Characterization of *Pectobacterium carotovorum* subsp. *odoriferum* causing soft rot of stored vegetables

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Abstract *Pectobacterium carotovorum* subsp. *odoriferum* has been generally considered to have a narrow host range and has been isolated most often from chicory. Research was conducted to identify 91 *Pectobacterium* spp. strains isolated from different vegetables in Europe, North and South America, Asia, and Africa, and to compare their ability to cause disease in chicory and potato. Among the 91 strains, 22 strains from Europe were identified as *P. c.* subsp. *odoriferum*. Based on phylogenetic analysis of 16S rDNA, *recA*, and *rpoS* gene sequences, strains isolated from stored vegetables clustered together with the type strain of *P. c.* subsp. *odoriferum* and clustered separately from the *P. c.* subsp. *carotovorum* isolates. Eleven strains previously identified as *P. c.* subsp. *carotovorum* were reclassified as *P. c.* subsp. *odoriferum*. All *P. c.* subsp. *odoriferum* isolates were able to cause soft rot symptoms on chicory and potato. Moreover, the symptoms on potatoes were more severe at temperatures from 15 to 37 °C with *P. c.* subsp. *odoriferum* isolates than with *P. atrosepticum* or *P. c.* subsp. *carotovorum* isolates. Tissue maceration by *P. c.* subsp. *odoriferum* isolates

was highest at 28 °C, and at that temperature tissue maceration was two-times greater for *P. c.* subsp. *odoriferum* isolates than for *P. c.* subsp. *carotovorum* isolates. Symptoms on inoculated chicory leaves were more severe with *P. c.* subsp. *odoriferum* (regardless of origin) than with other subspecies or species. To our knowledge, this is the first report that *P. c.* subsp. *odoriferum* occurs on a wide range of vegetables and has the ability to cause soft rot during potato storage.

Keywords *Pectobacterium carotovorum* subsp. *odoriferum* · *recA* · *rpoS* · 16S rRNA · Chicory · Potato · Soft rot

Introduction

Bacteria belonging to the genus *Pectobacterium* cause many diseases on a great variety of crops worldwide. *Pectobacterium* spp. have been isolated from monocotyledonous and dicotyledonous plants, soil, and water, and have also been found in association with a variety of invertebrates (Glasner et al. 2008). Some species, such as *P. atrosepticum*, *P. wasabiae*, *P. carotovorum* subsp. *carotovorum*, and *P. carotovorum* subsp. *brasiliense*, cause diseases on a variety of crops but *P. cacticida* and *P. betavascularum* have been isolated only from cacti and sugar beet, respectively (Alcorn et al. 1991; Gardan et al. 2003). The recently described species *P. aroidearum* preferentially infects monocots (Nabhan et al. 2013). *P. carotovorum* subsp. *odoriferum* has mostly been isolated from chicory in France

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(Samson et al. 1980; Gallois et al. 1992) and Japan (Lan et al. 2013) but can cause disease on hyacinth, celery, leek, and sugar beet (Gallois et al. 1992; Gardan et al. 2003). In recent years, strains of this subspecies were also isolated from okra in Malaysia (Nazerian et al. 2011), artichokes in Italy (Gallelli et al. 2009), Chinese cabbage in Korea (Thapa et al. 2011), and potato in Korea (Thapa et al. 2011) and Algeria (Yahiaoui-Zaidi et al. 2010). The identification of *P. c. subsp. odoriferum* has been based on RFLP analysis of *pelABCDE* genes, which encode pectate lyases (Darrasse et al. 1994) and 16S rRNA gene sequences (Hauben et al. 1998; Gardan et al. 2003). The identification of *P. c. subsp. odoriferum* has also been based on the *recA* gene (Waleron et al. 2002a), the *rpoS* gene (Waleron et al. 2002b), amplified fragment length polymorphism (AFLP) (Nabhan et al. 2012a), and multilocus sequence analysis (MLSA) (Ma et al. 2007; Kim et al. 2009; Nabhan et al. 2012b).

The first objective of this study was to identify newly isolated *Pectobacterium* strains and to verify the identities of *Pectobacterium* strains described in our previous work (Waleron et al. 2002a) in order to determine the presence of *P. c. subsp. odoriferum* on different vegetables. The second objective was to compare the ability of *P. c. subsp. odoriferum* isolated from chicory and other vegetables to induce disease symptoms on chicory leaves and potato tubers.

Materials and methods

Bacterial strains

Strains used in this study were listed in Table 1. Based on the research presented in this paper, the strains included three *Pectobacterium* species (*P. atrosepticum*, *P. betavascularum*, and *P. wasabiae*) and three subspecies (*P. c. subsp. carotovorum*, *P. c. subsp. odoriferum*, and *P. c. subsp. brasiliense*). Among the 22 *P. c. subsp. odoriferum* strains, 11 were isolated in France, eight in Poland, two in Serbia, and one in Switzerland. All Polish and Serbian strains had been isolated from stored vegetables rather than from plants growing in the field.

Bacterial strains were grown in tryptic soy broth (TSB Bio-Merieux) shake culture at 28 °C for 24 h. For long-term storage, strains were kept in 40 % glycerol (v/v) at –80 °C.

Biochemical and physiological tests

The identities of the 91 *Pectobacterium* strains were carried out with the following biochemical analyses, which are routinely used to differentiate *Pectobacterium* subspecies (Schaad et al. 2001): indol production; phosphatase and urease activity; reducing substances from sucrose; acid production from maltose, rhamnose, trehalose, and α -methyl-D-glucoside; lactose fermentation; growth at 37 °C; growth on NA containing 5 % NaCl; L-lysine and L-ornithine decarboxylase activity, and L-arginine hydrolase activity. As described later, the identities were also assessed by molecular analysis.

Potato tuber assay

The ability of 87 *Pectobacterium* strains to macerate potato tubers (see Table 1) was determined using a potato tuber assay. Surface-sterilized potato tubers were inoculated by inserting one pipette tip containing 25 μ l of bacterial suspension (2×10^7 cfu ml⁻¹) 10 mm into each tuber as described previously (Lojkowska and Kelman 1994). The tubers were then incubated at 28 °C and 95 % relative humidity for 72 h before the diameter of the rotting tissue in each tuber was measured. For 15 *P. c. subsp. odoriferum* strains isolated from chicory and other vegetables, additional tubers were inoculated and incubated at 10, 15, 18, and 37 °C as before. Each combination of strain and temperature was represented by three replicate tubers, and the experiment was performed three times. As a negative control, pipette tips inserted into tubers contained sterile water rather than a bacterial suspension.

Chicory assay

The ability to macerate chicory leaves was determined for 61 *Pectobacterium* strains (see Table 1). The inner surface of the chicory leaves was inoculated with 10 μ l of a bacterial suspension (10^8 cfu ml⁻¹), and the leaves were incubated in plastic bags at 28 °C and 95 % relative humidity for 24 h as described previously (Van Gijsegem et al. 2008). The 95 % humidity was created by placing a damp tissue in each bag. As a negative control, chicory leaves were inoculated with sterile water. Five leaves were inoculated per strain or control, and the experiment was performed twice. After

Table 1 Host plant, geographical origin, year of isolation, and pathogenicity on potato tubers tissue and chicory leaves tissue for selected *Pectobacterium* strains used in this study. The

identifications in this table are based on the results obtained in this paper. As indicated in the text, some of the identifications in the culture collections were incorrect

No.	Bacterial strain	Geographic origin	Year of isolation	Host	Diameter of rotting potato tissue ^a (mm)	Surface area of rotting chicory tissue ^b (cm ²)
<i>Pectobacterium carotovorum</i> subsp. <i>odoriferum</i> strains isolated from chicory						
1	IFB5285 (CFBP1878 ^T)	France	1979	Chicory	11.6	9.5
2	IFB5279	France	–	Chicory	nt	40.9
3	IFB5281	France	–	Chicory	11.0	14.6
4	IFB5283	France	–	Chicory	14.6	103.3
5	IFB5284	France	–	Chicory	nt	129.0
6	IFB5286	France	–	Chicory	19.0	115.8
7	IFB5287	France	–	Chicory	16.6	134.4
8	IFB5289	France	–	Chicory	17.8	102.1
9	IFB5293	Switzerland	1985	Chicory	28.0	85.6
<i>Pectobacterium carotovorum</i> subsp. <i>odoriferum</i> strains isolated from different vegetables						
10	IFB5282	France	1980	Leek	11.0	12.3
11	IFB5288	France	1982	Celery	18.0	107.2
12	IFB5290	France	–	Celery	17.0	70.9
13	IFB5291	Serbia	1999	Parsley	26.5	85.1
14	IFB5292	Serbia	1999	Parsley	26.0	119.7
15	IFB5294	Poland	1999	Celery	18.6	112.8
16	IFB5295	Poland	1999	Carrot	16.0	11.7
17	IFB5296	Poland	1999	Celery	17.6	137.4
18	IFB5297	Poland	1999	Carrot	12.6	2.2
19	IFB5298	Poland	1999	Carrot	17.8	7.2
20	IFB5299	Poland	1999	Onion	28.6	98.3
21	IFB5300	Poland	1995	Leek	20.0	104.8
22	IFB5301	Poland	2002	Celery	nt	nt
<i>Pectobacterium carotovorum</i> subsp. <i>carotovorum</i>						
23	IFB5263 (LMG2404) ^T	Denmark	1952	Potato	6.5	0
24	IFB5148	Hungary	1983	Potato	18.0	Nt
25	IFB5316	Finland	–	Potato	17.0	nt
26	IFB5152	Poland	1999	Rhubarb	25.0	nt
27	IFB5401	Poland	1995	Carrot	2.0	3.0
28	IFB5264	Poland	2002	<i>Solanum nigrum</i>	3.0	3.1
29	IFB5214	Poland	2002	<i>Solanum nigrum</i>	0	0.9
30	IFB5213	Poland	2002	<i>Solanum nigrum</i>	15.6	25.1
31	IFB5223	Poland	2002	<i>Solanum nigrum</i>	15.0	0
32–50	19 strains *	Poland	1996	Potato	0–13.5	0–16.5
<i>Pectobacterium carotovorum</i> subsp. <i>brasiliense</i>						
51	IFB5234	Brazil	2009	Potato	22.0	0.8
52	IFB5235	Brazil	2009	Potato	23.0	0.9
53	IFB5236	Brazil	2009	Potato	17.6	nt
54	IFB5243	Brazil	2009	Potato	27.5	nt
55	IFB5244	Brazil	2009	Potato	23.0	2.2
56	IFB5255	Brazil	2009	Potato	22.3	2.2
<i>Pectobacterium atrosepticum</i>						
57	IFB5050	Poland	1996	Potato	20.3	28.1

Table 1 (continued)

No.	Bacterial strain	Geographic origin	Year of isolation	Host	Diameter of rotting potato tissue ^a (mm)	Surface area of rotting chicory tissue ^b (cm ²)
58	IFB5007	Peru	1978	Potato	8.0	nt
59	IFB5010	Scotland, UK	1982	soil	11.0	nt
60	IFB5012	Israel	1955	Potato	10.8	nt
61	IFB5402	Poland	1996	Potato	21.0	nt
62	IFB5403	Poland	1996	Potato	24.0	nt
63	IFB5114	Poland	1996	Potato	24.0	nt
64	IFB5103	Canada	–	Potato	7.2	0
65	IFB5016	Scotland, UK	1982	soil	10.0	nt
66	IFB5017	Scotland, UK	1982	Potato	11.0	nt
<i>Pectobacterium betavascularum</i>						
67	IFB5269 (CFBP2122) ^T	USA	–	Sugar beet	17.0	0
68	IFB5270	USA	–	Sugar beet	3.8	nt
69	IFB5271	Mexico	–	Sunflower	15.6	0
70	IFB5272	La Reunion	–	Artichoke	4.6	nt
71	IFB5273	USA	–	Sugar beet	12.2	nt
72	IFB5274	California, USA	–	Sugar beet	6.0	nt
73	IFB5275	USA	–	Sugar beet	4.8	nt
74	IFB5276	Arizona, USA	–	Sugar beet	20.0	nt
<i>Pectobacterium wasabiae</i>						
75	IFB5302 (CFBP3304) ^T	Japan	1985	Horseradish	7.6	nt
76	IFB5303	Japan	1985	Horseradish	5.3	3.6
77	IFB5304	Japan	1985	Horseradish	8.0	nt
78	IFB5305	Japan	–	Horseradish	9.0	Nt
79	IFB5306	Japan	–	Horseradish	7.3	Nt
80	IFB5307	Japan	–	Horseradish	9.5	Nt
81	IFB5336	Serbia	1997	Sweet pepper	10.0	2
82	IFB5167	Poland	1996	Potato	18.3	2.5
83	IFB5317	Poland	1996	Potato	16.0	1.8
84	IFB5320	Poland	1996	Potato	20.0	nt
85	IFB5322	Poland	1996	Potato	17.6	0
86	IFB5324	Poland	1996	Potato	20.0	nt
87	IFB5325	Poland	1996	Potato	19.0	3.2
88	IFB5328	Poland	1996	Potato	20.0	nt
89	IFB5333	Poland	1996	Potato	20.0	nt
90	IFB5340	Poland	1996	Potato	15.0	0.9
91	IFB5342	Poland	1996	Potato	14.0	nt

*Strains described in Waleron et al. 2002 and Waleron et al. 2013

^aSurface-disinfested tubers were inoculated with the indicated strain and incubated at 28 °C for 72 h before the diameter of the rotting tissue was measured in each tuber. Values are means from three experiments with three replicate tubers per strain per experiment

^bChicory leaves were inoculated with the indicated strain and incubated at 28 °C for 24 h before the surface area of the rotten tissue was measured on each leaf. Values are means of two experiments with five replicate chicory leaves per strain per experiment

nt not tested

IFB Intercollegiate Faculty of Biotechnology, Gdansk, Poland; CFBP Collection Française des Bactéries Phytopathogènes, Angers, France; Invergowrie, Dundee, Scotland; LMG/BCCM Laboratorium voor Microbiologie Universiteit Gent/the Belgian Coordinated Collections of Microorganisms

Table 2 The diameter of rotting tissue in potato tubers inoculated with *Pectobacterium* strains and then incubated at 28 °C for 72 h

Species/subspecies	Number of tested strains	Diameter of rotting tissue per tuber (mm)	
		Mean ($\pm$ SD)	Range
All <i>P. c. subsp. odoriferum</i> strains tested	18	18.1 ($\pm$ 5.6)	11.0–28.6
<i>P. c. subsp. odoriferum</i> from chicory	7	16.9 ($\pm$ 5.7)	11.0–28.0
<i>P. c. subsp. odoriferum</i> from other vegetables	11	19.2 ($\pm$ 5.6)	11.0–28.6
<i>P. c. subsp. carotovorum</i>	28	12.5 ($\pm$ 6.3)	0–15.6
<i>P. c. subsp. brasiliense</i>	6	22.7 ($\pm$ 3.1)	18.0–27.5
<i>P. wasabiae</i> from horseradish and sweet pepper	7	7.8 ($\pm$ 1.6)	5.3–10.0
<i>P. wasabiae</i> from potato	10	17.2 ($\pm$ 2.4)	15.0–19.0
<i>P. atrosepticum</i>	10	14.7 ($\pm$ 6.7)	7.2–24.0
<i>P. betavascularum</i>	8	10.5 ($\pm$ 6.5)	3.8–20.0

24 h at 28 °C, the length and width of the rotten tissue was measured on each leaf, and the average area of rotten tissue was calculated to estimate disease severity.

PCR amplifications, sequencing, and phylogenetic analysis

recA, *rpoS*, and 16S rRNA genes were amplified as described previously (Waleron et al. 2002a, 2008, 2013). The nucleotide sequences of *recA*, *rpoS*, and

16S rRNA genes were determined directly from PCR fragments amplified by the PCR primers used. The obtained sequences were deposited in GenBank under the following accession numbers: AY217078, AY217080, AY217082, AY217084, AY217086, AY264786, AY264789, AY264792, KC584977, KC584986, KC584989, KC584996, KC584997, and KF704760 to KF704816.

BLAST analysis was used to obtain sequences of *recA*, *rpoS*, and 16S rRNA genes from other

Table 3 Comparisons of maceration of potato tubers caused by various *Pectobacterium* species, subspecies, and strains as determined by ANOVA and Tukey's test

Species, subspecies, strains	Species, subspecies, strains									
	all Pco strains	Pco chicory	Pco from other vegetables	Pcc	Pcbr	Pba	Pb	all Pw strains	Pw from horseradish	Pw from potato
all Pco strains	–	–	–	ns	ns	ns	$P<0.05$	ns	$P<0.01$	ns
Pco from chicory		–	ns	ns	ns	ns	ns	ns	$P<0.01$	ns
Pco from other vegetables			–	$P<0.05$	$P<0.05$	ns	ns	ns	$P<0.01$	ns
Pcc				–	$P<0.01$	ns	ns	ns	ns	$P<0.05$
Pcbr					–	$P<0.05$	$P<0.01$	$P<0.01$	ns	$P<0.05$
Pba						–	ns	ns	ns	ns
Pb							–	ns	ns	$P<0.05$
all Pw strains								–	–	–
Pw from horseradish									–	$P<0.01$
Pw from potato										–

Pco: *Pectobacterium c. subsp. odoriferum*. Pco chicory: *Pectobacterium c. subsp. odoriferum* strains isolated from chicory. Pco other vegetables: *Pectobacterium c. subsp. odoriferum* strains isolated from vegetables other than chicory. Pcc: *P. c. subsp. carotovorum*. Pcbr: *Pectobacterium c. subsp. brasiliense*. Pba: *Pectobacterium atrosepticum*. Pb: *Pectobacterium betavascularum*. Pw: *Pectobacterium wasabiae*. Pw horseradish: *Pectobacterium wasabiae* strains isolated from horseradish. Pw potato: *Pectobacterium wasabiae* strains isolated from potato. ns: not significant. $P<0.05$ or 0.01: significant at the 0.05 or 0.01 level

Pectobacterium strains that were most similar to those of *Pectobacterium carotovorum* subsp. *odoriferum*. The sequences were obtained from the following nine *Pectobacterium* genomes: *P. atrosepticum* (BX950851, ASAB00000000), *P. wasabiae* (CP001790, CP003415, AKVS00000000), *P. carotovorum* (ABVY01000000, ABVX01000000, CP003776), and *P. aroidearum* (CP001657). The sequences were aligned using the MUSCLE algorithm with the default settings in Geneious Pro 6.1.6 (www.geneious.com).

The Maximum Likelihood (ML) phylogenetic analyses were performed with MEGA 5 software (www.megasoftware.net). Bootstrapping was executed with 1,000 replications. The *recA*, *rpoS*, and 16S rRNA gene sequences of *Dickeya dadantii* 3937 (CP002038) were used as outgroups.

Statistical analysis

The data were subjected to one-way ANOVAs using SPSS 10.0. Means were compared by the Tukey's tests, and statistical significance was determined at 1 and 5 % levels.

Fig. 1 The genetic distance tree based on the 16S rRNA gene from *Pectobacterium* spp. Sequences obtained from the GenBank are described by accession numbers followed by strains numbers. Bootstrap values after 1,000 replicates are expressed as percentages. Bootstrap values < 50 % are excluded. *Dickeya dadantii* 3936 (CP002038) was included as an outgroup. The analysis used the consensus sequences of seven copies of the 16S rRNA gene from available genome sequences of: *P. atrosepticum* SCRI1043 (BX950851) and CFBP6276 (ASAB00000000); *P. wasabiae* CFBP3304 (AKVS00000000), WPP163 (CP001790), and SCC3193 (CP003415); *P. c. subsp. carotovorum* WPP14 (ABVY00000000) and PCC21 (CP003776); *P. c. subsp. brasiliense* (ABVX00000000); and *P. aroidearum* PC1 (CP001657). The consensus sequences are marked with stars

Results

Phenotypic characterization and classification of bacterial isolates

All 91 strains were Gram-negative rods, facultatively anaerobic, and negative for oxidase and urease. All 91 strains degraded pectate, produced indol, liquefied gelatin, and grew at 28 °C. The strains isolated from different vegetables were further identified using PCR assays with primers specific for *P. carotovorum* (Darrase et al. 1994),

Table 4 Effect of temperature on the diameter of rotting tissue in potato tubers inoculated with *P. carotovorum* subsp. *odoriferum* strains isolated from chicory and other vegetables

Strain	Diameter (± mm) of a rotting potato tuber tissue at different temperatures				
	10 °C	15 °C	20 °C	28 °C	37 °C
IFB5285 CFBP1878 ^T	1.8 (± 0.2)	4.5 (± 0.4)	11 (± 0.1)	11.6 (± 0.1)	8.8 (± 0.2)
IFB5283	0	0	7.2 (± 0.1)	14.6 (± 0.1)	9.5 (± 0.4)
IFB5286	2.1 (± 0.0)	5.9 (± 0.1)	15.4 (± 0.4)	19 (± 0.2)	12.7 (± 0.2)
IFB5287	nt	6.1 (± 0.1)	11.17 (± 0.2)	16.6 (± 0.3)	11.2 (± 0.1)
IFB5289	nt	7.9 (± 0.1)	11.3 (± 0.1)	17.8 (± 0.1)	10.9 (± 0.1)
IFB5293	3.0 (± 0.3)	6.0 (± 0.1)	7.5 (± 0.1)	28.0 (± 0.1)	20 (± 0.0)
IFB5291	0	8.7 (± 0.4)	11.0 (± 0.1)	26.5 (± 0.5)	13.3 (± 0.3)
IFB5292	0	9.3 (± 0.1)	11.0 (± 0.2)	26.0 (± 0.5)	12.3 (± 0.5)
IFB5294	3.3 (± 0.1)	2.0 (± 0.1)	nt	18.6 (± 0.1)	5.3 (± 0.2)
IFB5295	0	2.0 (± 0.2)	5.0 (± 0.1)	16.0 (± 0.1)	6.0 (± 0.2)
IFB5296	2.6 (± 0.1)	5.7 (± 0.4)	14.0 (± 0.1)	17.6 (± 0.2)	12.0 (± 0.3)
IFB5297	1.2 (± 0.1)	7.7 (± 0.1)	12.0 (± 0.1)	12.6 (± 0.1)	9.7 (± 0.1)
IFB5298	0	0	6.3 (± 0.1)	17.8 (± 0.1)	5.3 (± 0.1)
IFB5299	0	1.7 (± 0.3)	12.6 (± 0.2)	28.6 (± 0.1)	5.3 (± 0.1)
IFB5300	5.3 (± 0.1)	8.0 (± 0.1)	11.5 (± 0.1)	20.0 (± 0.1)	14.0 (± 0.1)

Values are means ± SD of three experiments with three replicate tubers per combination of strain and temperature in each experiment. Inoculated potato tubers were maintained for 72 h at the indicated temperature

P. atrosepticum (De Boer and Ward 1995), and *Dickeya* spp. (Nassar et al. 1996). All of tested strains except for *P. betavascularum* generated PCR products with the *P. carotovorum*-specific primers (Darrase et al. 1994). None of the isolates was positive in the PCR assay with *Dickeya* spp.-specific primers (Nassar et al. 1996). Ten strains were identified as *P. atrosepticum* using PCR assay with primers specific for this species (De Boer and Ward 1995).

The biochemical properties of the eight strains, IFB5294-IFB5301, isolated from carrot, celery, leek, and onion in Poland, as well as two strains from parsley in Serbia (IFB5291, IFB5292) and one Swiss strain (IFB5293) from chicory, were identical with those observed for *P. c. subsp. odoriferum* strains isolated in France from chicory, celery, and leek (IFB5279-IFB5290), except for the lack of tolerance to 5 % NaCl in the medium.

All tested strains were negative for acid production from D-arabitol, dulcitol, and sorbitol. They were unable to utilize malonate, citrate, L-arginine, L-ornitine, and L-lysine. However, they were catalase positive and produced acid from lactose, maltose rhamnose, and trehalose. Also, they grew at 37 °C. The biochemical characteristics indicated that strains from Poland, Serbia, and Switzerland are *P. c. subsp. odoriferum*.

The abilities of *P. c. subsp. odoriferum* strains and strains from the other *Pectobacterium* species and subspecies to macerate potato tubers and chicory leaves were compared (Table 1). The mean diameter of rotting tissue in inoculated potato tubers was 18.4 mm for *P. c. subsp. odoriferum*, 17.2 mm for *P. wasabiae* isolated from potato, 7.8 mm for *P. wasabiae* isolated from horseradish and sweet pepper, 10.5 mm for *P. betavascularum*, 11.6 mm for *P. c. subsp. carotovorum*, and 22.8 mm for *P. c. subsp. brasiliense*. Furthermore, the rotting in potato tubers caused by *P. c. subsp. odoriferum* was slightly higher for strains isolated from carrot, leek, parsley, and onion than for strains isolated from chicory (Table 2).

ANOVA and Tukey's tests were used to compare the statistical significance of the observed differences in the ability to macerate of plant potato tubers tissues by strains belonging to different species and subspecies (Table 3). Maceration was significantly greater for strains belonging to the subspecies *P. c. subsp. odoriferum* than for *P. betavascularum* strains and *P. wasabiae* strains isolated from horseradish. Moreover, maceration was significantly greater for *P. c.*

subsp. odoriferum strains isolated from chicory than for *P. wasabiae* strains isolated from horseradish, and was greater for *P. c. subsp. odoriferum* strains isolated from vegetables other than chicory than for strains *P. c. subsp. carotovorum* strains, and *P. wasabiae* strains isolated from horseradish, but it was less than for *P. c. subsp. brasiliense* strains. Maceration was also greater for *P. c. subsp. brasiliense* strains than for strains of *P. c. subsp. carotovorum*, *P. atrosepticum*, and *P. wasabiae* (Table 3).

Maceration of potato tubers by 15 *P. c. subsp. odoriferum* strains was determined at 10, 15, 18, and 37 °C. None of 15 strains was able to macerate efficiently potato tubers at 10 °C (Table 4). All strains induced soft rot symptoms on potato tubers at temperatures from 15 to 37 °C. Tissue maceration was greatest at 28 °C. No maceration was observed on control water-inoculated control tubers.

All *P. c. subsp. odoriferum* strains in this study were able to macerate of chicory leave tissue. Maceration of chicory leaves was similar for *P. c. subsp. odoriferum* strains isolated from carrot, leek, parsley, and onion vs. *P. c. subsp. odoriferum* strains isolated from chicory (Table 1). Of the 61 *Pectobacterium* strains used in this experiment, the average value of the rotten area of chicory leaf tissue was highest for *P. c. subsp. odoriferum* strains (Table 1).

Molecular characterization of *P. c. subsp. odoriferum* strains

The 26 *recA*, 22 *rpoS* and 23 16S rDNA gene sequences were obtained. The sequences of *P. c. subsp. odoriferum* strains isolated from chicory were compared with those of *P. c. subsp. odoriferum* strains isolated from other Vegetables.

A Blast analysis of the 10 16S rRNA gene sequences obtained from *P. c. subsp. odoriferum* strains isolated from other vegetables (i.e., not from chicory) with those available in GenBank indicated that strains isolated from other vegetables exhibited 100 % identity with the type strain of *P. c. subsp. odoriferum* CFBP1878^T and other *P. c. subsp. odoriferum* strains isolated from chicory.

The comparison of the *recA* and *rpoS* gene sequences of 14 of the 22 analyzed *P. c. subsp. odoriferum* strains showed that they were almost identical. Only one polymorphic position was observed in the amplified 735-bp fragment of *recA*, and two polymorphisms were observed in the 780-bp fragment of *rpoS* sequences.

The topologies of maximum likelihood trees based on 16S rRNA (Fig. 1), *recA* (Fig. 2), and *rpoS* (Fig. 3) genes were congruent. The Swiss strain, as well as Polish and

Serbian strains, isolated from different vegetables were clustered with the type strain of *P. c. subsp. odoriferum* CFBP1878^T and other *P. c. subsp. odoriferum* strains

Fig. 2 The consensus Maximum Likelihood tree based on the 735-bp fragment of the *recA* gene from *Pectobacterium* spp. Sequences obtained from the GenBank are described by accession numbers followed by strains numbers. Bootstrap values after

1,000 replicates are expressed as percentages. Bootstrap values < 50 % are excluded. *Dickeya dadantii* 3936 (CP002038) was included as an outgroup

Fig. 3 The consensus Maximum Likelihood tree based on the 780-bp fragment of the *rpoS* gene from *Pectobacterium* spp. Sequences obtained from the GenBank are described by accession numbers followed by strains numbers. Bootstrap values after 1,000 replicates are expressed as percentages. Bootstrap values < 50 % are excluded. *Dickeya dadantii* 3936 (CP002038) was included as an outgroup

isolated from chicory, and these clustered separately from the closely related subspecies *P. c.* subsp. *carotovorum* and *P. c.* subsp. *brasiliense*.

Discussion

In this study, we used several biochemical and molecular techniques to determine that *P. c.* subsp. *odoriferum* occurs on stored vegetables in Poland and Serbia. We have proved that the *P. c.* subsp. *odoriferum* subspecies was present in Poland already by 1995; however it was misidentified and classified as *P. c.* subsp. *carotovorum*. PCR-RFLP analysis of the *recA* gene was previously used to discriminate 18 different profiles among *P. carotovorum* strains (*recA* PCR-RFLP profiles 3–20) (Waleron et al. 2002a). Recently, strains assigned to *recA* PCR-RFLP profile 3 have been reclassified as *P. wasabiae* (Waleron et al. 2013). All *P. c.* subsp. *odoriferum* strains isolated from chicory were assigned to profile 22 *recA* PCR-RFLP (Waleron et al. 2002a). However, strains isolated from other vegetables and exhibiting biochemical characteristics typical of *P. c.* subsp. *odoriferum* strains were described as belonging to *recA* PCR-RFLP profile 13 (Waleron et al. 2002a). In the current study, the topologies of the maximum likelihood trees based on the 16S rRNA, *recA*, and *rpoS* genes clustered the Polish strains, one Swiss strain, and two Serbian strains, which belong to *recA* PCR-RFLP profile 13 (Waleron et al. 2002a) with *P. c.* subsp. *odoriferum*, and these strains clustered separately from the closely related subspecies *P. c.* subsp. *carotovorum* (Figs. 1, 2 and 3). The double-strand conformation polymorphism (DSCP) effect was the reason for assigning different *recA* PCR-RFLP profiles (13 and 22), for *P. c.* subsp. *odoriferum* strains (Waleron et al. 2002a) similarly as it was in case of *recA* PCR-RFLP profiles (3 and 23) for *P. wasabiae* strains from potato (Waleron et al. 2002a, 2013). According to our analysis of 16S rRNA, *recA*, and *rpoS* genes, those *P. c.* subsp. *carotovorum* strains that were previously identified as

profile 13 *recA* PCR-RFLP (Waleron et al. 2002a) should be reclassified as *P. c.* subsp. *odoriferum*. Our results agree with MLSA studies of Nabhan et al. (2012b), which showed that Swiss strain 582 (IFB5293) is *P. c.* subsp. *odoriferum*. Although our study clearly indicates that *P. c.* subsp. *odoriferum* strains are genetically homogeneous, the *P. c.* subsp. *odoriferum* strains isolated from chicory were phenotypically slightly different from those isolated from other vegetables and caused less potato tuber maceration. However, it is too early to draw a general conclusion because only a limited number of strains were analyzed. The most significant difference was that strains from chicory but not from other vegetables could grow on a medium containing 5 % NaCl. Phenotypic diversity among *P. c.* subsp. *odoriferum* strains was also documented by Lan et al. (2013). The atypical phenotypic features of some *P. c.* subsp. *odoriferum* strains (e.g., resistance to 5 % NaCl in the medium) together with the DSCP effect observed in the RFLP analysis of the chosen genes could explain their misidentification in the past.

To our knowledge, this is the first report showing that *P. c.* subsp. *odoriferum* can cause soft rot of different vegetables during storage. Given that ‘Witloof’ chicory is grown underground or indoors in the absence of sunlight, the environment during chicory storage is similar to that during the storage of potatoes and other vegetables. We also note that the symptoms produced by *P. c.* subsp. *odoriferum* on stored chicory are similar to those produced on potato and other vegetables in storage.

Contrary to the results of Nabhan et al. (2012b), this study demonstrates that, at least under laboratory conditions, *P. c.* subsp. *odoriferum* strains are able to induce soft rot of potato tubers. In fact, the ability to macerate potato tuber tissues at 28 °C was greater for *P. c.* subsp. *odoriferum* (and also for *P. c.* subsp. *brasiliense* and *P. wasabiae* strains from potato) than for other *Pectobacterium* species and subspecies. However, 28 °C is higher than the optimal temperature for maceration by *P. atrosepticum*. Our results indicate that *P. c.* subsp. *odoriferum* subspecies are able to cause potato tuber tissue maceration like other potato-infecting species, e.g., *P. atrosepticum*, *P. wasabiae*, and subspecies *P. c.* subsp. *carotovorum*, which have been reported to cause disease on different plant species. Potato growers and processors should be concerned with the possibility that *P. c.* subsp. *odoriferum* could be transferred to potato tubers during storage. In the case of seed potatoes, the planting of the infected materials (obviously not

tested for the presence of *P. c. subsp. odoriferum*) could result in losses. However it must be underlined that its ability to cause diseases symptoms on plants in field and storage conditions has to be confirmed.

P. c. subsp. odoriferum was first isolated in western Europe (Belgium, France, and Switzerland) (Gallois et al. 1992; Samson et al. 1980) and has since been reported in Poland, southern Europe (Gallelli et al. 2009), Africa (Yahiaoui-Zaidi et al. 2010), and Asia (Nazerian et al. 2011; Thapa et al. 2011; Lan et al. 2013). Although *P. c. subsp. odoriferum* strains have been isolated from potatoes in Korea (Thapa et al. 2011) and Algeria (Yahiaoui-Zaidi et al. 2010), the publications did not indicate when disease symptoms on potatoes were observed or the precise date of strain isolation.

P. c. subsp. odoriferum may also be present but incorrectly identified in other countries because distinguishing *P. c. subsp. odoriferum* from *P. c. subsp. carotovorum* is difficult. Methods relying on biochemical assays or restriction analysis of *pel* genes cannot always distinguish between these two groups. In addition, the use of MLSA does not always allow for the unambiguous identification of *P. carotovorum* (Nabhan et al. 2012b). The combination of biochemical and molecular methods allow for more effective differentiation of *P. c. subsp. odoriferum* from *P. c. subsp. carotovorum*.

In conclusion, the results presented here show that *P. c. subsp. odoriferum* strains can induce disease symptoms on potato and other vegetables in addition to chicory. Because *P. c. subsp. odoriferum* is widely distributed, its possible impact on vegetable production should be considered.

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