Eur J Plant Pathol (2019) 154:635–647
https://doi.org/10.1007/s10658-019-01687-1

Pectobacterium and *Dickeya* species detected in vegetables in Northern Ireland

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Accepted: 22 January 2019 / Published online: 7 February 2019

Abstract Destructive soft rot *Pectobacteriaceae* affect a number of vegetable crops and cause high economic loses in the field and storage. The diversity of *Pectobacterium* and *Dickeya* causing soft rot of vegetables in Northern Ireland is unknown. This study provides details of *Pectobacterium* and *Dickeya* spp. detected in vegetables from several locations in Northern Ireland in the years 2015–2017. Soft rot *Pectobacteriaceae* were identified based on DNA sequences. Thirty three strains were selected for further phylogenetic analysis based on the *recA* gene region. Results from the testing of over 3456 potato samples for plant health statutory purposes in the years 2005–2017 demonstrated that *Dickeya* spp. is not the major pathogen causing soft rot or blackleg in Northern Ireland. The most predominant species causing soft rot of vegetables in Northern Ireland were *Pectobacterium atrosepticum* and *Pectobacterium carotovorum* subsp. *carotovorum*. *Pectobacterium atrosepticum* was also detected on hosts other than potato. Testing of bacteria isolated from carrots led to the detection of *P. carotovorum* and *Dickeya* sp. This is the first study to provide knowledge about *Pectobacterium* and *Dickeya* spp. diversity causing soft rot of vegetables in Northern Ireland confirmed by real-time PCR and DNA sequences. This is also the first report of the detection of *D. aquatica* from a source other than water.

Keywords *Pectobacterium* · *Dickeya* · Soft rot Enterobacteriaceae · PCR detection · *rec*A sequencing

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s10658-019-01687-1) contains supplementary material, which is available to authorized users.

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Introduction

Soft rot *Pectobacteriaceae* (SRP; formerly soft rot *Enterobacteriaceae*) include the genera *Pectobacterium* and *Dickeya* (Adeolu et al. 2016) and are widespread globally (Bhat et al. 2010; Mansfield et al. 2012). SRP causes high loses particularly in potato production, estimated in the Netherlands at up to €30 million annually (Toth et al. 2011) with potential global losses of up to 30% (Agrios 2007) at an estimated cost of US$50–100 million every year in vegetables, fruits and ornamental plants (Pérombelon and Kelman 1980; Pérombelon 2002; Ma et al. 2007).

Previously known as *Erwinia* species (Winslow et al. 1920), the taxonomic reclassifications based on genetic differences within this genus led to the recognition of the genus *Pectobacterium* (Hauben et al. 1998) which was later separated into *Pectobacterium* and *Dickeya* (Samson et al. 2005). *Pectobacterium* currently includes ten formally described species and three subspecies

(Gardan et al. 2003; Nabhan et al. 2013; Khayi et al. 2016) with several recently described species including *Pectobacterium polaris* (Dees et al. 2017b), *Pectobacterium peruviense* (Waleron et al. 2017), *Pectobacterium punjabense* (Sarfraz et al. 2018), *Pectobacterium zantedeschiae* (Waleron et al. in press) and *Candidatus* Pectobacterium maceratum (Shirshikov et al. 2018) being proposed. The genus *Dickeya* currently includes eight species and two subspecies (Samson et al. 2005; Brady et al. 2012; Parkinson et al. 2014; van der Wolf et al. 2014; Tian et al. 2016). Recently, a number of reports have shown *Dickeya solani* and *Pectobacterium carotovorum* subsp. *brasiliense* to cause high losses in Western Europe (Nunes Leite et al. 2014; de Werra et al. 2015; Toth et al. 2011; van der Wolf et al. 2017). Moreover, several studies have confirmed that *Pectobacterium atrosepticum* and *Pectobacterium carotovorum* subsp. *carotovorum* are some of the predominant causes of soft rot and/or blackleg in temperate regions (de Boer et al. 2012; Dees et al. 2017a; Waleron et al. 2013; Elphistone 2016a, b). *Pectobacterium atrosepticum* is mainly associated with potato blackleg and soft rot in temperate climates (Pérombelon 2002; Skelsey et al. 2018; Oztruk et al. 2018) but its occurrence might not be limited to potato, as it has been isolated from other hosts such as sunflower (Bastas et al. 2009) and pepper (Stommel et al. 1996). *Pectobacterium carotovorum* subsp. *carotovorum* has a broad host range, including but not limited to carrots, potatoes, cabbage, lettuce and onions across a number of climatic zones including Europe (Peltzer and Sivasithamparam 1985; Waleron et al. 2002; Pérombelon 2002; Gardan et al. 2003; Toth et al. 2003; Crowhurst and Wright 1998; de Haan et al. 2008; van der Merwe et al. 2010; Cariddi and Sanzani 2013; Onkendi and Moleleki 2014; Moretti et al. 2016; Dees et al. 2017a; Naas et al. 2018; Oztruk et al. 2018). *Dickeya* spp. have been confirmed as the most destructive pathogens in terms of losses in Switzerland (Toth et al. 2011; Golanowska and Łojkowska 2016). The species *Dickeya dianthicola* is recommended for regulation as a pest in the European and Mediterranean Plant Protection Organisation (EPPO) (A2 pathogen; EPPO 2017). Therefore several countries have introduced national legislation to prevent the introduction of this species. In the UK, both Scotland and Northern Ireland have a 'nil' tolerance for *Dickeya* spp., and Northern Ireland has a 'nil' tolerance for *Pectobacterium* spp. (termed "blackleg") in pre-basic seed potatoes production and up to 1% tolerance for *Pectobacterium* spp. in basic seed potatoes (Anonymous 2010; Anonymous 2016).

The island of Ireland (Ireland and Northern Ireland) has a favourable climate for potato growing, with moderate temperatures, high humidity and a geographic location influenced by the Atlantic Ocean which restricts aphid populations and results in low levels of aphid-borne potato viruses (Proudfoot and Mccallum 1961). However, these conditions are also optimal for the spread of SRP (Pérombelon 2002). In Northern Ireland, potato is the second most important field crop after cereals (barley, wheat and oats) valued in 2017 to £23.8 million (Anonymous 2017). Overall, little is known about the diversity of SRP populations in Northern Ireland. Early observations indicated that blackleg disease may have been present in potato crops in Ireland as early as 1845 (Bourke 1966), with the first isolation of the putative organism *Pectobacterium atrosepticum* (listed as *Bacillus melanogenes*) by Pethybridge and Murphy (1911). *Pectobacterium atrosepticum* (recorded as *Erwinia carotovora* subsp. *atroseptica*) and *P. carotovorum* (recorded as *Erwinia carotovora*) have been recorded in Northern Ireland (Logan 1968; O'Neil and Logan 1975; Hossain 1986). *Pectobacterium atrosepticum* causing blackleg was listed as a large threat potato production in Northern Ireland by Logan et al. 1987. The presence of other species and subspecies of *Pectobacterium* in potato and other vegetables is unknown. Given the significance of potato and other vegetable (carrot and onion) production in Northern Ireland, with the total annual output of up to £42.5 million (Anonymous 2017), and the potential losses from SRP, the aim of this study was to investigate the diversity of *Pectobacterium* present in Northern Ireland and provide the first up to date report for over 30 years.

Materials and methods

Sample collection

Symptomatic and asymptomatic potato samples, and water samples from potato processing facilities are submitted to the Agri-Food and Biosciences Institute (AFBI) for detection of plant pests and pathogens on behalf of the Department of Agriculture, Environment and Rural Affairs (DAERA). These statutory samples are tested for the regulated bacterial potato pathogens *Clavibacter michiganensis* subsp. *sepedonicus*, *Ralstonia*

solanacearum, and *Dickeya* spp. During the years 2005–2017, 3456 statutory samples of symptomatic and asymptomatic material were tested for *C. michiganensis* subsp. *sepedonicus*, *R. solanacearum* with samples also tested for *Dickeya* spp. from 2010 onwards.

To investigate *Pectobacterium* diversity in Northern Ireland, in the years 2015 and 2016 a total 454 of these samples showing soft rot or blackleg symptoms were further analysed as part of this research. To supplement this data, a field survey was carried out in 2017 in four locations in Co. Londonderry and Co. Down in Northern Ireland. During this survey, potato samples with symptoms of blackleg were collected and tested for the presence of SRP. Carrots and onions grown in Northern Ireland were sourced from local growers and/or from local shops in Co. Down, Co. Antrim, Co. Armagh and Co. Londonderry and were also tested for SRP.

Preparation of samples from asymptomatic potato tuber extracts

Prior to analysis for detection of *Dickeya* spp. from statutory samples by real-time PCR with the specific primers ECH and/or SOL, DIC (Pritchard et al. 2013), potato extracts were prepared using the methodology adapted from Commission Directive 2006/63/CE for the control of *R. solanacearum* (Anonymous 2006). In brief, two hundred cores (5 mm) obtained from the stolon end of potato were transferred to a macerating container and 40 mL of macerating buffer containing Na_2HPO_4 (4.26 g), KH_2PO_4 (2.72 g) per litre was added, followed by 2 min of maceration at low speed in a homogeniser. Macerated samples were filter through a single layer muslin into a beaker. The filtrate was transferred into a centrifuge tube and centrifuged (200 rpm, 4–10 °C) for 10 min. The obtained supernatant was poured into a clean centrifuge tube and centrifuged (11,000 rpm, 4–10 °C) for 15 min. The supernatant was discarded and the pellet suspended in 1.5 mL of pellet buffer containing Na_2HPO_4 (2.7 g), NaH_2PO_4 (0.4 g) per litre. The sample was then enriched in pectate enrichment medium (PEM) in compact workstations for anaerobic and microaerophilic conditions for detection of *Dickeya* spp. for 48 h at 37 °C (Anonymous 2010c).

Isolation of SRP strains from symptomatic samples

A modified double layer crystal violet pectate (CVP) media was used for detection and isolation of SRP. The agar was prepared using a modification of the method previously reported by Bdliya et al. (2004) which excludes bromthymol blue and 2,3,5-triphenylterrazolium chloride and with the inclusion of Dipecta (Agdia-Bioford) as the pectate source. For the rotting vegetables, samples were taken from the macerated tissues about 1 cm inside the surface using a sterile scalpel, suspended in 10 ml of sterile water and vortexed for 5 min at 10x speed. A total of 100 μl of plant material extract was streaked onto a CVP agar plate, followed by streaking the same hockey stick on five subsequent plates – thus providing a dilution series of plates. The plates were incubated for 48 h at 28 °C for detection of *Pectobacterium* spp. and for 48 h at 37 °C for detection of *Dickeya* spp. from statutory samples after which they were assessed to determine the number of cavity or pit forming bacterial colonies of pink to red colour and with rod-shaped cells. Bacterial colonies that matched these characteristics were aseptically streaked onto nutrient agar (NA) plates (Oxoid) and incubated for 48 h at 25 °C to obtain pure cultures.

DNA extraction and molecular detection

For DNA extraction, bacterial cultures grown on NA were transferred into the lysis buffer of the Maxwell®16 cell LEV DNA Purification kit (Promega) following the manufacturer's instructions. A number of samples of extracts of asymptomatic potato were also lysed using the procedure above. Bacterial DNA were purified for 30–45 min using a Maxwell DNA Magnetic Particle Processor MX 3031 (Promega) using the cell purification program. DNA extracts were quantified using a NanoDrop 2000 (Thermo Scientific), and the concentrations of bacterial DNA extracts adjusted to between 10 and 20 ng. Purified bacterial DNA were stored at −20 °C until further analysis.

Real-time PCR was carried out using primers PEC, ECA, ECH, DIC and SOL (Pritchard et al. 2013). The real-time PCR assay was performed in a 12.5 μL reaction mixture comprising GoTaq Probe qPCR (QuantiNova) (6.25 μL), genomic DNA from bacterial culture ca. 10–20 ng (0.5 μL), primers (5 μM 0.375 μL), Nuclease-free H_2O (Qiagen) (4.74 μL). Amplification conditions were as follows: DNA was initially denatured at 95 °C for 2 min, followed by 40 cycles of denaturation 95 °C for 5 s, annealing at 50 °C for 5 s. PCR was carried out using a Prime Pro 48 real-time qPCR system

(Techne). For real-time PCR, positive results were regarded at critical threshold (C_t) values <36.

Conventional PCR was performed in a 12.5 μL reaction mixture comprising GoTaq PCR Mastermix (6.25 μL), genomic DNA from bacterial culture ca.10–20 ng (2.5 μL), primers Y1&Y2 (Darasse et al. 1994), Y45&Y46 (Frechon et al. 1998) (10 μM 0.50 μL), Nuclease- free H_2O (Qiagen) (2.75 μL). Amplification conditions were as follows: DNA was initially denatured at 95 °C for 5 min, followed by 37 cycles of denaturation 95 °C for 60 s, annealing at 67 °C for 60 s, and polymerisation at 72 °C for 60 s and finally 1 cycle at 72 °C for 10 min. PCR was carried out using a Standard Life Pro Thermocycler (Bioer). The obtained PCR products were visualised using QIAxcel Advanced System (Qiagen).

Amplification of the RecA multifunctional protein from the bacterial DNA extracts obtained was performed using primers EC-RecAR & EC-RecAF (Waleron et al. 2002). DNA amplification was performed in a 12.5 μL reaction mixture comprising GoTaq PCR Mastermix (6.25 μL), genomic DNA from bacterial culture ca. 10-20 ng (0.5 μL), primers (10 μM 0.50 μL), Nuclease- free H_2O (Qiagen) (4.75 μL). Amplification conditions were as follows: DNA was initially denatured at 95 °C for 5 min, followed by 32 cycles of denaturation 94 °C for 60 s, annealing at 47 °C for 60 s, and extension at 72 °C for 5 min and the final extension at 72 °C for 5 min. The procedure was carried out using a Life Pro Thermocycler (Bioer) RecA program for 2 h 42 min. The obtained PCR products were visualised using QIAxcel Advanced System (Qiagen). DNA extracts that amplified with RecA primers, and had a PCR product of 700-1000 bp were sent for sequencing.

Phylogenetic analysis and pairwise comparison

Sequencing of PCR amplicons was performed by a commercial sequencing service at Queens University Belfast Genomics Core Technology Unit using an Applied Biosystems 3730 (Thermo Fisher Scientific). The obtained sequences of the forward and reverse strands were assembled, aligned and trimmed using Geneious version 10.1.3 (Biomatters) software and compared using Basic Local Alignment Search Tool (BLAST) analysis with sequences available in GenBank (Altschul et al. 1990). The obtained sequences of *Pectobacterium* and *Dickeya* spp. were deposited in GenBank under following accession numbers: MH290883, MH315943, MH329871-MH329878, MH346375 - MH346385, MH481716 - MH481722, MH484268, MH688057 - MH688058, MK305814 and MK305815.

Obtained sequences (Table 1) were analysed for the species within the genus *Pectobacterium* and *Dickeya* spp. from across Northern Ireland by further phylogenetic analysis. Multiple alignment of the sequences was performed using CLC Main Workbench 8.0 (Qiagen) with reference sequences obtained from GenBank (National Centre of Biotechnology Information) i.e. *P. atrosepticum* (AY264783, KF704802), *P. c.* subsp. *carotovorum* (AY264799, AY264798, KY067403, MF314822) *P. c.* subsp. *brasieliense* (KP762587, KP762588), *P. c.* subsp. *odoriferum* (KF704811, KF704807), *P. wasabiae* (KC584992, KC584995 and AY264796), *D. aquatica* (JX273703), *D. zeae* (KY817909) and *Candidatus* P. maceratum (MK024779). Phylogenetic trees were generated using the Neighbor-joining method (Kimura 1980) using CLC Main Workbench 8.0 software. Bootstrapping was executed with 1000 replications. The bootstrap values <50% were excluded.

The multiple alignments were tested on five different models prior to construction of the tree to find the best-fit model for the final phylogenetic tree by the hierarchical likelihood ratio test (hLRT) and Bayesian Information Criterion (BIC). Both tests showed Kimura 80 as the best-fit model, thus a neighbour-joining tree was constructed based on that model (Fig. 1). The pairwise comparison of obtained multiple alignment was constructed (Tamura et al. 2004) based on percentage identity of Northern Irish strains with reference sequences obtained from GenBank using CLC Main Workbench 8.0 software.

Results

Statutory samples from asymptomatic and symptomatic potato tubers in Northern Ireland for *Dickeya* spp.

More than 3456 potato samples were tested for regulated bacterial pathogens between the years 2005–2017, 1891 originating in Northern Ireland and 1565 originating from other countries. Of these, *Dickeya* spp. were not detected in potato samples originating in Northern Ireland. *Clavibacter michiganensis* subsp. s*epedonicus* and *R. solanacearum* were not detected in any samples (Table 2).

Table 1 Details of *Pectobacterium* and *Dickeya* spp. isolated from potato and carrots in Northern Ireland identified by sequences of the *recA* gene fragment use in this study

Number	Accession number	Strain	Species[a]	Year of isolation	Location[b]	Host
1	MH329877	B2	*P. atrosepticum*	2017	Co. Antrim	potato stem
2	MH346384	F5	*P. atrosepticum*	2017	Co. Armagh	potato tuber
3	MH481719	L1	*P. atrosepticum*	2017	Co. L'derry	potato tuber
4	MH484268	P14	*P. atrosepticum*	2014	unknown	potato
5	MH481722	P16	*P. atrosepticum*	2014	unknown	potato
6	MH329878	P1B	*P. atrosepticum*	2016	Co. Antrim	potato tuber
7	MH481716	P2A	*P. atrosepticum*	2016	Co. Antrim	potato tuber
8	MH481717	P2B	*P. atrosepticum*	2016	Co. Antrim	potato tuber
9	MH290883	P4A	*P. atrosepticum*	2016	Co. Antrim	potato tuber
10	MH346385	P4B	*P. atrosepticum*	2016	Co. Antrim	potato tuber
11	MH481718	S11	*P. atrosepticum*	2017	Co. L'derry	potato tuber
12	MH346378	S12	*P. atrosepticum*	2017	Co. L'derry	potato tuber
13	MH346379	S15	*P. atrosepticum*	2017	Co. L'derry	potato tuber
14	MH346380	S16	*P. atrosepticum*	2017	Co. L'derry	potato tuber
15	MH346383	S18	*P. atrosepticum*	2017	Co. L'derry	potato tuber
16	MH346381	S21	*P. atrosepticum*	2017	Co. L'derry	potato tuber
17	MH481720	S24	*P. atrosepticum*	2017	Co. L'derry	potato tuber
18	MH346382	S26	*P. atrosepticum*	2017	Co. L'derry	potato tuber
19	MH481721	S27	*P. atrosepticum*	2017	Co. L'derry	potato tuber
20	MH346376	S3	*P. atrosepticum*	2017	Co. L'derry	potato tuber
21	MH346377	S4	*P. atrosepticum*	2017	Co. L'derry	potato tuber
22	MH346375	S5	*P. atrosepticum*	2017	Co. L'derry	potato tuber
23	MH329871	Ca1B	*P. c.* subsp. *carotovorum*	2016	Co. Down	carrot
24	MH329872	Ca2A	*P. c.* subsp. *carotovorum*	2016	Co. Down	carrot
25	MH329873	Ca2B	*P. c.* subsp. *carotovorum*	2016	Co. Down	carrot
26	MH315943	D0346	*P. carotovorum*	2016	Co. Down	carrot
27	MH329875	D0347	*P. carotovorum*	2016	Co. Down	carrot
28	MH329876	D0349	*P. c.* subsp. *carotovorum*	2016	Co. Down	carrot
29	MH329874	D0348	*P. carotovorum*	2016	Co. Down	carrot
30	MH688058	Ca3A	*D. aquatica*	2016	Co. Down	carrot
31	MH688057	Ca3B	*D. aquatica*	2016	Co. Down	carrot
32	MK305814	SR22	*P. c.* subsp. *carotovorum*	2014	unknown	potato tuber
33	MK305815	C2558	*P. c.* subsp. *carotovorum*	2015	Co. Antrim	potato tuber

[a] Identity based on phylogenetic analysis (Kimura 1980)

[b] Counties in Northern Ireland, UK

Symptomatic vegetables samples in years 2015–2017 for *Pectobacterium* spp.

From 454 samples tested, 164 samples from potatoes, carrots and onions originated from Northern Ireland were tested in years 2015–2017, using real-time PCR, conventional PCR and DNA sequencing. From all samples, 164 were confirmed as members of the genera *Pectobacterium* or *Dickeya* (Table 3). *Pectobacterium atrosepticum* was identified in 101 samples collected from vegetables between 2015 and 2017 (Table 3). Over the three years of sampling, *P. atrosepticum* was the most frequent species isolated from potato (97), also three from carrots and one from onion were confirmed with real-time PCR. *Pectobacterium carotovorum* was confirmed from twelve samples (Table 3). In Co. Down this species was identified in 83% of samples collected from carrots and onion, and in Co. Antrim 17% from

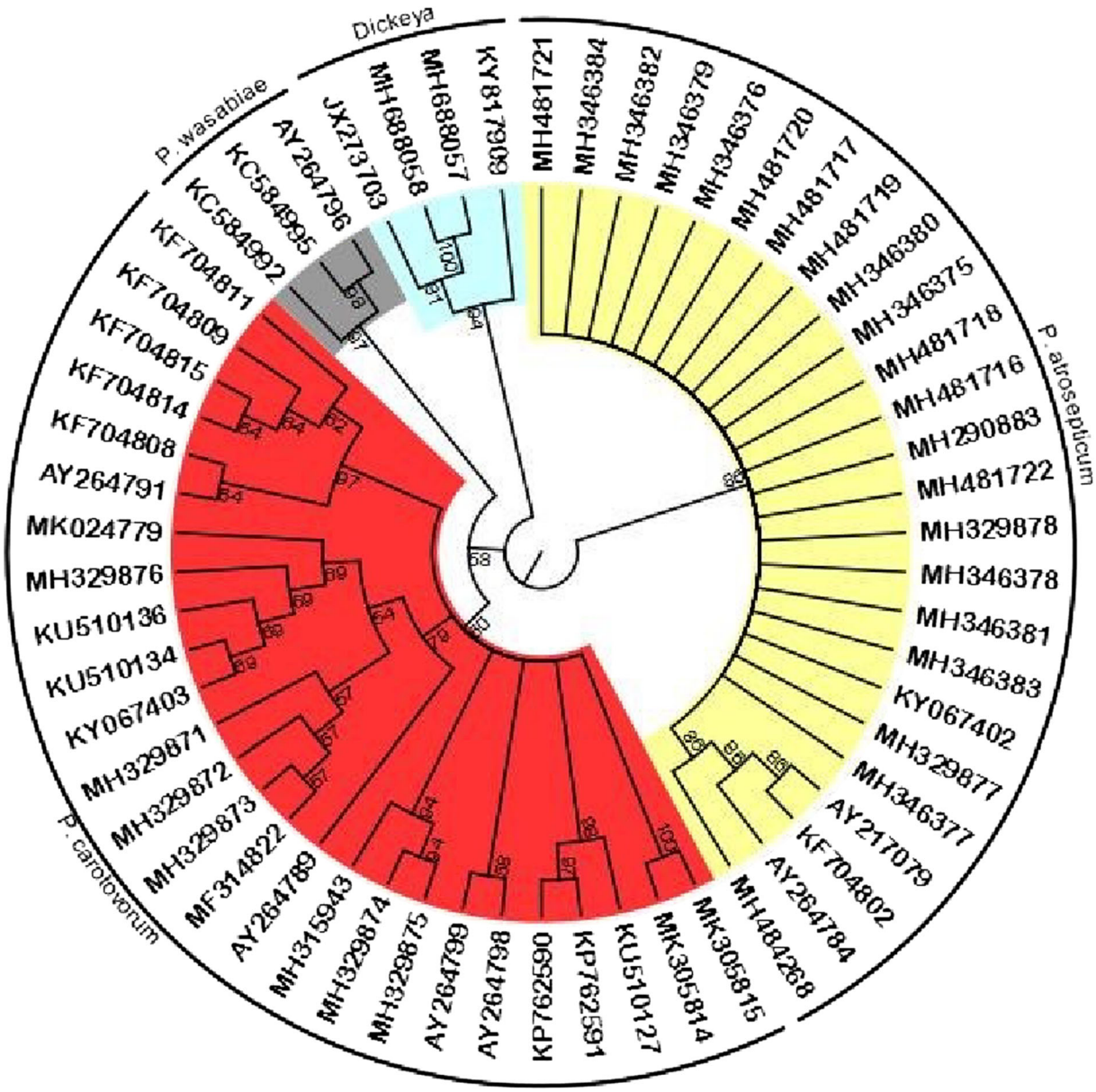

Fig. 1 The consensus of maximum likelihood phylogeny by neighbour-joining method (Kimura 1980) based on the fragment of the *recA* gene. *Pectobacterium* spp. and *Dickeya* spp. sequences detected in Northern Ireland and obtained from GenBank followed by accession numbers. Bootstrap values after 1000 replicates are expressed as percentages. Clusters with Bootstrap values <50% are collapsed. The strains identified as *P. atrosepticum* (yellow), *P. carotovorum* (red), *P. wasabiae* (grey) *Dickeya* spp. (blue)

potatoes. A single *Dickeya sp*. was identified from a potato sample by real-time PCR, and two carrots strains were identified as similar to *D. aquatica* by phylogenetic analysis. Real time PCR testing for *P. atrosepticum* and other SRP of 34 DNA extracts from asymptomatic tubers originating in Northern Ireland in 2016 found that 29 were positive for SRP.

Phylogenetic analysis

Phylogentic analysis was carried out on a panel of 33 strains (Table 1), previously identified by BLAST as *P. atrosepticum* (23), *P. c.* subsp. *carotovorum* (9), and *D. paradisiaca* (1). These strains grouped separately into three main clusters corresponding to *P. atrosepticum*, *P. carotovorum* and *Dickeya* spp. (Fig. 1). *Pectobacterium*

Table 2 Details of potato samples tested as part of statutory surveys for ring rot, brown rot and *Dickeya* spp. in Northern Ireland. Note that ring rot and brown rot have been not detected in Northern Ireland

Year	Number of samples		*Dickeya* spp.		*Dickeya solani*		*Clavibacter michiganensis subsp. sepedonicus and R. solanacearum* total
	NI[a]	Total[b]	NI[a]	Total[b]	NI[a]	Total[b]	
2005	161	273	nt	nt	nt	nt	0
2006	163	265	nt	nt	nt	nt	0
2007	277	404	nt	nt	nt	nt	0
2008	162	323	nt	nt	nt	nt	0
2009	214	335	nt	nt	nt	nt	0
2010	125	227	0	0	0	0	0
2011	153	270	0	3	0	3	0
2012	99	203	0	2	0	0	0
2013	111	334	0	0	0	0	0
2014	160	290	0	2	0	0	0
2015	128	259	0	1	0	1	0
2016	90	188	0	0	0	0	0
2017	48	85	0	0	0	0	0
Total	**1891**	**3456**	**0**	**9**	**0**	**4**	**0**

nt- not tested

[a] Samples originated from Northern Ireland

[b] Samples included Northern Irish stock and also imports

carotovorum grouped into four main clusters: three isolates from carrots with *P. carotovorum* subsp. *carotovorum* strain (MF314822) and one carrot isolate in separate cluster with new proposed species of *Candidatus* P. maceratum (MK024779). Furthermore, three of the Northern Irish strains isolated from carrots (D0346, D0347 and D0348) and two potato isolates grouped in a separate clusters. Northern Irish *P. atrosepticum* strains constituted the cluster with the most similar in BLAST analysis type of *P. atrosepticum* strain (KY067402) and one grouped in a separate sub-cluster with strains of type *P. atrosepticum* (AY217079, AY264784 and KF704802). Two of the Northern Irish strains Ca3A and Ca3B were grouped with the type strain of *D. aquatica* (JX273703) and *D. zeae* (KY817909) in the *Dickeya* cluster of *Pectobacterium* spp. phylogeny (Fig.1).

Discussion

In recent years, an increasing number of studies have detected *Dickeya* spp. in potato production in Europe (Toth et al. 2011; Potrykus et al. 2016). However detection of *Dickeya* spp. in countries that have implemented control programmes and legislation, such as Scotland and Norway is mostly linked to imported plant material (Skelsey et al. 2016; Dees et al. 2017a). In this study, detections of *Dickeya* spp. from statutory potato samples in years 2005–2017 in Northern Ireland were very rare and always linked to imported plant material. Moreover, so far *Dickeya* spp. have not been detected from potato grown in Northern Ireland analysed as statutory samples. This work therefore focused on investigating the diversity of *Pectobacterium* spp. rather than *Dickeya* spp. and samples obtained from field survey were not enriched at temperatures higher than 28 °C. As demonstrated by Potrykus et al. (2016), *Pectobacterium* spp. can overgrow *Dickeya* spp. during isolation at lower temperatures; however, *Dickeya* may have been present at low concentrations.

Two isolates from carrots in this study (Ca3A and Ca3B) showed high similarity to *D. aquatica*. Separate analysis has attributed these two Northern Irish strains to the SLC 2 clade defined by Parkinson et al. (2014) in the *Dickeya* cluster in a separate phylogeny of *Dickeya* spp. with a high bootstrapping value (Supplementary Fig. 1).

Table 3 Number of *Pectobacterium* and *Dickeya* spp. confirmed by molecular detection methods isolated on crystal violet pectate media or extracted from plant tissue from vegetables identified in years 2015–2017 from four locations in Northern Ireland

Year	Host	Number of samples																				Total				
		Co. Antrim				Total	Co. Down				Total	Co. L'Derry				Total	Co. Armagh				Total					Total (%)
		Pba[a]	Pc[b]	P[c]	D[d]		Pba[a]	Pc[b]	P[c]	D[d]		Pba[a]	Pc[b]	P[c]	D[d]		Pba[a]	Pc[b]	P[c]	D[d]		Pba[a]	Pc[b]	P[c]	D[d]	
2015																										
	potato	3	2	1	1	**7**	11	0	0	0	**11**	13	0	0	0	**13**	0	0	0	0	**0**	27	2	1	1	31
	carrot	0	0	0	0	**0**	0	0	0	0	**0**	0	0	0	0	**0**	0	0	0	0	**0**	0	0	0	0	0
	onion	0	0	3	0	**3**	0	0	0	0	**0**	0	0	0	0	**0**	0	0	3	0	**3**	0	0	6	0	6
Total (%)		**3(11)**	**2(67)**	**4(46)**	**1(100)**	**10**	**11(41)**	**0**	**0**	**0**	**11**	**13(48)**	**0**	**0**	**0**	**13**	**0**	**0**	**4(31)**	**0**	**3**	**27(72)**	**2(5)**	**7(30)**	**1(2)**	**37(23)**
2016																										
	potato	6	0	2	0	**8**	0	0	0	0	**0**	0	0	0	0	**0**	0	0	0	0	**0**	6	0	2	0	8
	carrot	0	0	0	0	**0**	3	9	15	2	**29**	0	0	0	0	**0**	0	0	0	0	**0**	3	9	15	2	29
	onion	1	0	5	0	**6**	0	1	2	0	**3**	0	0	0	0	**0**	0	0	0	0	**0**	1	1	7	0	9
Total (%)		**7(70)**	**0**	**7(42)**	**0**	**14**	**3(30)**	**10(100)**	**17(71)**	**2(4)**	**32**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**10(22)**	**10(22)**	**24(54)**	**2(6)**	**46(28)**
2017																										
	potato	1	0	1	0	**2**	23	0	9	0	**32**	34	0	3	0	**37**	6	0	4	0	**10**	64	0	17	0	81
	carrot	0	0	0	0	**0**	0	0	0	0	**0**	0	0	0	0	**0**	0	0	0	0	**0**	0	0	0	0	0
	onion	0	0	0	0	**0**	0	0	0	0	**0**	0	0	0	0	**0**	0	0	0	0	**0**	0	0	0	0	0
Total (%)		**1(2)**	**0**	**1(6)**	**0**	**2**	**23(38)**	**0**	**9(47)**	**0**	**32**	**34(53)**	**0**	**3(18)**	**0**	**37**	**6(9)**	**0**	**4(24)**	**0**	**10**	**64(79)**	**0**	**17(21)**	**0**	**81(49)**
Total (%)		**11(11)**	**2(17)**	**17(31)**	**1(33)**		**37(37)**	**10(83)**	**37(68)**	**2(66)**		**47(46)**	**0**	**3(6)**	**0**		**6(6)**	**0**	**8(15)**	**0**		**101(62)**	**12(8)**	**54(32)**	**3(1)**	
																							Total			**164**

Pba - *P. atrosepticum,* Pc - *P. carotovorum,* P - *Pectobacterium* spp., D - *Dickeya* spp.

[a] *P. atrosepticum* identified by real-time PCR primers ECA or/and *recA* gene sequences

[b] *P. carotovorum* identified by *recA* gene sequences

[c] Other *Pectobacterium* spp. identified by real-time PCR primers PEC or/and conventional PCR primers Y1&Y2

[d] *Dickeya spp*. identified by real-time PCR primers ECH or *recA* gene sequences

In 2008/09, a *Dickeya* sp. was isolated from water sources in Finland and Scotland which constituted a new clade SLC 2 which shows genetic differences to the known species of *Dickeya* (Laurila et al. 2008; Parkinson et al. 2009). Further work differentiated this new clade as a new species: *D. aquatica* (Parkinson et al. 2014). Research has shown that *D. aquatica* from water sources can cause rotting symptoms on potatoes in lab trials, but was not particularly virulent (Laurila et al. 2008, 2010). This study is the first to isolate *D. aquatica* from diseased plants in the field. The presence of this pathogen in Northern Ireland and its relation to soft rot symptoms is not well understood. Although samples were collected from symptomatic carrots, the irrigation water source (a local river in Northern Ireland) cannot be excluded as the source of contamination which could allow transmission to other hosts and constitute a potential threat in potato production regions. This pathogen may also be a recent introduction. This is an area where further research is needed.

Pectobacterium atrosepticum was the most frequently detected *Pectobacterium* species detected during surveys 2015–2017. This study builds on previous studies in Northern Ireland (Logan 1963, 1968; O'Neil and Logan 1975; Hossain and Logan 1983; Rhodes and Logan 1986, 1987; Wastie et al. 1988; Gans et al. 1991) and Ireland (Buttimer et al. 2018). The native range of *Pectobacterium atrosepticum* is unknown, however, the pathogen has potentially been causing blackleg in Ireland for over 100 years (Pethybridge and Murphy 1911; Bourke 1966). Similar to our findings, *P. atrosepticum* was also the most frequently detected species in temperate climates including Canada (de Boer et al. 2012), Norway (Dees et al. 2017a) and UK (Elphistone 2016a, b; Toth et al. 2016). In Scotland, most blackleg disease was caused by *P. atrosepticum* (Skelsey et al. 2016; Toth et al. 2016). In a seed potato survey between 2013 and 2015 in England, Wales and Scotland, *P. atrosepticum* constituted 89.4% of all positive samples (Elphistone 2016a). In the UK, 17 haplotypes of *P. atrosepticum* have been confirmed to be present, including testing of some strains from the 1930s (Elphistone 2016b). Within *P. atrosepticum* isolates from Northern Ireland, high genetic homogeneity occurred with high identity values (99.06–100% in pairwise comparison) to strain *P. atrosepticum* (KY067402) (Supplementary Fig. 2). High homogeneity of *P. atrosepticum* has also been found previously by Dees et al. (2017a).

Pectobacterium carotovorum subsp. *carotovorum* was recorded in Northern Ireland previously (Logan 1963, 1966, 1984; Hossain 1986). Logan (1963, 1966) reference to *Erwinia carotovora* and *Erwinia carotovora* pv. *'aroideae'* probably correspond to *Pectobacterium carotovorum* subsp. *carotovorum* and not *Pectobacterium aroidearum*, which is primarily a pathogen of monocotyledonous hosts (Nabhan et al. 2013) and most recently has been detected from potatoes (Moretti et al. 2016) and zucchini (Moraes et al. 2017). However, its presence has not been confirmed in phylogenetic analysis (data not shown). The *P. carotovorum* population in Northern Ireland was more heterogeneous than *P. atrosepticum* based on serological grouping (Hossain 1986), and our phylogenetic analysis agrees with this findings. Two of isolates from potatoes (SR22 and C2558) and three of the carrot strains (D0346, D0347 and D0348) grouped separately from others *P. c*. subsp. *carotovorum* and formed a separate clusters in the phylogenetic analysis (Fig.1). Further research including the analysis of more gene regions is needed to clarify the identities of these SRP. The use of more molecular markers, and a wider range of isolation methods (e.g. different temperatures), as suggested by Czajkowski et al. (2015), would likely reveal the presence of more diversity within and between the species of SRP detected in Northern Ireland, and build upon the research presented here.

In previous studies, *Pectobacterium carotovorum* strains originated from Ireland and Scotland were reclassified by Nabhan et al. (2013) and Waleron et al. (2013) as *P. wasabiae*. In this study, *P. wasabiae* was not detected. One isolate (from rotting carrot D0349) (Fig. 1) clustered together with the type strain of the proposed novel taxon *Candidatus* P. maceratum. Recently, this new genomospecies was described based on genomic distinction of five *P. c.* subsp. *carotovorum* strains and showed the most genetic similarity to *P. c.* subsp. *odoriferum* (Shirshikov et al. 2018). Work performed by Li et al. (2018) through whole genome sequences of 84 isolates of SRP shows high genetic similarity of *Candidatus* P. maceratum to *P. carotovorum* and *P. polaris*. Moreover, through phylogenetic analysis this species clustered together with other *P. c*. subsp. *carotovorum* suggesting than more isolates in GenBank might belong to this morphospecies (Li et al. 2018). Thus far, one study provides the details showing virulence and pathogenicity of *Candidatus* P. maceratum on potato and bittersweet (Waleron et al. 2019), therefore its

aggressiveness regarding other vegetables is not known. This study demonstrated that *Pectobacterium* spp. originating from Northern Ireland might be more diverse and further research should be performed to confirm occurrence of this new species.

The presence of three species of SRP in association with soft rot symptoms of vegetables as confirmed by PCR methods and phylogenetic analysis in Northern Ireland. This is the first published record of *Pectobacterium* and *Dickeya* spp. from Northern Ireland confirmed by DNA sequencing. This is the first study to document a confirmed isolation of *D. aquatica* from a symptomatic plant host in the field. Further research is needed to clarify the identity, distribution, and pathology of this taxa.

Acknowledgements The authors would like to thank the AFBI staff: B. Moreland, R. Swan, J. Pollock, J. Brazil, J. Morgan, J. Larkin, K. O'Neill, and F. McHardy for their assistance in the collection, preparation and analysis of the samples. The authors are grateful to the DAERA for provision of samples, field surveys and funding of this PhD project.

Funding The study was part of a PhD project funded by the DAERA in Northern Ireland PhD studentship.

Compliance with ethical standards The research does not involve human participants and/or animals.

Conflict of interest The authors declare that they have no conflict of interest.

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