Occurrence of *Pectobacterium wasabiae* in potato field samples

M. Waleron · K. Waleron · E. Lojkowska

Abstract During the growing seasons of 1996 and 1997, samples of potato stems and tubers with symptoms of blackleg and soft rot were collected in different regions in Poland. After growing to pure cultures on crystal violet pectate (CVP) medium, isolates of bacteria were identified as *Pectobacterium* spp. on the basis of their ability to degrade pectate and with the use of biochemical tests. About 43 % strains isolated from 122 different plant samples were identified as *Pectobacterium carotovorum* subsp. *carotovorum*, whereas the rest of the pectinolytic bacteria was identified as *Pectobacterium atrosepticum*. A recent screening of these isolates with *recA* PCR-RFLP allowed identification of 18 different RFLP groups within the tested *P. c.* subsp. *carotovorum* strains. The third largest group of the tested *P. c.* subsp. *carotovorum* strains (14 %), which were assigned to the profile 3 *recA* PCR-RFLP, was re-identified as *Pectobacterium wasabiae* (formerly *Erwinia carotovora* subsp. *wasabiae*) on the basis of *recA* and 16S rRNA genes sequences. About 50 % of *P. wasabiae* isolated from potato, in contrast to horseradish isolates of *P. wasabiae,* have an ability to grow at 37°C and some of them grow on media containing 5 % of NaCl. In a pathogenicity test with 11 strains of *P. wasabiae* these strains showed a high capacity to rot potato tubers.

Keywords *Pectobacterium wasabiae* · *recA* · Potato soft rot · Blackleg

Introduction

Potato (*Solanum tuberosum*) is one of the most important food crops in Poland and in the world (http://www.fao.org). Pectinolytic bacteria belonging to the genera *Pectobacterium* and *Dickeya* cause economically significant losses of potatoes, as well as of other horticultural and ornamental plants in the field and during storage. Both genera can be discriminated in the laboratory on the basis of biochemical, molecular and host range differences (Hauben et al. 1998; Gardan et al. 2003; Samson et al. 2005). Until recently *Pectobacterium atrosepticum* was considered as the most important pathogen of potato in temperate regions (Toth et al. 2003). However, isolation and identification of the pectinolytic bacteria from potato plants with symptoms of blackleg and soft rot in Poland in 1996–1997 indicated that *P. atrosepticum* was isolated only from 57 % of samples and *P. c.* subsp. *carotovorum* from 43 %. During this study no *Dickeya* strains were isolated (Sledz et al. 2000). Up to 2004 the exchange of plant material between Poland other countries was much more restricted than in recent years. Currently *Dickeya* spp., are also detected in Poland (Slawiak et al. 2009; Lojkowska et al. 2010). Among the *Pectobacterium* species, the number of reports concerning *P. wasabiae* is rising (Ma et al. 2007; Pitman et al. 2008, 2010; Baghaee-Ravari et al.

M. Waleron (✉) · K. Waleron · E. Lojkowska
Department of Biotechnology, Intercollegiate Faculty
of Biotechnology, University of Gdansk and Medical
University of Gdansk,
Kladki 24, 80-822 Gdansk, Poland
e-mail: goniawu@biotech.ug.edu.pl

K. Waleron
e-mail: waleron@biotech.ug.edu.pl

E. Lojkowska
e-mail: lojkowsk@biotech.ug.edu.pl

2011; Nabhan et al. 2012a,b; Rahmanifar et al. 2012; Moleleki et al. 2013). For the first time *P. wasabiae* has been isolated in the 1980s in Japan from Japanese horseradish (Goto and Matsumoto 1987). In 2001, the pathogen was isolated from blackleg-diseased potato plants in the USA (Ma et al. 2007) and later, the bacterium was reported in New Zealand (Pitman et al. 2008, 2010), Iran (Baghaee-Ravari et al. 2011; Rahmanifar et al. 2012); Syria (Nabhan et al. 2012a, b); South Africa and Zimbabwe (Ngadze et al. 2012; Moleleki et al. 2013), in Canada (De Boer et al. 2012) and in Europe (Nabhan et al. 2012b; Nykyri et al. 2012). The recent works of Nabhan et al. (2012b) and Nykyri et al. (2012) suggest that some of *P. carotovorum* strains, isolated since the 1960s in Ireland, Finland, and Germany were misidentified and now should be reclassified as *P. wasabiae*.

The objective of this study was to check the abundance of *P. wasabiae* in the collection of strains isolated in Poland and other countries by biochemical and molecular analyses and to verify the misclassification of earlier isolated *P. c.* subsp. *carotovorum* strains.

Material and methods

Bacterial strains

Two hundred and seven strains used in this study represent the five *Pectobacterium* species: *P. wasabiae* (7), *P. atrosepticum* (2), *P. betavasculorum* (1), *P. cacticidum* (1) and two subspecies: *P. c.* subsp. *carotovorum* (195) and *P. c.* subsp. *odoriferum* (1) (see Table 1). The *P. c.* subsp. *carotovorum* strains were isolated from different plants (the majority was isolated from potato), water, soil and insects, in various countries.

Bacterial strains were grown overnight in tryptic soy broth (TSB Bio-Merieux), in shaken culture, at 28 °C for 24 h. The *P. cacticidum* strain was grown at 43 °C, an optimal temperature for this species. For long-term storage, strains were kept in 80 % glycerol (v/v) at −80 °C.

Biochemical and physiological tests

The identity of the Polish *P. c.* subsp. *carotovorum* strains was confirmed by biochemical analyses, which are routinely used to differentiate *Pectobacterium*

subspecies (Goto and Matsumoto 1987; Schaad et al. 2001) such as: indol production, phosphatase activity, reducing substances from sucrose, acid production from maltose, and α-methyl-D-glucoside, lactose fermentation, growth at 37 °C, growth on NA containing 5 % NaCl and erythromycin sensitivity.

Potato tuber slice assay

The tuber macerating capacity of selected isolates was determined using a potato tuber slice assay. Potato slices (from surface sterilized potato tuber) were inoculated with 25 μl of bacterial suspensions (10^9 cfu ml^{-1}) and maintained at 27 °C and a relative humidity of 95 %, as described previously (Lojkowska and Kelman 1994). Diameter of rotting tissue was measured after 72 h. Experiments were performed in three repetitions and the means were calculated for each strain. Control tubers were treated in the same manner but sterile water was used instead of a bacterial isolate.

Plate assay for detection and measurement of pectolytic activity

Isolates were replicated on solid M63 medium (Miller 1972) containing polygalacturonic acid (Sigma). After 24 h incubation at 30 °C plates were stained by flooding them with 10 % (w/v) copper acetate, which forms a blue complex with the polymer, leaving clear haloes around colonies that produce pectolytic enzymes (Reverchon et al. 1985). The diameter of "halo" zones around bacterial colonies was measured. Experiments were performed in three repetitions and the means were counted for each strain.

PCR amplifications, sequencing and phylogenetic analysis

Amplification of the *recA* gene of 26 strains was performed directly from the cell lysates, as described previously (Waleron et al. 2002). The bacterial 16S rDNA of 15 strains was amplified using the universal primer 1492R and the domain *Bacteria*-specific primer 27F (Weisburg et al. 1991). The PCR conditions were as described previously (Reysenbach et al. 1992). The nucleotide sequences of *recA* and of 16S rRNA genes were

Table 1 Host plant, geographical origin, year of isolation, and *recA* PCR-RFLP profile for selected *Pectobacterium* strains used in this study

No.	IFB Number	Bacterial strain	Geographic origin	Year of isolation	host	*recA* PCR-RFLP profiles
Pectobacterium wasabiae						
1	IFB5302	CFBP3304	Japan	1985	horseradish	23
2	IFB5303	SCRI488	Japan	1985	horseradish	23
3	IFB5304	CFBP3308	Japan	1985	horseradish	23
4	IFB5305	SCRI917	Japan	?	horseradish	23
5	IFB5306	SCRI918	Japan	?	horseradish	23
6	IFB5307	SCRI919	Japan	?	horseradish	23
7	IFB5308	SCC3193	Finland	1980s	potato	3
Strains re-identified as *Pectobacterium wasabiae*						
8	IFB5317	1A1	Poland	1996	potato stem	3
9	IFB5318	1A3	Poland	1996	potato stem	3
10	IFB5319	1A4	Poland	1996	potato stem	3
11	IFB5320	15A3	Poland	1996	potato stem	3
12	IFB5321	16A2	Poland	1996	potato stem	3
13	IFB5322	16A6	Poland	1996	potato stem	3
14	IFB5323	16A13	Poland	1996	potato stem	3
16	IFB5324	17B3	Poland	1996	potato tuber	3
17	IFB5325	36A1	Poland	1996	potato stem	3
18	IFB5326	36A2	Poland	1996	potato stem	3
19	IFB5332	59A2	Poland	1996	potato stem	3
20	IFB5328	47A2	Poland	1996	potato stem	3
21	IFB5329	48B2	Poland	1996	potato tuber	3
22	IFB5330	54A1	Poland	1996	potato stem	3
23	IFB5309	SCRI103	Scotland	1977	potato tuber	3
24	IFB5310	SCRI140	USA	?	potato	3
25	IFB5337	SCRI249	Scotland	1982	potato	3
26	IFB5311	LA242	USA	1973	black leg potato	3
27	IFB5312	LA243	USA	1976	potato	3
28	IFB5313	LA246	USA	1975	potato	3
29	IFB5314	LA247	USA	1989	potato stem	3
30	IFB5315	LA400	USA	?	potato tuber	3
31	IFB5316	PH200	Finland	?	potato stem	3
32	IFB5334	IPO1082	Netherlands	1993	potato	3
33	IFB5335	IPO1084	Netherlands	1993	potato	3
34	IFB5336	59	Serbia	1997	sweet pepper	3
Pectobacterium atrosepticum						
35	IFB5050	16A1	Poland	1996	potato	1
36	IFB5103	SCRI1086	Canada	?	potato	2
Pectobacterium betavasculorum						
37	IFB5268	168	USA	?	?	21
Pectobacterium carotovorum subsp. *odoriferum*						
38	IFB5282	CFBP3259	France	1980	leek	22
Pectobacterium carotovorum subsp. *carotovorum*						
39–109		13 strains	Poland	1996	potato	4

Table 1 (continued)

No.	IFB Number	Bacterial strain	Geographic origin	Year of isolation	host	*recA* PCR-RFLP profiles
		18 strains	Poland	1996–2002	other plants	"
		16 strains[a]	Other countries		potato	"
		24 strains[a]	Other countries		other plants	"
110–141		8 strains	Poland	1996	potato	5
		3 strains	Poland	1996–2002	other plants	"
		8 strains[a]	Other countries		potato	"
		13 strains[a]	Other countries		other plants	"
141–206		19 strains	Poland	1996	potato	6–20
		16 strains	Poland	1996–2002	other plants	"
		16 strains[a]	Other countries		potato	"
		14 strains[a]	Other countries		other plants	"
Pectobacterium cacticidum						
207	IFB5363	ATCC49485	Arizona, USA	1962	opuntia	29

IFB Intercollegiate Faculty of Biotechnology, Gdansk, Poland, *CFBP* Collection Française des Bactéries Phytopathogénes, Angers, France; *SCRI* Scottish Crop Research Institute, Invergowie, Dundee, Scotland; *IPO* Plant Research International, Wageningen, The Netherlands

[a] Strains described in Table 1 Waleron et al. 2002

determined directly from PCR fragments amplified by the PCR primers used. Sequencing was carried out using an ABI PRISM Dye Terminator Cycle Sequencing Kit and ABI3730XL DNA Sequencer (Perkin-Elmer) according to the manufacturer's instructions. The obtained sequences were assembled, trimmed and deposited in GenBank under accession numbers: AY217078 to AY217080, AY217082, AY217084 to AY217086, AY264786, AY264789, AY264792, AY264795 AY264796, AY264799, AY264800, KC584976 to KC584990, KC584991 to KC585000.

For comparison, sequences of the *recA* and 16S rRNA genes were searched in the NCBI database using software BLASTn (http://www.ncbi.nlm.nih.gov/). The sequences from *Pectobacterium* genomes (AKVS01000000, ABVY01000000, ABVX01000000, CP003415, BX950851, CP001790, CP003776) and the most similar sequences obtained from GenBank according to the BLAST analysis, were aligned using the MUSCLE algorithm with the default settings in Geneious Pro 5.4.6 (www.geneious.com).

Maximum Likelihood (ML) and Neighbour-Joining (NJ) phylogenetic analyses were performed with the MEGA 5 software (www.megasoftware.net). Bootstrapping was executed with 1000 replications.

The *recA* and 16S rRNA genes sequences of *Dickeya dadantii* 3937 (CP002038) were used as an out-group.

Results

Characterization and classification of bacterial isolates

During the growing seasons of 1996–97, 122 samples of potato stems and tubers with symptoms of blackleg and soft rot, each representing a different field, were collected from different regions of Poland. Pectinolytic bacteria were isolated and about 1500 isolates were identified as *Pectobacterium* spp. on the basis of biochemical and phenotypic tests: the isolates were Gram-negative rods, oxidase negative, facultative anaerobes, grew at 28 °C and able to degrade pectate. Further characterization of these isolates, using PCR assays with primers specific for *Dickeya* spp., *P. atrosepticum* and *P. carotovorum* was carried out as described by Nassar et al. (1996), De Boer and Ward (1995) and Darrasse et al. (1994) respectively. About the 600 strains (43 %) strains isolated from rotting plant tissue were assigned to *P. c.* subsp. *carotovorum* by their biochemical features and PCR (Sledz et al.
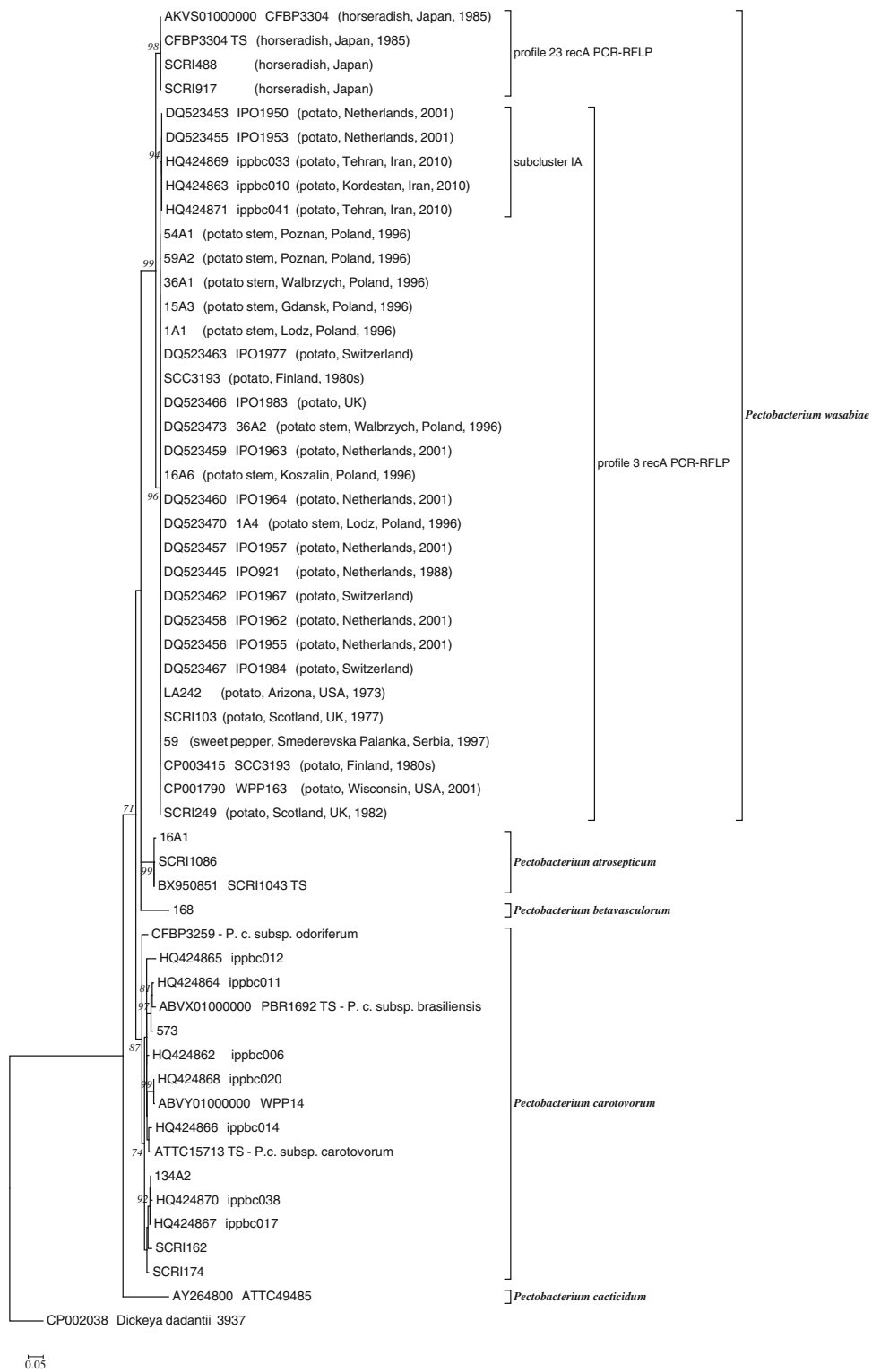
Fig. 1 The Maximum Likelihood tree based on 730 bp recA gene fragment from *Pectobacterium* genus. Sequences obtained from the GenBank are described by accession numbers followed by strains numbers. Bootstrap values after 1000 replicates are expressed as percentages. Bootstrap values<50 % were cut off. Dickeya dadantii 3936 (CP002038) was included as an outgroup

2000). For further analysis in the present study, 50 out of 600 strains indicating some diversity in biochemical features (phosphatase activity, reducing substances from sucrose, acid production from maltose, and growth at 37 °C, growth on NA containing 5 % NaCl) were chosen.

Characterization of *P. c.* subsp. *carotovorum* isolates by *recA* PCR-RFLP

Application of *recA* PCR-RFLP to study the genetic diversity of those 54 strains and 37 strains isolated from vegetables as well as 104 strains of *P. c.* subsp. *carotovorum* obtained from 18 different countries, and from four continents (Europe, Australia, North and South America) (see Table 1) indicated a high diversity within the group of bacteria studied. The *recA*-PCR RFLP analysis of 195 *P. c.* subsp. *carotovorum* strains allowed for discrimination of 18 different profiles. The most numerous profiles 4 and 5 grouped strains isolated from different host plants and other sources from all over the world. The third largest group of the tested *P. c.* subsp. *carotovorum* strains (14 %) was assigned to the profile 3 *recA* PCR-RFLP (Waleron *et al.* 2002).

recA and 16S rDNA—based phylogenetic analyses

The *recA* and 16S rDNA genes sequences of the strains tested before, as described above, were obtained. The sequences were compared with those of *P. wasabiae* strains isolated from potato (Nykyri et al. 2012) and horseradish (Goto and Matsumoto 1987). A Blast analysis of the sequences obtained with those available in GenBank indicated that strains, described earlier as *recA* PCR-RFLP profile 3 exhibited a 99 % identity with the *recA* gene of *P. wasabiae* strains isolated from potato (CP003415, CP001790). As a result, 14 % of strains earlier identified as *P. c.* subsp. *carotovorum* should were re-classified as *P. wasabiae*.

The maximum likelihood (ML) tree based on the *recA* gene clustered Polish isolates from profile 3 *recA* PCR-RFLP and other *P. wasabiae* strains isolated from potato, with *P. wasabiae* SCC3193 (CP003415) and readily distinguishing them from the closely related subspecies *P. c.* subsp. *carotovorum* (Fig. 1). Moreover, *P. wasabiae* strains isolated from potato varied (distance 2.8–3.2 %) from those isolated from
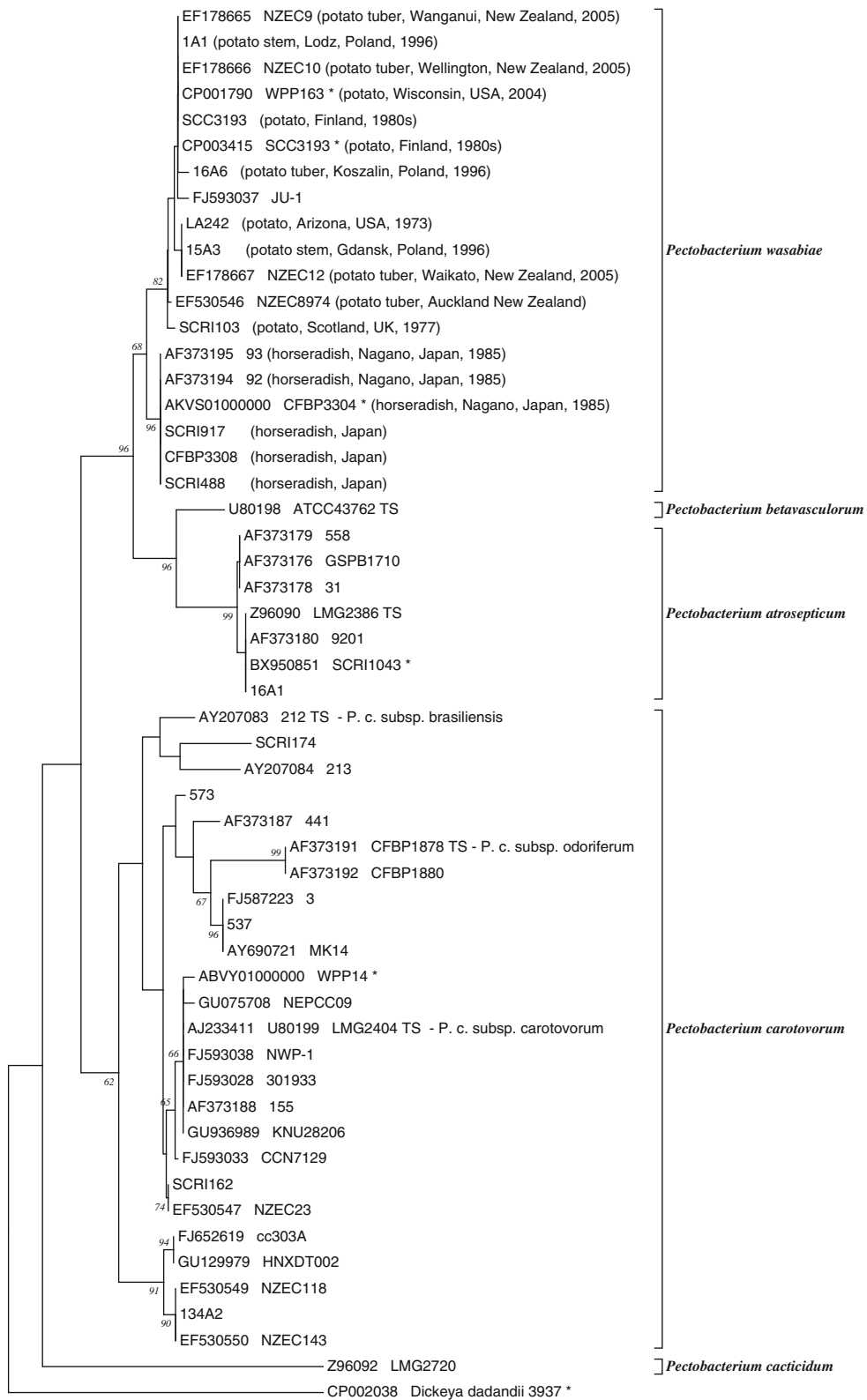
Fig. 2 The genetic distance tree based on the 16S rRNA gene from *Pectobacterium* genus. Sequences obtained from the GenBank are described by accession numbers followed by strains numbers. Bootstrap values after 1000 replicates are expressed as percentages. Bootstrap values<50 % were cut off. Dickeya dadantii 3936 (CP002038) was included as an outgroup. For the phylogeny, the consensus sequences of seven copies of the 16S rRNA gene from available genome sequences of *P. wasabiae* strains CFBP3304 (AKVS01000000), WPP163 (CP001790) and SCC3193 (CP003415) were used. The consensus sequences were marked with stars

horseradish (Fig. 1) and were clearly separated from those retrieved from horseradish due to 22 polymorphic positions present within 730 bp fragment of *recA* gene. Among those polymorphisms, 16 were unique for strains isolated from horseradish and caused the conformation change of RFLP fragments, which influence on their electrophoretic mobility in non-denaturing acrylamide gels, leading to double-strand conformation polymorphism (DSCP) (Hagerman 1990). The DSCP effect was the reason for assigning different *recA* PCR-RFLP profiles (3 and 23), for *P. c.* subsp. *carotovorum* and *P. wasabiae* strains (Waleron et al. 2002). The remaining six polymorphic positions have allowed the differentiation of two strains from the Netherlands and three strains from Iran, from other *P. wasabiae* strains isolated from potato (Fig. 1).

Characterization of *P. wasabiae* isolates

The topology of a distance tree based on 16S rRNA gene sequences confirmed that strains assigned earlier as profile 3 *recA* PCR-RFLP of *P. c.* subsp. *carotovorum* are more related with *P. wasabiae* than with other *P. c.* subsp. *carotovorum* strains (Fig. 2) again. The genetic distance to the *P. wasabiae* strains from horseradish (0.1–0.6 %) was significantly lower than to the *P. carotovorum* strains (1.5–2.3 %). Moreover the *P. wasabiae* strains from horseradish were separated from those isolated from potato (Fig. 2).

Phenotypic features of *P. wasabiae* strains were compared using traditional microbiological methods, which are routinely used to differentiate *Pectobacterium* subspecies (Schaad et al. 2001). All *P. wasabiae* strains were negative for indol production, phosphatase activity, reducing substances from sucrose, and negative for acid production from maltose, and α-methyl-D-glucoside. However, about 50 % of *P. wasabiae* strains isolated from potato did grow on NA at 37 °C and seven of

EF178665　NZEC9 (potato tuber, Wanganui, New Zealand, 2005)

1A1 (potato stem, Lodz, Poland, 1996)

EF178666　NZEC10 (potato tuber, Wellington, New Zealand, 2005)

CP001790　WPP163 * (potato, Wisconsin, USA, 2004)

SCC3193　(potato, Finland, 1980s)

CP003415　SCC3193 * (potato, Finland, 1980s)

16A6　(potato tuber, Koszalin, Poland, 1996)

FJ593037　JU-1

LA242　(potato, Arizona, USA, 1973)

15A3　(potato stem, Gdansk, Poland, 1996)

EF178667　NZEC12 (potato tuber, Waikato, New Zealand, 2005)

EF530546　NZEC8974 (potato tuber, Auckland New Zealand)

SCRI103　(potato, Scotland, UK, 1977)

Pectobacterium wasabiae

82

68

AF373195　93 (horseradish, Nagano, Japan, 1985)

AF373194　92 (horseradish, Nagano, Japan, 1985)

AKVS01000000　CFBP3304 * (horseradish, Nagano, Japan, 1985)

96

SCRI917　(horseradish, Japan)

CFBP3308　(horseradish, Japan)

SCRI488　(horseradish, Japan)

96

U80198　ATCC43762 TS　*Pectobacterium betavasculorum*

AF373179　558

AF373176　GSPB1710

AF373178　31

96

99

Z96090　LMG2386 TS

AF373180　9201

BX950851　SCRI1043 *

16A1

Pectobacterium atrosepticum

AY207083　212 TS - P. c. subsp. brasiliensis

SCRI174

AY207084　213

573

AF373187　441

99

AF373191　CFBP1878 TS - P. c. subsp. odoriferum

AF373192　CFBP1880

67

FJ587223　3

537

96

AY690721　MK14

ABVY01000000　WPP14 *

GU075708　NEPCC09

AJ233411　U80199　LMG2404 TS　- P. c. subsp. carotovorum

66

FJ593038　NWP-1

FJ593028　301933

65

AF373188　155

GU936989　KNU28206

FJ593033　CCN7129

SCRI162

74

EF530547　NZEC23

94

FJ652619　cc303A

GU129979　HNXDT002

91

EF530549　NZEC118

134A2

90

EF530550　NZEC143

62

Pectobacterium carotovorum

Z96092　LMG2720　*Pectobacterium cacticidum*

CP002038　Dickeya dadandii 3937 *

0.005

them grew on NA containing 5 % of NaCl unlike all horseradish strains used in this studies. The pectinolytic activity, measured as the diameter of the halo in the plate assay, of the *P. wasabiae* strains isolated from potato was higher than those of *P. wasabiae* strains isolated from horseradish and *P. c.* subsp. *carotovorum* strains, 13.5 mm, 5.8 mm, 10.0 mm, respectively. The tuber macerating capacity, and pectinolytic activity of a potato and horseradish isolates of *P. wasabiae* were also compared. The mean diameter of rotting tissue measured after inoculation of potato tuber slices with *P. wasabiae* strains isolated from potato was higher than those obtained for *P. c.* subsp. *carotovorum* and almost two times higher than those noted for strains isolated from horseradish, 14.5 mm, 12.5 mm, 8.8 mm, respectively.

Discussion

In this study the occurrence of *P. wasabiae* in Poland and other countries was checked by using several biochemical, physiological and molecular techniques. However, only molecular techniques allowed for identification of *P. wasabiae* strains isolated from potato.

We proved that *P. wasabiae* has been present on potato plants in Poland as well as Finland, Netherlands, Serbia, Scotland and the USA for many years. Our results are in agreement with MLSA studies of Nabhan et al. (2012b), which showed that a Polish strain, 1A1 (IFB5317) could be identified as *P. wasabiae*.

We consider that out of 195 analysed *P. c.* subsp. *carotovorum* strains, about 14 %, which were previously identified as profile 3 *recA* PCR-RFLP should be reclassified as *P. wasabiae*.

The 26 out of the 27 strains belonging to profile 3 (excluding strain IFB5336 from sweet pepper) originated from potato. Thus, about 20 % of strains classified as *P. c.* subsp. *carotovorum* in earlier studies and originated from potato and able to cause blackleg or soft rot actually are *P. wasabiae* species. Four *P. wasabiae* strains were isolated from potato tubers and 15 from stems. In the case of seven remaining strains the part of potato plant from which bacteria were isolated is unknown. In Canada, *P. wasabiae* strains were isolated only from stems and they represented 16 % of *Pectobacteria* causing stem rot of potatoes (De Boer et al. 2012). In New Zealand,

South Africa and Zimbabwe the pathogen represented only about 5 % of soft-rotting *Pectobacteria* detected on potatoes. However, in New Zealand *P. wasabiae* was searched only in seed tubers (Pitman et al. 2008) and because of this the number of detected *P. wasabiae* strains may be underestimated.

Our studies clearly indicate that *P. wasabiae* strains isolated from potato are different from those isolated from horseradish and exhibit a higher level of diversity than the latter ones; however, only a limited number of horseradish strains were analysed. The heterogeneity within the strains of *P. wasabiae* was observed also when other genes like *acnA, mdh, proA* and *gapA* were analysed (Pitman et al. 2010; Baghaee-Ravari et al. 2011; De Boer et al. 2012; Moleleki et al. 2013). In addition the *P. wasabiae* strains from potato exhibited phenotypic diversity. The mean tuber macerating capacity and the activity of pectinolytic enzymes from *P. wasabiae* strains from potato was significantly higher than from *P. wasabiae* strains from horseradish and a little higher than activity observed for the *P. c.* subsp. *carotovorum* strains. Similar observations were reported by Pitman et al. (2008) and Moleleki et al. (2013).

About 50 % of *P. wasabiae* strains isolated from potato was able to grow at 37 °C and seven on NA containing 5 % NaCl, whereas strains isolated from horseradish in our hands failed to do so entirely. Exactly the same results were obtained by Nykyri et al. (2012) for two strains of *P. wasabiae* SCC3193 and CFBP3304. The ability to grow at 37 °C and growth in 5 % NaCl of some *P. wasabiae* strains from potato can have been the cause of misidentifications in the past. For example the growth of bacteria in 37 °C was the reason for Pitman et al. (2008) and Moleleki et al. (2013) to classify strains growing in this temperature as *P. c.* subsp. *carotovorum* or *P. c.* subsp. *brasiliensis*. Taking into account the fact that 50 % of Polish *P. wasabiae* isolates from potato were able to grow in 37 °C, the contribution of *P. wasabiae* isolates to disease occurrences in New Zealand and South Africa could also be different from that reported (Pitman et al. 2008; Moleleki et al. 2013).

The occurrence of *P. wasabiae* was noticed all over the world and it is present in Europe, Asia, Africa, Australia and both Americas. According to our results *P. wasabiae* occurred on potato plants already in 1990s in Poland and in the Netherlands; *P. wasabiae* strains IFB5311, IFB5312, IFB5313 were isolated in

the USA in early 1970s. The pathogen was then recognized in the early 1980s in Finland and Scotland. Our studies confirmed the greater number of strains isolated in Poland and other countries; and that *P. wasabiae* is not a new pathogen but one that was already much longer present than we expected, but went unnoticed due to the limited classification methods available/followed in the laboratories involved. Nabhan et al. (2012b) reported three *P. wasabiae* strains, among them strain SCRI 102 that was isolated in 1962 in Ireland. Also De Boer et al. (2012) re-identified four *P. wasabiae* strains originated from USA, among 200 of *Pectobacterium* spp. strains isolated during 1970–1985 in Canada, USA, Peru, Scotland and the Netherlands. Our results and data from the literature indicate that *P. wasabiae* species has been present and isolated though unnoticed (due to misidentification) for a long time.

References

Baghaee-Ravari, S., Rahimian, H., Shams-Bakhsh, M., Lopez-Solanilla, E., Antúnez-Lamas, M., & Rodríguez-Palenzuela, P. (2011). Characterization of *Pectobacterium* species from Iran using biochemical and molecular methods. *European Journal of Plant Pathology, 129*, 413–425.

Darrasse, A., Priou, S., Kotoujansky, A., & Bertheau, Y. (1994). PCR and Restriction Fragment Length Polymorphism of a *pel* gene as a tool to identify *Erwinia carotovora* in relation to potato diseases. *Applied and Environmental Microbiology, 60*, 1437–1443.

De Boer, S. H., & Ward, L. J. (1995). PCR detection of *Erwinia carotovora* subsp. atroseptica associated with potato tissue. *Phytopathology, 85*, 854–858.

De Boer, S. H., Li, X., & Ward, L. J. (2012). *Pectobacterium* spp. associated with bacterial stem rot syndrome of potato in Canada. *Phytopathology, 102*, 937–947.

Gardan, L., Gouy, C., Christen, R., & Samson, R. (2003). Elevation of three subspecies of Pectobacterium carotovorum to species level: Pectobacterium atrosepticum sp. nov., Pectobacterium betavasculorum sp. nov. and Pectobacterium wasabiae sp. nov. *International Journal of Systematic and Evolutionary Microbiology, 53*, 381–391.

Goto, M., & Matsumoto, K. (1987). *Erwinia carotovora* subsp. *wasabiae* subsp. nov. isolated from diseased rhizomes and fibrous roots of Japanese horseradish (*Eutrema wasabi* Maxim.). *International Journal of Systematic Bacteriology, 37*, 130–135.

Hagerman, P. J. (1990). Sequence-directed curvature of DNA. *Annual Review of Biochemistry, 59*, 755–781.

Hauben, L., Moore, E. R. B., Vauterin, L., Steenackers, M., Mergaert, J., Verdonck, L., et al. (1998). Phylogenetic position of phytopathogens within the *Enterobacteriaceae*. *Systematic and Applied Microbiology, 21*, 384–397.

Lojkowska, E., & Kelman, A. (1994). Comparison of the effectiveness of different methods of screening for bacterial soft rot resistance of potato tubers. *American Potato Journal, 71*, 99–113.

Lojkowska, E., Potrykus, M. & Slawiak, M. (2010). Molecular characterization and pathogenicity of *Dickeya* strains isolated recently from potato plants in Poland. In: *Proceedings of the 12th International Conference on Plant Pathogenic Bacteria*, 7–11 June 2010, St. Denis, Reunion. P4–92 (pp. 158).

Ma, B., Hibbing, M. E., Kim, H. S., Reedy, R. M., Yedidia, I., Breuer, J., et al. (2007). The host range and molecular phylogenies of the soft rot enterobacterial genera *Pectobacterium* and *Dickeya*. *Phytopathology, 97*, 1150–1163.

Miller, J. H. (1972). *Experiments in molecular genetics*. Cold Spring Harbor: Cold Spring Harbor Laboratory Press.

Moleleki, L. N., Onkendi, E. M., Mongae, A., & Kubheka, G. C. (2013). Characterisation of *Pectobacterium wasabiae* causing blackleg and soft rot diseases in South Africa. *European Journal of Plant Pathology, 135*, 279–288.

Nabhan, S., De Boer, S. H., Maiss, E., & Wydra, K. (2012a). Taxonomic relatedness between *Pectobacterium carotovorum* subsp. *carotovorum*, *Pectobacterium carotovorum* subsp. *odoriferum* and *Pectobacterium carotovorum* subsp. *brasiliense* subsp. nov. *Journal of Applied Microbiology, 113*, 904–913.

Nabhan, S., Wydra, K., Linde, M., & Debener, T. (2012b). The use of two complementary DNA assays, AFLP and MLSA, for epidemic and phylogenetic studies of pectolytic enterobacterial strains with focus on the heterogeneous species *Pectobacterium carotovorum*. *Plant Pathology, 61*, 498–508.

Nassar, A., Darrasse, A., Lemattre, M., Kotoujansky, A., Dervin, C., Vedel, R., et al. (1996). Characterization of *Erwinia chrysanthemi* by pectinolytic isozyme polymorphism and restriction fragment length polymorphism analysis of PCR- amplified fragments of *pel* genes. *Applied and Environmental Microbiology, 62*, 2228–2235.

Ngadze, E., Brady, C. L., Coutinho, T. A., & van der Waals, J. E. (2012). Pectinolytic bacteria associated with potato soft rot and blackleg in South Africa and Zimbabwe. *European Journal of Plant Pathology, 134*, 533–549.

Nykyri, J., Niemi, O., Koskinen, P., Nokso-Koivisto, J., Pasanen, M., Broberg, M., et al. (2012). Revised phylogeny and novel horizontally acquired virulence

determinants of the model soft rot phytopathogen *Pectobacterium wasabiae* SCC3193. *PLoS Pathogens, 8*, e1003013.

Pitman, A. R., Wright, P. J., Galbraith, M. D., & Harrow, S. A. (2008). Biochemical and genetic diversity of pectolytic enterobacteria causing soft rot disease of potatoes in New Zealand. *Australasian Plant Pathology, 37*, 559–568.

Pitman, A. R., Harrow, S. A., & Visnovsky, S. B. (2010). Genetic characterisation of *Pectobacterium wasabiae* causing soft rot disease of potato in New Zealand. *European Journal of Plant Pathology, 126*, 423–435.

Rahmanifar, B., Hasanzadeh, N., Razmi, J., & Ghasemi, A. (2012). Genetic diversity of Iranian potato soft rot bacteria based on polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis. *African Journal of Biotechnology, 11*, 1314–1320.

Reverchon, S., Hugouvieux-Cotte-Pattat, N., & Robert-Baudouy, J. (1985). Cloning of genes encoding pectolytic enzymes from a genomic library of the phytopathogenic bacterium, *Erwinia chrysanthemi*. *Gene, 49*, 215–224.

Reysenbach, A. L., Giver, L. J., Wickham, G. S., & Pace, N. R. (1992). Differential amplification of rRNA genes by polymerase chain reaction. *Applied and Environmental Microbiology, 58*, 3417–3418.

Samson, R., Legendre, J. B., Christen, R., Fischer-Le Saux, M., Achouak, W., & Gardan, L. (2005). Transfer of *Pectobacterium chrysanthemi* (Burkholder et al. 1953) Brenner *et al.* 1973 and *Brenneria paradisiaca* to the genus *Dickeya* gen. nov. as *Dickeya chrysanthemi* comb. nov. and *Dickeya paradisiaca* comb. nov. and delineation of four novel species, *Dickeya dadantii* sp. nov., *Dickeya dianthicola* sp. nov., *Dickeya dieffenbachiae* sp. nov. and *Dickeya zeae* sp. nov. *International Journal of Systematic and Evolutionary Microbiology, 55*, 1415–1427.

Schaad, N. W., Jones, J. B., & Chun, W. (2001). *Laboratory guide for identification of plant pathogenic bacteria* (3rd ed.). St. Paul: APS Press.

Slawiak, M., Lojkowska, E., & van der Wolf, J. M. (2009). First report of bacterial soft rot on potato caused by *Dickeya* sp. (syn. *Erwinia chrysanthemi*) in Poland. *Plant Pathology, 58*, 794.

Sledz, W., Jafra, S., Waleron, M., & Lojkowska, E. (2000). Genetic diversity of *Erwinia carotovora* strains isolated from infected plants grown in Poland. *OEPP/EPPO Bulletin, 30*, 403–407.

Toth, I. K., Bell, K. S., Holeva, M. C., & Birch, P. R. J. (2003). Soft rot Erwinia: from genes to genomes. *Molecular Plant Pathology, 4*, 17–30.

Waleron, M., Waleron, K., Podhajska, A. J., & Lojkowska, E. (2002). Genotyping of bacteria belonging to the former *Erwinia* genus by PCR-RFLP analysis of *recA* gene fragment. *Microbiology, 148*, 583–595.

Weisburg, W. G., Barns, S. M., Pelletier, D. A., & Lane, D. J. (1991). 16S ribosomal DNA amplification for phylogenetic study. *Journal of Bacteriology, 173*, 697–703.