

Co-occurrence of latent *Dickeya* and *Pectobacterium* species in potato seed tuber samples from northern Finland

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Recent methodological developments have uncovered the etiological diversity of the potato blackleg and soft rot Pectobacteriaceae. At least five species in the genera *Dickeya* and *Pectobacterium* have been confirmed to cause blackleg on potatoes in Finland. The bacteria are seed borne and remain latent in the tuber until conditions favourable for growth, multiplication and infection prevail. Tubers could be infected by one or more of these species. This short communication is based on the results of molecular detection data collected for more than 14 years from potato seed lots produced in Finland. Diagnostic PCR assay specific to *Dickeya solani*, *Pectobacterium atrosepticum*, *Pectobacterium carotovorum*, *P. brasiliense* and *P. parmentieri* revealed that potatoes are infected by one or more of these species; it also revealed that single species infection is more common than multiple colonization. An event of simultaneous occurrences of different strains from the *Pectobacterium* species appears to be more frequent than that observed between *Dickeya* and *Pectobacterium* species. The absence of co-occurrence of *Dickeya solani* and *Pectobacterium atrosepticum* is intriguing.

Key words: blackleg, soft rot, seed potato, *Dickeya*, *Pectobacterium*, co-occurrence, molecular detection

Introduction

The enterobacteria in the genera *Dickeya* and *Pectobacterium* belonging to the recently established Pectobacteriaceae family (Adeolu et al. 2016) are the primary causes of blackleg and soft rot on potatoes. According to the survey by the journal of Molecular Plant Pathology (Mansfield et al. 2012), *Dickeya* and *Pectobacterium* spp. have been ranked among the top ten bacterial plant pathogens in terms of economic and scientific importance.

Advances in molecular biology and genomics have revealed high genetic diversity in both genera. As a result, the taxonomy of *Dickeya* and *Pectobacterium* has undergone significant revision in recent years. This includes the establishment of new species and transfer of strains to new species (Samson et al. 2005, Pasanen et al. 2013, van der Wolf et al. 2014, Khay et al. 2016, Dees et al. 2017, Motyka et al. 2017, Portier et al. 2020). Species previously indistinguishable from each other and misclassified as *P. carotovorum* have now been readily identified by using molecular methods (Nykyri et al. 2012, Khayi et al. 2016, Potrykus et al. 2016, Pritchard et al. 2016). The observed etiological diversity causes diagnostic complexity and increases the cost of laboratory seed testing. Since these species cause symptoms on potatoes indistinguishable from each other, visual inspection as a method of seed certification and pathogen identification is currently not reliable.

To date polymerase chain reaction (PCR) is widely used for species-specific detection and diagnosis of the blackleg Pectobacteriaceae (Degefu et al. 2009, Czajkowski et al. 2015). As a result, as many as six species, namely *D. dianthicola*, *D. solani*, *P. atrosepticum*, *P. brasiliense*, *P. carotovorum* and *P. parmentieri* (formerly *P. wasabiae*), are known as main and common causes of blackleg and soft rot in Europe including Finland (Laurila et al. 2010, Toth et al. 2011, Tsrer et al. 2011, Moleleki et al. 2012, Czajkowski et al. 2015, Degefu et al. 2013, Potrykus et al. 2016, Zoledowska et al. 2018). New species of *Pectobacterium*, namely *P. punjabense* (Sarfras et al. 2018), *P. polaris* (Dees et al. 2017) and most recently *P. parvum* (Pasanen et al. 2020) have been discovered in Finland, but information on the prevalence and economic importance of these emerging new species on potato production in Finland is very limited or lacking. The bacteria are seed borne and survival outside the host plant is very poor.

Studies have revealed that temperature plays an important role in growth and pathogenic differentiation of the blackleg Pectobacteriaceae (Pérombelon and Kelman 1980, Pérombelon et al. 1987, Pérombelon 1992, Tsrer et al. 2009, Duarte et al. 2004, van der Merwe et al. 2010, Tsrer et al. 2013, Golanowska et al. 2017). From an *in vitro* study, du Raan et al. 2016 reported that there are differences in optimal temperatures among the species and isolates within the species. However, most of the reports available to date regarding the optimal temperature are

generated from *in vitro* studies, not supported by greenhouse or field data. Despite these, there is not much dispute that temperature plays an important role in species selection and pathogenicity of the potato blackleg pathogens (du Raan 2016 and references therein).

According to years of ecological and epidemiological studies, it has become evident that blackleg is a disease complex or a disease syndrome of a worldwide threat to potato production and a disease which has become cumbersome and costly to diagnose and control (Degefu et al. 2016). In complex diseases, more than one species is involved in the expression of the characteristic disease symptom on the plant tissue and plant organ they invade and it is a widely accepted current concept that such plant pathogens interact either synergically or antagonistically (Le May et al. 2009). Therefore, it is reasonable to argue that the same mechanism governs the blackleg Pectobacteriaceae and potato pathosystem in field infections and in laboratory enrichment culturing. However, detailed investigation of these phenomena and the impact on potato health and diagnostics is needed.

Moreover, reports have started to emerge that *D. solani* and *P. carotovorum* produce bacteriocin and bacteriocin-like substances which are inhibitory to other closely related bacteria (Holtmark et al. 2008, Garland et al. 2013, Kazemi-Zaromi et al. 2015). Currently, more efforts are being made to identify more of these substances. However, information on the level of toxicity of these secreted substances and their ability to counteract the other related species is lacking.

The aim of this study is to analyse the occurrence/co-occurrence of the commonly known destructive blackleg and soft rot enterobacteria as a step towards obtaining a basic understanding of the meaning of the etiological diversity of the pathosystem, as one development tool for the diagnosis and integrated management of the disease.

Materials and methods

Seed lots submitted for certification and samples collected during annual surveys from the High Grade seed potato production area of the municipality of Tyrnävä in northern Finland (64°45'52.88" N 25°39'8.28" E) were subjected to species-specific diagnostic PCR detection and identification tests. Pre-PCR and post-PCR procedures were as described in Degefu et al. (2009). Both real-time qPCR and conventional or endpoint PCR were employed for the detection of the latent bacteria in the seed tubers. A working sample of one hundred tubers randomly picked from the total of two hundred submitted tuber samples were tested for latent *Dickeya* and *Pectobacterium* species using species-specific primers and probes (Tables 1 & 2). Primers were used in the endpoint PCR (Table 1) and primers and probes were used in real-time PCR (Table 2). The PCR programs and reaction mixes were as described in Degefu et al. (2009). Seed lots which tested positive for the bacteria were identified and the species detected in each sample (single species or co-occurring) were recorded. Tests made for *P. brasiliense* and *P. parmentieri* were limited to the last three or four years of the project period since the two species had been appearing on potatoes in Finland since 2015. Therefore, there was not sufficient data from these new and emerging species to make meaningful comparisons.

Results

Fourteen years (2004–2018) of PCR-based detection and diagnosis of latent soft rot and blackleg enterobacteria, namely *Dickeya* and *Pectobacterium* spp. in seed potatoes cultivated in Finland, revealed that potatoes are infected by one or more species of the bacteria. The test results from 1058 *Dickeya* and *Pectobacterium* spp.-positive samples revealed an interesting single species and simultaneous or co-colonization scenarios. The results demonstrated that single species infection is more common (82% of samples) than multiple species or co-infection (Table 3). In an event of co-colonization of tubers, *Pectobacterium-Pectobacterium* co-occur more frequently than the *Dickeya-Pectobacterium* combination. *P. atrosepticum* and *P. carotovorum* co-occurred more frequently, followed by *D. solani* and *P. carotovorum*. To a lesser extent, co-habitation of all three (*D. solani*, *P. atrosepticum* and *P. carotovorum*) was also noted. Only one case of *D. solani-P. atrosepticum* was detected during the fourteen-year test period. Tests from potato stem segments with blackleg symptoms also revealed similar scenarios, but the study was carried out extensively on tubers.

Table 1. Primers used in endpoint PCR

Specificity	Primer	Sequence 5' - 3'	Annealing temp	References
<i>Dickeya</i> spp.	ADE 1	GATCAGAAAGCCGAGCCAGAT	72 °C	Nassar et al. 1996
	ADE2	TGTGGCCGATCAGGATGTTTTGTCGTGC		
<i>P. atrosepticum</i>	Eca 1F	CGGCATCATAAAAACACG	55 °C	De Boer and Ward 1995
	Eca 2R	GCACACTTCATCCAGGGA		
<i>P. brasiliense</i>	BR1F	GCGTGCCGGGTTTATGACCT	62 °C	Duarte et al. 2004
	L1R	CAGGGCATCCACCGT		
<i>P. parmentieri</i> (formerly <i>wasabiae</i>)	PhF	GGTTCAGTGCCTCAGGAGAG	60 °C	De Boer et al. 2012
	PhR	GCGGAGAGGAAGCGGTGAAG		
<i>P. carotovorum</i>	Ec001F	GGTTACGATCAGCG TCTCG	60 °C	MTT, unpublished
	Ec001R	GATGTGCCGATGCCGATAC		
	EXPCC F	GAACCTCGACCGCCGACCTTCTA	60 °C	Kang et al. 2003
	EXPCCR	GCCGTAATTGCCTACCTGCTTAAG		

Table 2. Primers and probes used in real time quantitative PCR (QPCR)

Specificity	Primer/probe	Sequence 5' - 3'	References
<i>D. solani</i>	SOL-C-F	GCCTACACCATCAGGGCTAT	Pritchard et al. 2013
	SOL-C-R	ACACTACAGCGGCATAAAC	
	SOL-C-P	FAM-CCAGGCCGTGCTCGAAATCC-TAMRA	
<i>D. dianthicola</i>	DIA-A-F	GGCCGCCTGAATACTACATT	Pritchard et al. 2013
	DIA-A-R	TGGTATCTCTACGCCATCA	
	DIA-A-P	FAM-ATTAACGGCGTCAACCCGGC-TAMRA	
<i>P. atrosepticum</i>	ECA-F	CGGCATCATAAAAACACGCC	De Boer and Ward 1995
	ECA-R	CCTGTGTAATATCCGAAAGGTGG	
	ECA-P	FAM-ACATTCAGGCTGATATCCCCCTGCC-TAMRA	
<i>P. brasiliense</i>	PBR-F	Not released	van der Waals
	PBR-R	Not released	
	PBR-P	Not released	
<i>P. parmentieri</i>	PwF1	TCTGTTCAATGTCAACGCAGGTA	van der Wolf , unpublished
	PwR1	AGGTAACCGCAATTTGCTCAA	
	PwP1	FAM-TGTGCGCAACCTG-MGB	

Discussion

During the last three decades, blackleg in Finland has undergone significant transformation with respect to the diversity of the causal bacteria (Harju and Kankila 1993, Degefu 2015, Degefu et al. 2013, Degefu and Virtanen 2015, Degefu 2016). The *Dickeya* and *Pectobacterium* species, which have not been known in Finland, have emerged as consistent threats to potato production (Degefu et al. 2013). Climate change is believed to be the major factor for the observed new scenario. The northward extension of some tropical and sub-tropical species and the southward expansion of the cooler and temperate species have been considered the reasons for the changing face and global dimension of blackleg and soft rot on potatoes. Advances in molecular diagnostics facilitated the monitoring, detection, and diagnosis of the disease. As a result, during the last fourteen years the introduction, establishment, and incidence of *Dickeya* and *Pectobacterium* on potatoes in Finland, northern Finland in particular, have been closely monitored. While *P. atrosepticum* and *P. carotovorum* have long been known as blackleg and soft rot bacteria on potatoes in Finland, *D. solani*, *P. brasiliense*, and *P. parmentieri* are some of the recently emerged or re-emerged and potentially destructive potato pathogens in Finland.

This PCR-based detection study confirmed that potatoes could be infected by one or more of these known blackleg or soft rot-causing pathogens, causing characteristic symptoms that are indistinguishable from each other. It has also become evident that infection by single species is more common than colonization by multiple species, suggesting that the bacteria compete to varying degrees. This is consistent with the common understanding that

co-occurring pathogens interact either synergistically or antagonistically in their ecological niche (Le May et al. 2009, Lamichhane and Venturi 2015). The data collected from about a decade and half of molecular (PCR) diagnostics and detection of latent *Dickeya* and *Pectobacterium* species in potato seed lots produced in Finland revealed interesting co-occurrence phenomena (Table 3). The near lack (only one) of co-occurrence of *D. solani* and *P. atrosepticum* during the fourteen years of investigation (Table 3) is intriguing. The phenomenon was consistent throughout the fourteen years of investigation, so it is unlikely that it occurred by chance. The most recent genomic study (Motyka-Pomagruk et al. 2021) revealed some peculiar gene pools specific to *D. solani*, which might contribute to the occupation of an isolated ecological niche by this particular species.

Table 3. Occurrence of latent *Dickeya* and *Pectobacterium* species in potato seed lots produced in Finland

Species identification	Number of positive seed lots	Percentage out of total samples
<i>Dickeya solani</i> (DS)	114	11.0
<i>Pectobacterium atrosepticum</i> (PCA)	201	19.0
<i>Pectobacterium carotovorum</i> (PCC)	550	52.0
PCA + PCC	115	11.0
DS + PCC	53	5.0
DS + PCA + PCC	24	2.23
DS + PCA	1	0.09

The two species exhibit contrasting temperature optimums for pathogenicity and growth (Pérombelon and Kelman 1980, Pérombelon et al. 1987, du Raan et al. 2016). *P. atrosepticum*, which has been the major cause of blackleg on potatoes in Finland and other cooler regions of northern Europe, thrives best at cooler temperatures, lower than 25 °C, although there are reports that it can survive at temperatures of up to 37 °C (Pérombelon et al. 1979, Pérombelon 1992). On the other hand, *D. solani* is more prevalent and causes severe blackleg outbreaks at temperatures above 25 °C (Elphinstone and Toth 2007, Degefu et al. 2013). It is, therefore, reasonable to argue that growing season temperature has a significant impact on pathogenicity differentiation and species selection, specifically in these two species. However, reports from several studies indicate that the cardinal, optimal and growth range temperatures of the blackleg enterobacteria appeared to overlap (du Raan et al. 2016), making it difficult to draw a well-defined demarcation and justify temperature as a sole mechanism for such strong species selection (Pérombelon et al. 1979, Molina and Harrison 1980, Pérombelon and Kelman 1980, Pérombelon et al. 1987, du Raan et al. 2016, Abdullah et al. 2017). For example, the growth temperature range for *P. atrosepticum* is 18–31 °C, while that of *D. solani* is 24–42 °C.

Another important concept which draws attention is the unique ability of *D. solani* to outcompete or overtake the *Pectobacterium* species and its relatively higher aggressiveness in causing disease symptoms from lower inoculum levels compared to other species (Toth et al. 2011). The reason for these abilities is not fully known. Bacteriocins and bacteriocin-like substances reviewed in the introduction have been implicated as possible factors. According to current knowledge, bacteriocins are not reported from *P. atrosepticum*. This might explain the reason for the unique phenomena—the lack of co-occurrence of *D. solani* and *P. atrosepticum* observed in this study. On the other hand, the co-occurrence event observed among *D. solani*, *P. atrosepticum* and *P. carotovorum*, and *P. carotovorum* and *D. solani*, although not very frequent (Table 3), suggests that bacteriocin and bacteriocin-like substances might play a role in the co-habitation ability among these species. It is rather tempting to suggest that in these three species' co-occurrence, the presence of *P. carotovorum* might play a role in buffering the selection strength of the system due to its ability to produce bacteriocin. However, the role and mechanisms of action of toxins and bacteriocins of *D. solani* and *P. carotovorum* are less investigated. Therefore, at this stage experimental evidence is lacking to confirm the assumptions.

The observed strong species selection between *D. solani* and *P. atrosepticum* might take place in the field because of growing season temperature or as a result of chemical or bacteriocin actions at the time of *in vitro* enrichment culturing in the PCR detection procedure (Degefu et al. 2009). It is, therefore, a subject of investigation that is vital for advancement in the development of diagnostics for assessment of the prevalence of the bacteria in potatoes.

In conclusion, this research note provides a starting point for investigations on the interactions among the potato-blackleg *Pectobacteriaceae* pathosystem in an effort to find innovative solutions for effective diagnosis and integrated management of these pathogens which have become consistent global threats to potato production.

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