Population Structure and Biodiversity of *Pectobacterium parmentieri* Isolated from Potato Fields in Temperate Climate

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Abstract

Pectobacterium parmentieri (formerly *Pectobacterium wasabiae*) is a newly established species of pectinolytic plant-pathogenic bacteria responsible for the symptoms of soft rot and blackleg on potato. In this work, we describe biodiversity and the population structure of *P. parmentieri* strains isolated during two consecutive growing seasons from the seed potato fields in Poland. About 450 samples of diseased potato tubers, potato plants, or accompanying weeds were collected throughout the country and tested for the presence of *P. parmentieri* by molecular identification methods. We found that *P. parmentieri* strains commonly occur in almost all regions of Poland. Furthermore, these isolates constituted significant fraction of pectinolytic bacteria from seed potato fields because 16% (2013) and 13% (2014) of the analyzed plant samples were infected with *P. parmentieri*. Subsequently, a detailed characterization of the obtained strains was conducted basing on repetitive sequences profiling, *recA*-gene-based phylogeny, and

phenotypic features. By applying repetitive extragenic palindromic sequence-based polymerase chain reaction (REP-PCR), we revealed the presence of five distinct genomic profiles among *P. parmentieri* strains, with profile I being the most abundant (approximately 44%). The performed *recA* gene-based phylogenetic analysis divided *P. parmentieri* isolates into two distinct clades, although the strains originating from different years did not group separately. Evaluation of the phenotypic traits playing crucial roles for the virulence of pectinolytic bacteria (namely, pectinase, cellulase and protease activities, and siderophore production, in addition to potato tissue maceration, swimming, and swarming motility) indicated some differences among the characterized strains. To the best of our knowledge, this is the first study that describes biodiversity and the population structure of *P. parmentieri* isolated in two growing seasons under temperate climate conditions and, hence, illustrates high heterogeneity within this species.

Pectobacterium parmentieri (formerly virulent *P. carotovorum* subsp. *carotovorum* or *P. wasabiae*) is a plant-pathogenic, soft-rotting, pectinolytic bacterium that used to be classified to the *Enterobacteriaceae* family (de Haan et al. 2008; Khayi et al. 2016; Nykyri et al. 2012; Waleron et al. 2013). Due to the recent comprehensive genome scale taxonomic analysis, this species has been transferred to the *Pectobacteriaceae* fam. nov. within the order *Enterobacterales* (Adeolu et al. 2016). Microorganisms currently belonging to *P. parmentieri* underwent several taxonomic revisions during the previous years. At first, they had been incorporated into the genus *Erwinia*, which included *Erwinia carotovora* subsp. *carotovora*, *E. carotovora* subsp. *atroseptica*, and *E. chrysanthemi* among other non-pectinolytic bacterial phytopathogens. Later, Hauben et al. (1998) established the genus *Pectobacterium* that included four species: *P. cactacidum*, *P. chrysanthemi*, *P. cyripedi*, and *P. carotovorum*, the latter species consisting of the following subspecies: *P. carotovorum* subsp. *atrosepticum*, *P. carotovorum* subsp. *betavascularum*, *P. carotovorum* subsp. *carotovorum*, *P. carotovorum* subsp. *odoriferum*, and *P. carotovorum* subsp. *wasabiae*. Subsequently, three subspecies of *P. carotovorum* were elevated into the species level; namely, *P. atrorepticum*, *P. odoriferum*, and *P. wasabiae* (Gardan et al. 2003; Nykyri et al. 2012).

Regarding *P. wasabiae*, it was isolated for the first time in the late 1980s from symptomatic Japanese horseradish (Goto and Matsumoto 1987) and was not considered to be a potato pathogen. Neither the occurrence of *P. wasabiae* on potato nor its presence on territory outside of Japan has been reported before 2009. In 2010, Pitman et al.

(2010) demonstrated that *P. wasabiae* was capable of causing disease symptoms on potato plants in New Zealand. Subsequently, *P. carotovorum* subsp. *carotovorum* SCC3193, a model strain widely used in molecular studies (Eriksson et al. 1998; Koskinen et al. 2012), was reclassified to *P. wasabiae* SCC3193 (Nykyri et al. 2012). This event triggered further renaming of *P. carotovorum* subsp. *carotovorum* strains deposited in international collections as *P. wasabiae* (De Boer et al. 2012; Nabhan et al. 2012; Sławiak et al. 2013; Waleron et al. 2013). Most recent comprehensive analysis based on DNA-DNA hybridization (DDH), genome average nucleotide identity (gANI), and average nucleotide identity (ANI) values calculated *in silico* on *P. wasabiae* genomes resulted in reclassification of all potato-originated *P. wasabiae* isolates into a newly established species, *P. parmentieri* (Khayi et al. 2016).

P. parmentieri strains are gram-negative rod-shaped necrotrophs that destroy plant tissue components through the activity of plant cell-wall-degrading enzymes (PCWDE) such as pectinases, cellulases, and proteases secreted via type I or II secretion systems (Chatterjee et al. 1995; Hugouvieux-Cotte-Pattat et al. 1996; Liu et al. 1999; Charkowski et al. 2012). Pectinases play a major role in the virulence of soft-rotting pathogens because they cleave pectins, which are the branched polysaccharides present in the primary cell walls of terrestrial plants. These enzymes are divided into pectate lyases and polygalacturonases, basing on differences in their activities: pectate and pectin lyases degrade highly esterified pectins into small molecules via β -elimination mechanism, while polygalacturonases hydrolyze the α -1,4 glycosidic bonds between galacturonic acid residues (Pickersgill et al. 1998). Other important pathogenicity factors include cellulases that hydrolyze 1,4- β -D-glycosidic linkages in cellulose and hemicellulose, yielding shorter polysaccharides, oligosaccharides, or monosaccharides (Py et al. 1991). Proteases also deserve to be mentioned, because they disrupt the host plant's protoplasts via degradation of transmembrane proteins (Marits et al. 1999). In addition, an effective spread of the invading pathogen through the plant vascular system, often referred as motility of the strain, is undoubtedly essential for the development of disease symptoms (Toth et al. 2003). Moreover, efficient production of the iron-scavenging molecules provides for adequate amount of essential cofactors involved in almost all life-supporting processes (Ishimaru and Loper 1992).

High economic impact of all *Pectobacterium* spp. was emphasized by listing the members of this genus among the top 10 bacterial plant

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GenBank accession numbers KY889066 to KY889139 correspond to the *recA* gene sequences reported here.

*The e-Xtra logo stands for “electronic extra” and indicates that seven supplementary figures are published online.

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pathogens (Mansfield et al. 2012). The above-mentioned attribution was based not only on severe damage noted in potato production sector but also on a broad host range of these microorganisms. In general, *Pectobacterium* spp. were reported to cause soft-rotting symptoms on 16 dicot plant families in 11 orders and 11 monocot families in 6 orders (Ma et al. 2007). Particularly, *P. parmentieri* was described as a pathogen of a similarly broad host range as *P. carotovorum* (Pitman et al. 2010), therefore affecting such divergent vegetables and crops as potato, carrot, cabbage, maize, sugar beet, calla lily, and star of Bethlehem (Yishay et al. 2008). In addition, there were multiple reports published on the occurrence of *P. parmentieri* in various climatic zones. The presence of this pathogen was confirmed in Germany, Finland, Poland, Norway, Switzerland, The Netherlands, New Zealand, Canada, the United States, and the Republic of South Africa (De Boer et al. 2012; Dung et al. 2012; Nabhan et al. 2012; Moleleki et al. 2013; Pasanen et al. 2013; Pitman et al. 2010; Rosenzweig et al. 2016; Sławiak et al. 2013; Suárez et al. 2017; Waleron et al. 2013). More detailed analyses suggested that *P. parmentieri* strains constitute significant fraction of soft-rotting pectobacteria isolated in Poland (approximately 20%) (Waleron et al. 2013) or Canada (16%) (De Boer et al. 2012) whereas, in New Zealand, South Africa, or Zimbabwe, they amounted to about 5%.

Despite the fact that the appearance of *P. parmentieri* was confirmed in numerous countries, no comprehensive study on the population structure and biodiversity of *P. parmentieri* strains has been conducted. Thus, the work presented here summarizes a detailed survey focusing on the monitoring and characterization of *P. parmentieri* strains isolated from seed potato plantations in Poland.

Materials and Methods

Bacterial strains, media, and culture conditions. *P. parmentieri* strains isolated from seed potato plantations on the territory of Poland in 2013 and 2014 are listed in Table 1. The reference strains used in this study are included in Table 2. All investigated bacteria were grown on crystal violet pectate (CVP) (Hyman et al. 2001) or Luria broth agar (LA) media at 28°C, unless otherwise stated. Concerning liquid cultures, bacteria were grown in lysogeny broth (LB) at 28°C with 120 rpm agitation (Incur-Shaker Mini; Benchmark Scientific).

Collection of the potato plants, potato tubers, and weeds. All plant samples were collected from potato seed plantations from May to September in the growing seasons of 2013 and 2014 by the inspectors of the Regional Inspectorates of Plant Health and Seed Inspection Service in Poland. Obtained plant material included 441 samples (201 in 2013 and 240 in 2014) of blackleg-affected potato plants (117 in 2013 and 133 in 2014), tubers exhibiting the symptoms of soft rot (64 in 2013 and 79 in 2014), and a few accompanying weeds (20 in 2013 and 28 in 2014). The collected samples were placed in sealed plastic bags and sent to the Laboratory of Plant Protection and Biotechnology of the Intercollegiate Faculty of Biotechnology, University of Gdansk and Medical University of Gdansk.

Isolation and identification of *P. parmentieri* strains. Approximately 1 g of plant tissue was homogenized in Bioreba extraction bag (Bioreba), while flooded with 10 ml of phosphate-buffered saline (pH 7.2). Aliquots of 100 µl from 10⁻⁵ and 10⁻⁶ serial dilutions of the homogenates were plated on CVP medium. Later, cavity-forming colonies were picked up and repeatedly grown first on CVP and then on LA medium in order to obtain axenic cultures. Afterward, cell lysates prepared by freezing and thawing of dense bacterial suspensions were subjected to multiplex polymerase chain reaction (PCR) (Potrykus et al. 2014b). All lysates that gave specific amplicons with the ExpcR and ExpcF primers (Kang et al. 2003) were used for subsequent single PCR with PhF and PhR primers, which are specific for the identification of *P. parmentieri* (De Boer et al. 2012). All strains designated as *P. parmentieri* were frozen in 40% glycerol and stored for further use at -80°C.

Genomic characterization of *P. parmentieri* strains. Bacterial genomic DNA was isolated by utilizing the Genomic Mini AX Bacteria Kit (A&A Biotechnology) and diluted to the final concentration of 10 ng µl⁻¹.

Repetitive sequence-based PCR analysis. Genomic profiles of *P. parmentieri* strains were obtained by implementing the repetitive

sequence-based (REP) PCR, as described by Versalovic et al. (1991). Preliminary studies were performed with the use of BOX, ERIC, and REP primers and, for further analysis, REP 1 R-I and REP 2-I primers were selected. The resulting amplicons were separated by electrophoresis in 1% agarose (Basica) gel in 0.5× Tris-borate-EDTA buffer at 50 V for 4 h, visualized, and documented with the Gel Doc imaging system (Bio Rad Laboratories Inc.) supported by the Quantity One Software (Bio Rad Laboratories Inc.). The obtained fingerprints were analyzed by Bionumerics 6.5 software (Applied Maths). All of the reference strains used for comparison purposes are listed in Table 2.

***recA* gene-based phylogenetic analysis.** Amplification of the 730-bp fragment of the *recA* gene was carried out by following the previously described protocol (Waleron et al. 2002) and involved 78 *P. parmentieri* strains collected during both years of study. The generated amplicons were sequenced from both ends by Genomed Ltd. and were edited manually afterward with the use of CLC Main Workbench 6.5 software (Qiagen). The 590-bp *recA* gene fragment was selected for further studies involving the construction of a *recA* gene-based phylogenetic tree (neighbor-joining algorithm with the Jukes-Cantor nucleotide distance measure) by implementing CLC MainWorkbench 6.5. Moreover, the *recA* gene sequences of *P. parmentieri* SCC3193, isolated in Finland; five strains isolated in Poland in 1996 (IFB5317, IFB5320, IFB5325, IFB5332, and IFB5553); and each one from the United States (WPP163), Canada (CFIA1002), Scotland (SCRI103), and France (CFBP8475^T), in addition to *P. wasabiae* (CFBP3304) from Japan, were downloaded from the GenBank database and included in the conducted analysis. The bootstrap value was set on the level of 100 replicates. *Dickeya solani* IFB0099 was used as an outgroup.

Phenotypic analysis of *P. parmentieri* strains. Overnight bacterial cultures in LB were centrifuged for 10 min at 6,500 rpm and washed twice in Ringer's solution to remove the residuals of the medium. The prepared bacterial suspensions were adjusted to an optical density at 600 nm of 0.1 (approximately 10⁸ CFU ml⁻¹) and used for inoculation of the plate assays listed below, with a volume of 2 µl. All of the tests were incubated at 28°C for 48 h, unless otherwise stated. The experiments were carried out in three biological repetitions, including four technical replicates each.

Pectinase activity was tested on M63Y PGA (polygalacturonate) plates (Reverchon et al. 1986). After 48 h of incubation, this medium was stained with an aqueous solution of 10% copper acetate. Degradation of PGA resulted in the appearance of whitish halo zones around bacterial colonies, which were measured in order to assess the total pectinase activity.

Cellulase activity was analyzed on M63 medium supplemented with glycerol at 2 g liter⁻¹ and carboxymethylcellulose at 10 g liter⁻¹ (Wood 1980). After 48 h of incubation, the plates were flooded with Congo Red solution at 10 mg ml⁻¹ for 10 min and subsequently washed with 1 M NaCl for 5 min. The halo diameter appearing around the colonies indicated the activity of cellulase and was subsequently measured.

Protease activity was evaluated on medium containing skim milk at 12.5 g liter⁻¹ (Ji et al. 1987). Diameters of the clear halos around the colonies reflecting the activity of proteases were measured after 24 h of incubation.

Siderophore production capacity was determined on chrome azurol S-agar plates (Schwyn and Neilands 1987). Diameters of the orange halo zones around the colonies corresponding to siderophore production rate were measured after 72 h of incubation.

Swarming motility was assessed by inoculating nutrient agar (NA) plates solidified with 0.6% agar (Harshey 2003). Diameters of the colonies were measured after 24 h of incubation.

Swimming motility was assessed by inoculating NA plates solidified with 0.3% agar (Harshey 2003). Diameters of the colonies were measured after 24 h of incubation.

Potato slice maceration assay was performed on 'Lord' potato tubers (average size = 60 to 70 mm). Tubers were washed under running water and sterilized with 10% commercially available bleach for 20 min. Later, the tubers were thoroughly washed in water and dried in the laminar flow cabinet. Subsequently, all of the tubers were aseptically cut crosswise into 10-mm-thick slices, in which two holes (5 by 5 mm) per slice were drilled. The potato slices were placed in plastic boxes

(200 by 320 by 75 mm) covered with a layer of moistened Whatman filter paper. Afterward, each hole in every potato slice was inoculated with 50 µl of bacterial suspension (10^8 CFU ml⁻¹, prepared as described above). Incubation lasted 48 h at 28°C. After this time, the rotten tissue diameters were measured. As a negative control, 0.85% NaCl was used. The experiment was repeated twice, involving eight technical replicates.

Statistical analysis. Statistical significance of differences observed in phenotypic assays (i.e. enzymatic activities, siderophore production, motility, and ability to macerate potato tissue) was analyzed by R, version 3.3.2 (2016-10-31) (R Core Team 2014). Levene's test was applied for testing the equality of variances and the Shapiro-Wilk's test was implemented for evaluating normality of the data. The differences among group means were tested by the analysis of variance, followed by Tukey's honestly significance test. *P* value < 0.05 was used.

Statistical analysis on *recA* gene sequences was conducted in order to quantitatively evaluate the genetic biodiversity between strains

isolated from the same plant in each year according to the previously described protocol (Mengoni and Bazzicalupo 2002). To measure variance among the selected isolates, 650-bp fragments of *P. parmentieri recA* gene sequences were used. For this purpose, analysis of molecular variance (AMOVA) by Arlequin 3.5 (Excoffier et al. 2005) was applied.

Results

***P. parmentieri* strains commonly occur on potato seed plantations in Poland.** *Pectobacterium* spp. were isolated from 230 plant samples (101 and 129 in years 2013 and 2014, respectively). *P. parmentieri* strains were detected in approximately 16% of all tested plant samples in 2013 whereas, in 2014, they amounted to 13%. The total number of isolated strains was similar between the years of study, equaling 47 in 2013 and 38 in 2014. The *P. parmentieri* isolates originated from blackleg-affected potato stems (24 in 2013 and 29 in 2014), potato tubers with the symptoms of soft rot (17 in 2013

Table 1. *Pectobacterium parmentieri* strains isolated from potato fields in Poland

Number	Strain ^b	Host plant	Year ^c	REP ^d	SNP in <i>recA</i> gene sequence from ATG codon ^a		
					SNP1, 138 bp, GA	SNP2, 540 bp, TA	SNP3, 642 bp, CT
1	IFB5400	Potato stem	2013	II	...	...	...
2	IFB5491	Potato tuber	2013	I	...	...	...
3	IFB5493	Potato tuber	2013	II	+	+	+
4	IFB5404	Potato stem	2013	III	NE	NE	NE
5	IFB5497 ^e	Potato stem	2013	II	NE	NE	NE
6	IFB5498 ^e	Potato stem	2013	II	...	...	...
7	IFB5405	Potato stem	2013	I	...	...	...
8	IFB5406	Potato tuber	2013	I	...	...	...
9	IFB5407	Potato stem	2013	I	...	...	...
10	IFB5408	Potato stem	2013	I	...	+	...
11	IFB5409	Potato stem	2013	III	+	+	...
12	IFB5410	Potato stem	2013	I	...	+	...
13	IFB5411	Potato stem	2013	I	...	+	...
14	IFB5412	Potato stem	2013	I	...	...	...
15	IFB5413	Potato stem	2013	I	...	...	...
16	IFB5414	Weed	2013	IV	...	+	...
17	IFB5415	Potato stem	2013	I	...	...	...
18	IFB5416	Potato stem	2013	I	...	+	+
19	IFB5417	Soil	2013	II	+	+	+
20	IFB5418	Potato stem	2013	III	...	+	...
21	IFB5419	Weed	2013	II	+	+	+
22	IFB5420	Potato stem	2013	II	...	...	...
23	IFB5421	Potato tuber	2013	IV	...	...	...
24	IFB5422	Potato tuber	2013	IV	...	...	...
25	IFB5423	Potato stem	2013	III	...	...	...
26	IFB5424	Potato stem	2013	I	...	...	...
27	IFB5425	Potato stem	2013	III	...	...	...
28	IFB5426	Potato stem	2013	IV	...	...	...
29	IFB5427	Weed	2013	I	...	+	...
30	IFB5428	Weed	2013	IV	...	...	...
31	IFB5429	Weed	2013	I	...	+	...
32	IFB5430	Potato tuber	2013	II	...	...	...
33	IFB5431	Potato tuber	2013	II	...	...	...
34	IFB5432	Potato tuber	2013	III	+	+	+
35	IFB5433	Potato tuber	2013	III	+	+	+
36	IFB5434	Potato tuber	2013	III	+	+	+
37	IFB5435	Potato tuber	2013	I	...	+	...
38	IFB5436	Potato tuber	2013	I	...	+	...
39	IFB5437	Potato tuber	2013	I	...	+	...
40	IFB5441	Potato tuber	2013	II	+	+	+

(continued on next page)

^a The *recA* sequence of *P. parmentieri* SCC3193 was used as a reference; + indicates the presence of single-nucleotide polymorphism (SNP) at a given position, NE = not established due to lack of amplification, and NT = not tested.

^b IFB = the collection of bacterial plant pathogens at the Intercollegiate Faculty of Biotechnology University of Gdansk and Medical University of Gdansk, Gdansk, Poland.

^c Year of isolation.

^d Repetitive sequence-based (REP) profile.

^e Strains were described previously by Dees et al. (2017).

and 4 in 2014), asymptomatic weeds (5 in 2013 and 5 in 2014), and soil (1 in 2013). In this work, out of 85 *P. parmentieri* isolates, we focused on 78 (40 from 2013 and 38 from 2014; Table 1). In 16 cases (3 in 2013 and 13 in 2014), more than one *P. parmentieri* strain of diverse genotypic feature was isolated from a certain plant sample (Table 3).

Genomic characterization of *P. parmentieri* strains. By comparison of various REP-PCR fingerprinting methods incorporating either BOX, REP, or ERIC primers, we defined REP profiling as the most useful tool for further grouping of *P. parmentieri* strains. The obtained REP genomic profiles included 8 to 10 bands of 750 to 3,000-bp in size. Five distinct profiles (numbers I to V) were distinguished among the tested *P. parmentieri* strains by utilizing Bionumerics 6.5 (Applied Maths) (Fig. 1). REP profile I was the most abundant, because it was observed among approximately 44% of the strains isolated in both 2013 and 2014 (Fig. 2). This pattern was also characteristic for the reference strain *P. parmentieri* SCC3193 isolated in

Finland. The incidence of profiles II and III was similar among the strains originating from the consecutive years. In contrast, REP profile IV was observed only among strains isolated in 2013, while REP profile V exclusively among the ones from 2014 (Fig. 2). As shown for *P. wasabiae* CFBP3304, all of the tested *P. wasabiae* strains isolated from Japanese horseradish exhibited a distinct REP profile. Interestingly, *P. parmentieri* strains isolated from the same potato plant indicated different REP profiles in several cases (Table 3).

All of the isolated *P. parmentieri* strains grouped into two separate clades on the basis of phylogenetic analysis performed on a 590-bp *recA* gene fragment (Fig. 3). Regardless of the year of isolation, *P. parmentieri* from 2013 and 2014 could be found in both clades, although the majority of the strains originating from 2014 constituted clade 1 (30 of 38), whereas the ones from 2013 separated almost equally into clades 1 and 2. Remarkably, *P. parmentieri* CFBP 8475^T type strain isolated in France was present in clade 2. In addition, the reference strain *P. parmentieri* SCC3193 grouped within clade 1, together

Table 1. (continued from previous page)

Number	Strain ^b	Host plant	Year ^c	REP ^d	SNP in <i>recA</i> gene sequence from ATG codon ^a		
					SNP1, 138 bp, GA	SNP2, 540 bp, TA	SNP3, 642 bp, CT
41	IFB5442	Potato tuber	2013	I	...	+	...
42	IFB5443	Potato tuber	2013	I	+	+	+
43	IFB5500 ^e	Potato stem	2013	III	NT	NT	NT
44	IFB5501 ^e	Potato stem	2013	I	NT	NT	NT
45	IFB5507 ^e	Potato stem	2013	II	NT	NT	NT
46	IFB5511 ^e	Potato stem	2013	II	NT	NT	NT
47	IFB5513 ^e	Potato tuber	2013	I	NT	NT	NT
48	IFB5597	Potato stem	2014	I	...	...	...
49	IFB5598	Potato stem	2014	I	...	...	...
50	IFB5599	Weed	2014	I	...	...	...
51	IFB5600	Potato stem	2014	I	...	...	...
52	IFB5601	Potato stem	2014	II	...	...	...
53	IFB5602	Weed	2014	I	...	...	...
54	IFB5603	Potato stem	2014	II	...	...	...
55	IFB5604	Potato stem	2014	V	...	...	...
56	IFB5605	Potato stem	2014	I	...	+	...
57	IFB5606	Potato stem	2014	II	...	...	...
58	IFB5607	Potato stem	2014	I	...	...	...
59	IFB5608	Potato stem	2014	I	...	...	...
60	IFB5609	Potato stem	2014	I	...	...	...
61	IFB5610	Potato stem	2014	I	...	...	...
62	IFB5611	Potato stem	2014	V	NE	NE	NE
63	IFB5612	Potato stem	2014	II	...	...	...
64	IFB5613	Potato stem	2014	I	...	...	...
65	IFB5614	Potato stem	2014	I	...	...	...
66	IFB5616	Potato stem	2014	V	...	...	...
67	IFB5617	Potato stem	2014	II	...	...	...
68	IFB5618	Potato stem	2014	I	...	...	...
69	IFB5619	Potato stem	2014	III	...	...	...
70	IFB5620	Potato stem	2014	V	...	...	...
71	IFB5621	Potato tuber	2014	III	...	...	...
72	IFB5622	Potato tuber	2014	I	...	+	...
73	IFB5623	Potato stem	2014	II	+	+	+
74	IFB5624	Potato stem	2014	III	...	...	...
75	IFB5625	Potato tuber	2014	II	...	...	...
76	IFB5626	Potato tuber	2014	I	...	+	...
77	IFB5627	Weed	2014	V	...	+	...
78	IFB5628	Weed	2014	III	...	...	...
79	IFB5629	Potato stem	2014	III	...	...	...
80	IFB5630	Weed	2014	II	...	...	...
81	IFB5631	Potato stem	2014	III	...	...	...
82	IFB5632	Potato stem	2014	III	...	...	...
83	IFB5633	Potato stem	2014	III	...	...	...
84	IFB5634	Potato stem	2014	I	...	+	...
85	IFB5635	Potato stem	2014	I	...	+	...

with *P. parmentieri* from the United States, Canada, and Scotland, and several strains isolated in Poland in 1996 (Fig. 3).

Regarding single-nucleotide polymorphisms (SNP), we were able to distinguish three SNP regions in the analyzed 590-bp fragment of the *recA* gene: namely, G-A transversion at the 138-bp position, counting from the beginning of ATG codon (observed in 12.8% of the tested strains); T-A transition at 540-bp (35.9% cases); and C-T transversion at 642-bp (characteristic for 11.54% of *P. parmentieri* isolates). The above mentioned 540-bp T-A transition was more common among strains isolated in 2013 (52.5% of the analyzed strains) than those originating from 2014 (18.4% events). Moreover, G-A transversion at 138-bp and C-T transversion at 642-bp were described more frequently among the strains from the 2013 population (22.50%) than from 2014 (2.63%) (Table 1).

In 8 of 17 cases, we obtained bacterial isolates varying in any genotypic feature in other words, different REP profile or SNP in *recA* gene sequence (Table 3) from the same plant material therefore, biodiversity among these strains was determined by measuring molecular diversity indexes. Within the 650-bp *recA* gene fragment analyzed, we determined three transitions and two transversions among strains isolated from the same plant in year 2013, whereas four transitions and five transversions were noted for the strains from 2014 sharing the same plant origin. This observation pointed to higher genomic variability among the latter isolates.

Phenotypic characterization of *P. parmentieri* strains. Crucial virulence determinants of soft-rotting pectinolytic bacteria, including pectinase, cellulase, and protease activities; siderophore production; and ability to macerate potato tissue, swim, and swarm, were evaluated for all of the investigated *P. parmentieri* strains (Supplementary Figs. S1, S2, S3, S4, S5, S6, and S7). Mean values of each phenotypic trait determined for the *P. parmentieri* populations from 2013 and 2014 were calculated and compared with the results collected for *P. parmentieri* SCC3193, *P. wasabiae* CFBP3304, *P. carotovorum* subsp. *carotovorum* IPO200, *P. atrosepticum* LMG2386, and *D. solani* IFB0099 (Fig. 4).

Interestingly, we observed higher variability in the pectinase and cellulase activities among distinct *P. parmentieri* strains from 2014 than the ones from 2013. Notably, 13 of 38 strains isolated in 2014 did not exhibit any cellulolytic activity. In the case of three *P. parmentieri* isolates (IFB5611, IFB5632, and IFB5635), lack of cellulolytic and low pectinolytic activities highly correlated with the

impaired ability to macerate potato tissue. Ten other strains that indicated lack of cellulolytic activity did not show lower virulence than the rest of the *P. parmentieri* isolates.

The average pectinase and cellulase activities between the 2013 and 2014 *P. parmentieri* populations were not significantly different (Fig. 4). It is worth noticing that *P. parmentieri* SCC3193 showed significantly lower pectinase activity than the strains isolated in Poland. However, in terms of cellulases activity, the observed differences were statistically insignificant. Focusing on *D. solani* IFB0099, this strain exhibited higher activities of both pectinases and cellulases than the *Pectobacterium* spp. strains (Fig. 4A and B) and also the highest virulence (Fig. 5).

Individual *P. parmentieri* strains varied in protease activity, with no dependence on the year of isolation. Furthermore, the mean protease activity of the population from 2013 was significantly higher than the one calculated for isolates from 2014 (Fig. 4C). Interestingly, *P. parmentieri* SCC3193 showed no protease activity. In general, all of the analyzed *P. parmentieri* strains showed significantly lower mean protease activities than *P. wasabiae* CFBP3304, *P. carotovorum* subsp. *carotovorum* IPO200, and *D. solani* IFB0099 (Fig. 4C). In only a few cases (*P. atrosepticum* LMG2386 and *P. parmentieri* IFB5629 and IFB5611), lack of the protease activity could have been correlated with the decreased maceration capacity on potato.

Regarding the capacity of *P. parmentieri* to efficiently chelate iron ions, we observed significant differences in siderophore production rate between the individual strains from 2013 and 2014. In general, strains from 2013 showed significantly lower siderophore production than the strains isolated in 2014. Corresponding to what was observed for cellulase and pectinase activities, highly virulent *D. solani* IFB0099 showed the highest production of siderophores, whereas the reference strains *P. parmentieri*, *P. wasabiae*, and *P. atrosepticum* revealed no siderophore production (Fig. 4D). In the case of only two reference strains (*P. atrosepticum* LMG2386 and *P. wasabiae* CFBP3304), a lower siderophore production rate could not have been correlated with the decreased potato maceration capacity.

Concerning swimming and swarming abilities, individual *P. parmentieri* isolates from 2014 were more motile than those isolated in 2013. In addition, isolates from 2014 exhibited higher variation in swimming motility than the strains originating from the preceding year. Additionally, significantly higher motility was noted for the entire 2014 population than for the one from 2013 (Fig. 4E and F).

Table 2. References of pectinolytic bacteria used in this study

Species, number	Strain ^a	Host, location	Year ^b	REP ^c	GenBank <i>recA</i> accession	Reference
<i>Pectobacterium parmentieri</i>						
1	CFBP8475 ^T RNS08.42.1A	Potato	2008	NT	NZ_CP015749.1	Khayl et al. 2016
2	CFIA1002	Potato stem, Canada	2007	NT	NZ_JENG01000009.1	De Boer et al. 2012
3	WPP163	Potato, United States	2008	NT	CP001790.1	Ma et al. 2007
4	SCRI103	Potato tuber, Scotland	1977	NT	AY264795.1	Waleron et al. 2002
5	SCC3193	Potato, Finland	1980s	I	NC_017845	Nykyri et al. 2012
6	IFB5317	Potato stem, Poland	1996	NT	KC584996.1	Waleron et al. 2013
7	IFB5320	Potato stem, Poland	1996	NT	KC584997.1	Waleron et al. 2013
8	IFB5325	Potato stem, Poland	1996	NT	KC584998.1	Waleron et al. 2013
9	IFB5332	Potato stem, Poland	1996	NT	KC585000.1	Waleron et al. 2013
10	IFB5553	Potato stem, Poland	1996	NT	AY217080.1	Waleron et al. 2013
<i>P. wasabiae</i>						
11	CFBP3304	Horseradish Japan	1985	VI	NZ_CP015750	Goto and Matsumoto 1987
<i>P. carotovorum</i> subsp. <i>carotovorum</i>						
12	IPO200	Potato, The Netherlands	1974	VII	NT	PRI
<i>P. atrosepticum</i>						
13	LMG2386	Potato stem	1957	NT	NT	BCCM
<i>Dickeya solani</i>						
14	IFB0099	Potato stem, Poland	2005	NT	NT	Slawiak et al. 2009

^a IFB = Intercollegiate Faculty of Biotechnology, University of Gdansk and Medical University of Gdansk, Gdansk, Poland; SCRI = Scottish Crop Research Institute, Scotland; CFBP = Plant-Associated Bacteria Collection, France; IPO = Plant Research International (PRI), Wageningen, The Netherlands; and LMG = Belgian Coordinated Collections of Microorganisms (BCCM), Belgium.

^b Year of isolation.

^c Numbers indicate the attributed repetitive sequence-based (REP) profile; NT = not tested.

Although the highest swarming ability of all of the investigated strains was attributed to *P. parmentieri* SCC3193, it had lower swimming motility than the isolates obtained in from 2014 (Fig. 4E and F). However, the noted higher swimming or swarming motility of the strains could not have been correlated with increased virulence on potato, at least in this experimental setup.

Potato maceration abilities, which can be linked with the virulence of soft-rotting phytopathogens, indicated that distinct *P. parmentieri* isolates from 2013 degraded potato tissue to the similar extent, whereas the strains from 2014 significantly differed from one another in this capacity. Although the strains from 2013 exhibited higher maceration ability from the isolates originating from 2014, the observed variation was not statistically significant. In spite of showing higher maceration than the *P. parmentieri* isolates from 2014, *P. parmentieri* SCC3193 showed lower potato maceration capacity than the strains from 2013. Notably, *D. solani* IFB0099 revealed the highest potato maceration ability under the implemented conditions (Fig. 5).

Discussion

In this study, a 2-year survey on the occurrence of *P. parmentieri* on seed potato plantations in Poland is presented. We have demonstrated the ubiquitous presence of this pathogen in a temperate climate on the territory located mostly within the North European Plain. This statement is supported by the fact that the *P. parmentieri* strains were detected in 16% (2013) and 13% (2014) of all analyzed plant samples. Even though the appearance of bacteria currently classified to this taxon has been broadly documented in European countries since the 1990s (Dees et al. 2017; Nabhan et al. 2012; Pasanen et al. 2013; Waleron et al. 2013), there was no study focusing on genotypic and phenotypic biodiversity of the country-scale populations of *P. parmentieri* originating from the consecutive growing seasons. Thus far, scientific interest was attributed mainly to molecular or biochemical identification methods (De Boer et al. 2012; Waleron et al. 2002), reclassification of isolates deposited in international collections (Nykyri et al. 2012; Waleron et al. 2013), their phylogenetic relatedness (Pasanen et al. 2013; Pitman et al. 2010; Waleron et al. 2013), and their ability to cause disease symptoms (Pasanen et al. 2013). The research presented here provides extensive description of genomic features and virulence determinants defined for 78 *P. parmentieri* isolates of divergent plant and geographical origin and, as a consequence, contributes significantly to broadening knowledge about this newly established plant pathogen.

Concerning genotypic characterization of *P. parmentieri* isolates, five various REP genomic profiles were assigned to the tested isolates. The implemented REP fingerprinting method was proven to be distinctive enough to reveal subtle differences among individual *P. parmentieri* strains from the territory of Poland. Interestingly, REP profile I characterized the highest fraction of *P. parmentieri* strains analyzed in both years. Because the same REP pattern I was also attributed to *P. parmentieri* SCC3193, a common origin of the strains differing in isolation time (approximately 30 years) and from either Finland or Poland might be suspected. In addition, implementation of REP-PCR allowed us to establish a specific profile for *P. wasabiae*, different from the profiles assigned to *P. parmentieri*.

Among other genotyping methods applied for characterization of *Pectobacterium* spp. thus far, PCR restriction fragment length polymorphism (RFLP) (Moleleki et al. 2013; Waleron et al. 2002), random amplified polymorphic DNA PCR (Maki-Valkama and Karjalainen 1994), multilocus sequence analysis (MLSA), and fluorescent amplified fragment length polymorphism (fAFLP) (Pitman et al. 2010) should be listed. For instance, Waleron et al. (2002, 2013) implemented PCR-RFLP on a *recA* 730-bp gene fragment to describe the genotypic relatedness between 19 different species of the former genus *Erwinia*. In the growing season 1996–97, 600 strains of *P. carotovorum* (43% of all pectinolytic isolates) were obtained from soft rot- or blackleg-affected potato plants from the territory of Poland. Taking into account that 14 of 50 *P. carotovorum* strains were assigned with *recA* PCR-RFLP profile 3, typical for the current *P. parmentieri* strains, it can be concluded that this plant

pathogen was frequently isolated from potato fields in Poland even 20 years ago (Waleron et al. 2002, 2013). However, the *recA* PCR-RFLP showed only limited discriminant abilities for investigation of within-species variation because one single profile was reported for bacteria now belonging to *P. parmentieri*. A similar approach, undertaken by Moleleki et al. (2013), amplified the 16S-23S intergenic transcribed spacer (ITS) region of atypical *Pectobacterium* spp. originating from South Africa and subsequently cut the resulting DNA fragments with two restriction enzymes. The applied procedure enabled differentiation of *P. parmentieri* isolates from other *Pectobacterium* spp., with the exception of *P. wasabiae*, although it did not seem distinctive enough for evaluation of within-species variation. In addition, focusing on only one gene might not reveal diversification that could have been disclosed from a genome-wide repetitive sequences analysis. Also, utilization by Moleleki et al. (2013) and Waleron et al. (2013) of either two or four separate restriction enzyme digestions increases the required time and cost of the whole analysis.

To the best of our knowledge, the study presented here is the first to describe, in detail, the phylogenetic relatedness between numerous individual *P. parmentieri* strains. We observed two distinct clades within this species on the basis of a 590-bp fragment of the highly conserved housekeeping *recA* gene. In the reported grouping, the majority of the *P. parmentieri* isolates ($n = 21$) from 2013 constituted clade 2, together with only few ($n = 7$) strains from 2014 and the *P. parmentieri* type strain CFBP 8475^T. The other clade (clade 1)

Table 3. Characteristics of the *Pectobacterium parmentieri* originating from the same plant

Year, plant sample	Strain	Host plant	REP ^a	SNP in <i>recA</i> sequence ^b
2013				
1	IFB5427	Weed	I	No difference
	IFB5429	Weed	I	No difference
	IFB5428	Weed	V	No difference
2	IFB5430	Potato tuber	II	No difference
	IFB5431	Potato tuber	II	No difference
3	IFB5432	Potato tuber	III	138 bp, G-A
	IFB5433	Potato tuber	III	642 bp, C-T
	IFB5434	Potato tuber	III	642 bp, C-T
2014				
1	IFB5599	Weed	I	No difference
	IFB5602	Weed	I	No difference
2	IFB5601	Potato stem	II	No difference
	IFB5603	Potato stem	II	No difference
3	IFB5604	Potato stem	V	540 bp, T-A
	IFB5605	Potato stem	I	540 bp, T-A
4	IFB5606	Potato stem	II	540 bp, T-A
	IFB5607	Potato stem	I	540 bp, T-A
5	IFB5608	Potato stem	I	540 bp, T-A
	IFB5609	Potato stem	I	540 bp, T-A
6	IFB5612	Potato stem	II	No difference
	IFB5614	Potato stem	I	No difference
7	IFB5616	Potato stem	V	No difference
	IFB5617	Potato stem	II	No difference
8	IFB5625	Potato tuber	II	No difference
	IFB5621	Potato tuber	III	No difference
9	IFB5622	Potato tuber	I	No difference
	IFB5626	Potato tuber	I	No difference
10	IFB5624	Potato stem	III	No difference
	IFB5629	Potato stem	III	No difference
11	IFB5628	Weed	III	No difference
	IFB5630	Weed	II	No difference
12	IFB5631	Potato stem	III	No difference
	IFB5633	Potato stem	III	No difference
13	IFB5634	Potato stem	I	No difference
	IFB5635	Potato stem	I	No difference

^a Repetitive sequence-based (REP) profile.

^b SNP = single-nucleotide polymorphisms.

was formed by over a dozen strains obtained in 2013 ($n = 16$), most of the *P. parmentieri* isolates from 2014 ($n = 30$), 5 strains isolated in Poland in 1996, in addition to several reference strains from international collections that had been obtained from such various countries as Finland (SCC3193), Canada (CFIA1002), Scotland (SCRI103), or the United States (WPP163).

Our results support the diversification described by Khayi et al. (2016), owing to the fact that both the genome-to-genome distance and DDH estimations between the *P. parmentieri* CFBP8475^T and other *P. parmentieri* isolates (SCC3193, CFIA1002, and WPP163) pointed to similar genomic distance between the strains separated into two *recA*-based clades. Additionally, the *in silico* calculated gANI and the distance tree of concatenated sequences of *rpoD*, *gyrB*, *recA*, *rpoS*, *fusA*, *dnaX*, *gyrA*, *purA*, *dnaN*, *gapA*, and *rplB* indicated the highest genomic distance between the *P. parmentieri* CFBP8475^T and the other investigated *P. parmentieri* strains (namely, SCC3193 and WPP163). Apart from the work of Khayi et al. (2016), *P. parmentieri* had not been differentiated from *P. wasabiae* strains in any of the previously conducted studies. Nevertheless, the observations made by De Boer et al. (2012) and Waleron et al. (2013) clearly support the division, proposed here, of *P. parmentieri* strains into two discrete clades. In detail, *P. parmentieri* strains isolated in Poland in 1996 clustered together with the reference strains SCC3193, SCRI103, and

WPP163 outside subcluster IA of the maximum-likelihood-based phylogenetic tree constructed on 730-bp *recA* gene fragment (Waleron et al. 2013). Moreover, the heavily branched neighbor-joining cladogram on the *proA* gene sequence and the concatenated maximum-likelihood tree on both *mdh* and *gapA* partial gene sequences constructed by De Boer et al. (2012) and Moleleki et al. (2013), respectively, imply even more complex phylogenetic relationships within this taxonomic group and account for possible further rearrangements within *P. parmentieri* species.

In the present study, we decided to focus on individual SNP as one of the measures for genotypic variation within the *recA* gene fragment, studied in detail, of *P. parmentieri*. We described three SNP at positions of 138, 540, and 642-bp observed in the 22.5, 52.5, and 22.5% fractions, respectively, of the *P. parmentieri* population from 2013. Regarding the *P. parmentieri* population from 2014, the above-listed SNP were noted for 2.6, 18.4, and 2.6% of the investigated isolates. Both the unequal distribution of the isolates from 2013 and 2014 within the distinct clades of the constructed phylogenetic tree and the above-shown observation on polymorphic sites point to assumption that the *P. parmentieri* population in 2013 was genetically more heterogeneous than the one from 2014.

As is commonly known, biodiversity is the biological insurance against loss or poor performance of a species and provides for specific

Fig. 1. Repetitive sequence-based (REP) profile analysis of *Pectobacterium* spp. strains. Lanes 1 to 4, REP profile I (IFB5405, IFB5408, IFB5597, and IFB5598); lanes 5 to 8, REP profile II (IFB5400, IFB5417, IFB5601, and IFB5617); lanes 9 to 12, REP profile III (IFB5404, IFB5418, IFB5619, and IFB5621); lanes 13 and 14, REP profile IV (IFB5414 and IFB5421); lanes 15 and 16, REP profile V (IFB5604 and IFB5616); lane 17, *Pectobacterium parmentieri* SCC3193; lane 18, *P. wasabiae* CFBP3304; lane 19, *P. carotovorum* subsp. *carotovorum* IPO200; lane 20, negative control (NC). MM = GeneRuler 1-kb DNA Ladder (Thermo Scientific).

Fig. 2. Comparison of the distribution of specific repetitive sequence-based (REP) profiles among *Pectobacterium parmentieri* populations from 2013 and 2014.

advantages under any environmental fluctuations (e.g. temperature) (Naeem and Li 1997). Wang et al. (2016) have tested the impact of coldness and warmth on species richness in a large-scale field experiment and confirmed that changes in temperature affect variation among bacterial communities. Taking into account these statements, we analyzed the agrometeorological data collected in Poland in 2013 and 2014 (Central Statistical Office of Poland 2016). In winter, significantly lower air temperature (P approximately 4.2×10^{-13}) occurred in 2012–13 than in 2013–14, with the means equaling $-1.8 \pm 1.3^\circ\text{C}$ and $+2.3 \pm 1.1^\circ\text{C}$, respectively. Also in 2012–13, higher precipitation of 39.8 ± 5.5 mm was noted (P approximately 2.5×10^{-8}) in comparison with 27.1 ± 5.4 mm recorded in 2013–14. The above-presented data might be one of the reasons for higher variability observed among the strains collected in vegetation season of 2013. Average temperatures collected for the summer seasons in 2013 and 2014 were also juxtaposed; however, no statistically significant differences were noted ($P < 0.05$).

In order to evaluate biodiversity among *P. parmentieri* isolates from the same plant samples, we used AMOVA analysis. It revealed that strains originating from 2014 exhibited more transitions and transversions in the *recA* gene than the strains isolated in 2013. In general, transversions occurred more frequently than transitions. Also, Yap et al. (2004) reported unexpected variability among *P. carotovorum* (including *P. parmentieri* at that time) isolates from single host obtained from one region and season. Factors other than the potato host plant have been suggested as the driving force of evolution within this species.

Because the investigated *P. parmentieri* strains exhibit high genotypic variation, the phenotypic divergence reported here is not unexpected. A more intriguing trend was previously observed for *D. solani* strains, among which only one REP, BOX, and ERIC profile and 100% homology of housekeeping genes sequences were described thus far (Golanowska et al. 2016). Despite their homogeneity on the genotypic level, these isolates significantly vary in their abilities to produce PCWDE or siderophores or in motility (Golanowska et al. 2016; Potrykus et al. 2014a, 2016). In terms of pectinase production, all of the analyzed *P. parmentieri* isolates exhibited relatively high activity of these enzymes, except for two strains from 2014 (IFB5635 and IFB5611). Waleron et al. (2013) reported that the *P. wasabiae* isolated from horseradish showed significantly lower pectinase activities than the *P. parmentieri* strains obtained from potato, which is consistent with the presented results. Additionally, regarding all PCWDE tested, the *P. parmentieri* isolates from 2014 exhibited greater diversification in the enzyme production rates than the strains obtained in 2013. Importantly, there were few *P. parmentieri* strains (mostly from 2014) that did not demonstrate any proteolytic or cellulolytic capacities. Previously, Dees et al. (2017), while comparing the annual populations of pectinolytic bacteria in Poland and in Norway, demonstrated that a *P. parmentieri* strain (IFB5511) was incapable of cellulose degradation. Although the capacity to produce siderophores is frequently encountered among plant-pathogenic bacteria (Leong and Neilands 1982) and was exhibited by all strains of *P. parmentieri* isolated in Poland, there were three reference strains *P. parmentieri* SCC3193, *P. wasabiae* CFBP3304, and *P. atropeticum* LMG2386 deprived of this trait. Regarding motility, *P. parmentieri* isolates from 2014 were significantly more motile in the case of either swimming or swarming than the isolates from 2013 or the investigated reference strains (except for *P. parmentieri* SCC3193).

Most of all, every tested *P. parmentieri* isolate was capable of macerating potato tissue. High maceration capacity of the current *P. parmentieri* strains was reported earlier by Waleron et al. (2013). It is worth noting that *P. wasabiae* CFBP3304 exhibited the lowest virulence. These outcomes support the fact that *P. parmentieri* used to be named highly virulent *P. carotovorum* subsp. *carotovorum* (de Haan et al. 2008). However, Dees et al. (2017) observed lower maceration ability of the tested *P. parmentieri* strains in comparison with other species of pectinolytic bacteria. Generated disagreement could be attributed to varying experimental setups: inoculation of potato slices versus whole potato tubers and aerobic versus anaerobic

conditions. The anaerobic conditions established by Dees et al. (2017) might be more favorable for other pectinolytic species (*P. carotovorum* subsp. *carotovorum* or *D. solani*) than for *P. parmentieri*; however, further research in this field is required.

Fig. 3. Phylogenetic analysis based on *recA* gene sequences of *Pectobacterium parmentieri* strains isolated in Poland. Reference strains included in this study but also originating from other European countries are as follows: *P. parmentieri* isolated in the United States (WPP163), Canada (CFIA1002), Scotland (SCR1103), and France (RNS08.42.1A); *P. wasabiae* CFBP3304 (Japan); and six strains of *P. parmentieri* in 1996 on the territory of Poland (IFB5317, IFB5320, IFB5325, IFB5332, and IFB5553). *Dickeya solani* IFB0099 (Poland) was used as an outgroup. The tree was generated with a neighbor-joining algorithm with the Jukes-Cantor nucleotide distance measure CLC MainWorkbench 6.5 software (Qiagen). Bootstrap was set on the level of 100 replicates. IFB = the collection of the Intercollegiate Faculty of Biotechnology University of Gdansk and Medical University of Gdansk, Gdansk, Poland.

Fig. 4. Phenotypic characteristics of *Pectobacterium parmentieri* strains isolated in Poland. Main traits connected with *P. parmentieri* virulence have been compared: pectinase cellulase, protease activities, siderophore production, swimming and swarming ability. The top and bottom lines of the boxplot refer to upper and lower quartiles, respectively. The ends of the whiskers reach minimum and maximum values. The dark diamond represents the mean and the black bar depicts the median. Outliners are marked by circles.

Fig. 5. Comparison of the potato tissue maceration ability of the tested *Pectobacterium* and *Dickeya* strains. The top and bottom lines of the boxplot refer to upper and lower quartiles, respectively. The ends of the whiskers reach minimum and maximum values. The dark diamond represents the mean and the black bar depicts the median. Outliners are marked by circles.

To the best of our knowledge, this is the first study focusing on extensive investigation of two *P. parmentieri* populations that originated from consecutive growing seasons under temperate climate conditions. High heterogeneity within this newly established species was proven on the basis of genotypic and phenotypic characterization of upper and lower 78 *P. parmentieri* strains isolated from potato fields. Because the implemented control measures for potato soft rot and blackleg involve only preventive methods, performing monitoring studies in addition to broadening knowledge on biodiversity of *P. parmentieri* now seems crucial.

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