum*
Multiplex PCR	Potrykus *et al*. 2014	Differentiated common most potato blackleg and soft rot pathogens – but did not detect Pcb; does not distinguish Pc from Pw; reference strain DNA is needed for gel interpretations between different laboratories
Rep-PCR profiles	Oskiera *et al*. 2017	Good species resolution; reference strain DNA is needed for gel interpretations between different laboratories
PCR-RFLP analysis	Waleron *et al*. (2002)	Good species resolution; reference strain DNA is needed for gel interpretations between different laboratories
Multi Locus Sequence Tagging	Waleron *et al*. (2013)	Target genes need to be informative
Pulse field gel electrophoresis	Kim *et al*. 2009	High resolution to subspecies level; reference strain DNA is needed for gel interpretations between different laboratories
Full or partial genomic DNA sequence analysis	Auch *et al*. 2010 Shirshikov *et al*. 2018	Used for taxonomic studies and able to distinguish phylogenetic species
Potato tuber maceration assay	Dees *et al*. 2017	Used with genetic testing

There are many published records where consensus of several methods is used to resolve biosecurity issues. For instance, Stead *et al*. (2010) first recorded *Dickeya zeae* in the UK using FAME, rep-PCR and *rec*A sequence phylogeny from herbarium isolates lodged as *Dickeya chrysanthemi*.

Given that many *Dickeya* and *Pectobacterium* taxa have high genetic variability there a risk that relying on species specific primers and a single primer assay for their detection

will lead to errors. For instance a primer set (dia-A) used in the USA failed to detect *D. dianthicola* in an outbreak of blackleg because the bacterial strain involved had a deletion that eliminated the primer target site (Pritchard *et al*. 2013).

Recent guideline have been published for using genome data in prokaryote taxonomy following the adoption of cheaper and faster DNA sequencing and analysis technologies (Chun *et al*. 2018). Species delineation thresholds were set for pairwise calculations of average nucleotide identity (ANI) at 95 to 96% and for digital DNA-DNA hybridization (dDDH) at 70%. Meier-Kolthoff *et al*. (2014) had previously set a threshold to distinguish prokaryote subspecies by dDDH is 79%. This approach was recently used by Shirshikov *et al*. (2018) who analysed sequences covering 44-58% of total genomes for five bacterial isolates that had previously been placed in *P. carotovorum*. They determined that these potato and cabbage isolates formed a distinct clade which they proposed the name '*Canditatus Pectobacterium maceratum'*. The closest taxa were *P. carotovorum subsp. carotovorum* and *P. carotovorum* subsp. *odoriferum* which had maximum ANI and dDDH values below the thresholds listed above.

Australian diagnostic laboratory capability

All key Australian state government diagnostic laboratories have capacity to undertake basic bacterial isolations, molecular biology and some specialised equipment used as confirmation tests. However, not all diagnostic service providers are currently focussed on detection of *Dickeya* and *Pectobacterium* soft rot pathogens so successful adoption of appropriate diagnostic protocols may require shared competency training.

One significant issue raised by Australian potato industry representative was that current commercial diagnostic testing for blackleg and soft rot pathogens in Australia is prohibitively expensive. For example one state government laboratory charges $660 per sample for a diagnosis compared with US$35 if they were to send samples to the USA. This is an undesirable situation from a commercial perspective and from an Australian biosecurity intelligence perspective.

Current best management practices

There are no simple solutions to controlling bacterial plant diseases. Australian horticulture does not permit use of antibiotics and where they have been used overseas a rapid development of resistance in bacterial populations ensued or their use has been discontinued due to other environmental health concerns. Similarly disinfection of potato tubers by steaming, hot dry air, UV radiation and various inorganic salts and antimicrobials have been investigated and were found to have some efficacy under controlled experimental conditions but have not been proven effective in practice (Czajkowski *et al*. 2011). There are no single genes known that confer resistance to these bacterial pathogens and given their wide genetic diversity and propensity to acquire new genes it is unlikely conventional breeding programs will yield anything more than varieties with reduced susceptibility to certain bacterial strains. Current overseas research with novel genome editing technologies such as CRISPR and breeding programs based on diploid potato lines may provide future promise.

Currently, Australian (and internationally) growers rely on the few key management options available to them:

- Using certified seed which has been inspected for disease symptoms during their production (or indexed for bacterial pathogens in some jurisdictions);
- Planting whole seed rather than cut seed particularly in regions where tuber soft rot and blackleg diseases have been a problem (such as in warm or wet soils);
- Scheduling planting to avoid periods when extremely wet or high temperatures prevail;
- Using long crop rotations, particularly for seed production
- Avoiding risks of cross-contamination between alternative host crops such as ornamental bulbs or vegetable crops grown in the same fields or using machinery or personnel that could spread bacteria on a farm. Note that there is evidence that some of these bacterial pathogens can be spread by certain flies so there may need to be biosecurity consideration required if for example bulbs are grown within the vicinity of a potato seed crop.
- Potato processing companies use modern cool stores (such as Dutch environment control technologies) for potatoes which have led to significantly reduced postharvest losses from tuber breakdown due to soft rot bacteria.
- Careful management of plant nutrition – maintaining optimal Calcium & Magnesium levels in plants, and optimal soil moisture through irrigation management and adequate drainage are well-known management strategies to help plants resist blackleg and soft rot infections.

Summary of discussions with potato industry representatives*

- Blackleg is widespread but currently not a significant disease issue in Australian potato production. Since the detection of *D. dianthicola* in WA there has been an increased awareness that there are different bacterial causes of blackleg in potatoes and there is some trepidation about government regulatory consequences if exotic species are detected on their properties or in their region.
- There is a significant but sporadic problem with 'slimy stem (stalk)' disease in the Lockyer Valley and Atherton Tablelands which is mostly due to infection under favourable environmental (windy and wet) conditions during production around flowering time. It is thought that this disease is mostly due to an opportunistic infection by *Pectobacterium* species (confirmed as *P. carotovorum* and *P. carotovorum* subspecies *brasiliense*) that enter wounded stem tissue under wet conditions. This problem was exacerbated in the past when growers had kept tubers with latent bacterial infections as seed for a further cropping cycle. They now use certified seed for each season's production with better outcomes. There have also been cases in the Atherton Tablelands where *P artosepticum* has been detected in blackleg affected plants that were associated with one seed lot but not another grown in the same field. This has fueled suspicion of seed being the source of this disease.
- Export trade of seed potatoes (such as with Indonesia) will likely be affected if Australian confirms presence of certain blackleg and soft rot bacteria under their more recent given names since many of our trading partners may still be using names such as *Erwinia* spp.
- Interstate trade of potatoes (and other host commodities) may also be affected if there is no national consensus and protocols for surveillance and diagnostics of these bacteria under their current valid names.

*Industry representatives did not wish to be identified

Further Research and Recommendations

Component 1: Update records of soft rot & potato blackleg pathogens in Australia

(i)	Characterise bacterial isolates lodged in Australian collections to understand the genetic diversity of soft rot and potato blackleg pathogens present in the past
(ii)	Survey potato, ornamental and other horticultural industries for key soft rot and blackleg-causing bacteria

There are over 100 isolates from soft rot and potato blackleg bacteria held in Australian culture collections – most are in herbaria in Victoria, NSW and Queensland (Drs J. Edwards, A Manners and J. Bailey pers. comm.). With only a few exceptions, most of these isolates were lodged prior to taxonomic revisions described here. It would also be prudent to ensure fresh isolates were collected and characterised from major production areas across Australia to update these records. Collection of isolates from other horticultural plants could be leveraged from other current R&D projects. Examples that could be considered are: the current nursery industry biosecurity project; and the vegetable industry area-wide management of bacterial and viral disease project. A study could extend to testing imported ornamental bulbs with any positive findings used to inform Australian Government quarantine authorities. Overseas experience described above pertaining to global spread of exotic bacterial pathogens supports the need for such an audit.

Component 2: Improved diagnostics capacity and industry biosecurity

(i)	Validate diagnostic protocols for detection and differentiation of soft rot and blackleg-causing bacteria
(ii)	Publish agreed national diagnostic protocols for detection of exotic soft rot bacterial species and strains that are deemed to be biosecurity threats

In addition to the published studies listed in Table 3 there are several overseas laboratories that are developing improved diagnostic tests that will detect species or subspecies of *Dickeya* and *Pectobacterium* soft rot bacteria. Key Australian plant bacteriologists are aware of these developments and have collaborative links with overseas researchers (Drs R. Mann and T. Chapman pers. comm.). Australian horticultural industries are well-placed to capture these latest developments and have them validated for use in Australia. A validation process is necessary in tests for exotic pathogens to mitigate risks that they produce false-positives to endemic or indigenous bacteria that are related but distinct from exotic threats.

Component 3: Review policies for seed potato certification and guidelines for entry and distribution of vegetative-propagated horticultural commodities

(i)	Review seed potato certification protocols and quarantine requirement to mitigate risks of spreading key blackleg and soft rot-causing bacteria with vegetatively-propagated horticultural material.

This component will be informed from the results obtained in completing components 1 and 2 above. The global emergence of biosecurity threats and examples of breaches cited in this review suggest that these risks are likely to be considerable.

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