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Assessment of recent outbreaks of *Dickeya* sp. (syn. *Erwinia chrysanthemi*) slow wilt in potato crops in Israel

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Abstract Suspected *Dickeya* sp. strains were obtained from potato plants and tubers collected from commercial plots. The disease was observed on crops of various cultivars grown from seed tubers imported from the Netherlands during the spring seasons of 2004–2006, with disease incidence of 2–30% (10% in average). In addition to typical wilting symptoms on the foliage, in cases of severe infection, progeny tubers were rotten in the soil. Six strains were characterised by biochemical, serological and PCR-amplification. All tests verified the strains as *Dickeya* sp. The rep-PCR and the biochemical assays showed that the strains isolated from blackleg diseased plants in Israel were very similar, if not identical to strains isolated from Dutch seed potatoes, suggesting that the infection in Israel originated from the Dutch seed. The strains were distantly related to *D. dianthicola* strains, typically found in potatoes in Western Europe, and were similar to biovar 3 *D. dadanti* or *D. zeae*. This is the first time that the presence of biovar 3 strains in potato in the Netherlands is described. One of the strains was used for pathogenicity assays on potato cvs Nicola and Mondial. Symptoms appeared 2 to 3 days after stem inoculation, and 7 to 10 days after soil inoculation. The control plants treated with water, or plants inoculated with *Pectobacterium carotovorum*, did not develop any symptoms with either method of inoculation. The identity of *Dickeya* sp. and *P. carotovorum* re-isolated from inoculated plants was confirmed by PCR and ELISA.

Keywords *Solanum tuberosum* · *Erwinia* · Latent infection · Seed tuber

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Introduction

Erwinia chrysanthemi disease on potato in Israel had been previously reported only once, in the 1980s (Lumb et al. 1986), in plants grown from seed tubers imported from the Netherlands. However, since 2004 the disease is occurring more frequently, causing economic damage.

Erwinia chrysanthemi was recently reclassified into six *Dickeya* sp. (Samson et al. 2005). Different *Dickeya* spp. cause disease on different hosts, under different climatic conditions. The new classification distinguishes the *Dickeya* spp. from the other potato soft rot *Erwinia carotovora* spp., recently classified into the genus *Pectobacterium* (Samson et al. 2005). *Dickeya* spp. is recognised as an important pathogen of potato (Pérombelon 2002) in many areas, including Northern Europe. It was recently reported in Spain (Palacio-Bielsa et al. 2006) and in Finland (Joutsjoki et al. 2005). The important *Dickeya* spp. causing disease on potato are (biovar 3) strains of *D. dadantii* and *D. zeae*, which are pathogens of potato in warm climates, and the more temperate-adapted (biovar 1and 7) strains of *D. dianthicola* that appear to be spreading on potato in Europe (Samson et al. 2005). Disease symptoms are variable, including pre-emergence tuber decay in semi-arid areas (Cother 1980), extensive stem rotting in tropical areas (de Lindo et al. 1978), wilt associated with limited stem rot in cool-temperate regions (Tanii and Baba 1971), and wilt without stem rot in Israel (Lumb et al. 1986). Highly humid environmental conditions tend to reduce desiccation and favour soft rot of infected tissues (Lumb et al. 1986; Serfontein et al. 1991). Disease symptoms in the recent outbreaks in Israel first appear as wilt of the top leaves, which spreads to the lower ones, followed by desiccation. Usually, discolouration of the vascular system in the stem base is observed, followed by external darkening. In severe infections, the stem or whole plant dries out. Symptoms are usually associated with soft rot of the mother tuber, and sometimes (depending on the level of infection), daughter tubers are also rotten.

The objectives of the present study were to confirm the identity of *Dickeya* as the causal organism of slow wilt in potato crops grown from seed imported from the Netherlands, and to assess the impact of *Dickeya*-infections on disease expression in Israel.

Materials and methods

Isolation

Diseased potato plants showing wilt of the top leaves with or without stem pith necrosis, usually with brown internal necrosis at the stem base, were collected from commercial potato plots (Table 1). Stems were surface-sterilised in 0.3% (*v*/*v*) NaOCl for 3 min, washed in running tap water, and dried in the laminar

Table 1 Year, cultivar, quantity of seed lots, disease incidence and number of symptomatic plants or tubers collected from which *Dickeya* sp. was isolated

Year	Cultivar	Quantity (ton)	Disease Incidence in commercial fields (%)[a]	No. of plant samples
2004	Desiree			2
	Mondial			20
		2165	10	
2005	Mondial			9 (+3 tuber samples)
	Desiree			7
	Sapphire			2 (+3 tuber samples)
	Quincy			1
	Lady Crystal			1
	Nicola			1 tuber sample
	Spunta			1
		1824	5–30, average 8.2	
2006	Mondial			4 (+1 tuber sample)
	Desiree			7
	Nicola			1
	Sante			1
	Quincy			2
	Platina			1 tuber sample
	Vivaldi			1
		3317	2–30, average 10	

[a] Disease incidence was evaluated by officers of the Plant Protection Israeli Services (PPIS)

flow cabinet. Ten segments (each 20 mm long) taken from the stem base of each plant were macerated with 6 ml sterile distilled water (SDW) in an Ultraturrax blender (Janke Kunkel) for 60 to 90 s at 4°C. Tubers with no visible symptoms collected from affected stems were surface-sterilised in 0.3% NaOCl for 3 min and rinsed with SDW. Segments (5–7 g) taken from the stolon end (including both the vascular bundles and the peel) were ground as described above. The suspensions were streaked on modified crystal violet pectate (CVP) selective medium (Pérombelon and Hyman 1986) and incubated at 27°C and 33.5°C for 48–72 h.

Characterisation of strains

Single colonies obtained on CVP medium were purified by repeated sub-culturing on nutrient agar (NA, Difco), and selected strains were further characterised. *Pectobacterium atrosepticum* (Pa; strain SCRI 1043) and *P. carotovorum* subsp. *carotovorum* (Pcc; strain SCRI 193), obtained from L. Hyman (SCRI, UK), were included for comparison in biochemical, physiological, molecular and serological tests. All strains were kept at −80°C in 30% (*v*/*v*) glycerol, or in water at ambient temperature. For characterisation, 24 to 48 h-old cultures on NA were used.

Biochemical identification and classification of strains

The strains were identified by standard bacteriological methods; (Cother and Powell 1983; Dickey 1979) using the following tests: indole production from tryptophan, production of phosphatase, and sensitivity to erythromycin (Cother et al. 1992; Janse and Spit 1989; Pérombelon and van der Wolf 1998). The strains were also classified using the microtiter plate assay described by Palacio-Bielsa et al. (2006) with few modifications as described below. This included growth at 39°C, 41°C and 25°C (control) on nutrient broth (NB, OXOID; Dye 1969), anaerobic hydrolysis of arginine (Moeller 1955), and polysaccharide inulin utilisation in phenol red peptone water (inulin extracts were used from chicory and dahlia; in a 0.3% final concentration). Eight carbon sources were tested by acidification/alkalisation on liquid Ayers, Rupp and Johnson medium (Ayers et al. 1919) with bromothymol blue mixed with different 0.3% carbohydrates: (−)-D-arabinose, 5-keto-D-gluconate, mannitol, (+)-D-melibiose, (+)-D-raffinose and (−)-D-tartrate, β-gentiobiose and (+)-L-tartrate. The different basal media (150 µl) were dispensed on a sterile culture microplate (Greiner bio-one, Cellstar), and 15 µl of bacterial suspension of 10^8 cells ml^{-1} of each strain to be analysed were added per well. Plates which contained arginine, were covered with a layer (100 µl) of sterile glycerol to obtain anaerobic conditions. All 96-well microplates were wrapped in parafilm, and incubated at 25°C for 72 h, except for (−)-D-arabinose, for which an incubation period of 96 h was used. Plates were observed every 24 h and tests were completed after 120 h of incubation. Tests were performed three times in separate microplates and experiments, and each time four wells per assay were used. Strains IPO 981 (biovar 7), IPO 982 (biovar 1) and IPO 2017 (biovar 3) were used as controls.

Serological identification

Enzyme-linked immunosorbent assay (ELISA) was performed in microplates (NuncMaxiSorp™ Brand products; two wells per strain), using polyclonal antibodies (Neogen Europe) according to the manufacturer's instructions. The absorbance was measured with an automatic reader at 405 nm (A_{405}) and, after 1 h, A_{405} values over twofold the mean value of negative controls (supplied by the commercial ELISA sets) were considered to be positive. *Dickeya dianthicola* supplied by the commercial ELISA sets, and Pa and Pcc reference strains (obtained from SCRI) were also included in these tests.

PCR identification

Bacterial genomic DNA was extracted from either bacterial suspensions in SDW (10^8 cells ml^{-1}), prepared from 24 h cultures in NB medium, or pellets of filtered homogenised plant material that was centrifuged for 2 min at 12,000×*g*. The DNA was extracted and purified using a GenElute™ bacterial genomic DNA kit as described by the manufacturer (Sigma-Aldrich). PCR was performed in 25 µl reaction volumes containing 12.5 µl reaction buffer—Reddy Mix PCR Master Mix (ABgene House) containing 1.25 U DNA polymerase, 75 mM Tris–HCL pH 8.8, 20 mM $(NH_4)_2SO_4$, 1.5 mM $MgCl_2$, 0.01% (*v*/*v*) Tween^R 20, 0.2 mM each of dATP, dCTP, dGTP, and dTTP—5 pmol each of the *D. dianthicola*-specific

primer pectate lyase-encoding gene cluster, *pel*ADE (ADE1/ADE2; Nassar et al. 1996) and 12.5 µl of DNA or bacterial suspensions. DNA amplification was performed using a Biometra T- Gradient Thermoblock. PCR conditions consisted of 2 min at 94°C, followed by 35 cycles of 45 s at 94°C and 45 s at 72°C, 2 min at 72°C, and a final extension at 72°C for 3 min (PCR product size 450 bp). PCR was also performed with general primers G1 and L1 (Toth et al. 2001), under conditions of 5 min at 94°C, 25 cycles of 1 min at 94°C, 2 min ramp to 55°C; 7 min at 55°C; 2 min ramp to 72°C; 2 min at 72°C, and a final extension at 72°C for 7 min (PCR product size: Ech—440, 590 bp and Pcc—540, 575 bp). Amplified DNA fragments (12.5 µl) were run on a 1.5% agarose gel at 80 V for 1 h and visualised under UV light following ethidium-bromide staining. Pa and Pcc reference strains were also included.

Rep-PCR genomic fingerprinting identification and characterisation

Bacterial genomic DNA was isolated from bacterial suspensions in SDW (10^8 cells ml^{-1}), prepared from 48 h cultures on TSA medium (Tryptone Soya Agar, Oxoid). The DNA was purified using the silica beads method (Frechon et al. 1998). The PCR conditions were used as described by Rademaker et al. (1997), with few modifications as described below. Primers REP1R (5′-IIIICGICGICATCIGGC-3′) and REP2I (5′-ICGICTTATCIGGCCTAC-3′) were used (Versalovic et al. 1991). The PCR mixture (27.075 µl) consisted of: 1× PCR buffer [16.6 mM $(NH_4)_2SO_4$, 67 mM Tris–HCl, pH 8.8, 10.05 mM $MgCl_2$, 6.7 µM EDTA and 30 mM β-mercapto-ethanol], 1.13 mM dNTPs, 3.69 µM of each primer, 3 U Taq DNA polymerase (Roche Diagnostics GmbH, Germany) and 10 µl of template DNA. PCR amplifications were performed in a DNA Engine Peltier thermal cycler (Bio-Rad) with an initial denaturation (95°C, 7 min) followed by 40 cycles of denaturation (94°C, 1 min), annealing (40°C, 2 min), and extension (65°C, 8 min) with a single final extension step (65°C, 16 min). The PCR products were analysed on 1.5% agarose gel containing ethidium bromide. Patterns were analysed using the Quantity One programme (Bio-Rad). A phylogenetic tree was constructed using the UPGMA (un-weighted pari-group method using arithmetic means) tree building method.

Pathogenicity tests

Potato plantlets (6 weeks-old) of cvs Mondial and Nicola were grown in 20 cm diameter pots with sterile sand in a temperature-controlled greenhouse (28–30°C/22–24°C day/night temperatures). Inoculations were carried out either by injecting 10 µl bacterial suspension (10^8 cells ml^{-1}) into the stem at the third node from the stem base (the injection point was immediately covered with a parafilm strip) or by soil inoculation: 20 ml of the bacterial suspension was added to each pot in four holes. Only one representative *Dickeya* sp. strain (G-87) was used for the pathogenicity test (Table 2). Control plants were inoculated as already described

Table 2 *Dickeya* spp. and *Pectobacterium* strains used in the study

Strain	Species	Source	Location	Date	Crop/Cultivar
G-87	*Dickeya* sp.	Israel	Nir Itshak	22/2/06	Potato, Sapphire
G-115	*Dickeya* sp.	Israel	Kisufim	23/4/06	Potato, Sante
G-118	*Dickeya* sp.	Israel	Nevatim	24/4/06	Potato, Desiree
G-120	*Dickeya* sp.	Israel	Or Haner	2/5/06	Potato, Quincy
G-121	*Dickeya* sp.	Israel	Kerem Shalom	2/5/06	Potato, Quincy
G-122	*Dickeya* sp.	Israel	Kisufim	11/5/06	Potato, Sante[a]
1991	*D. dianthicola*	the Netherlands			Potato
IPO 981	*D. dianthicola*	the Netherlands			Potato
IPO 982	*D. dianthicola*	the Netherlands			Potato
IPO 2222	*Dickeya* sp.	the Netherlands			Potato
IPO 2225	*Dickeya* sp.	the Netherlands			Potato
IPO 2017	*Dickeya* sp.	the Netherlands			Hyacinth
SCRI 1043	*Pectobacterium atrosepticum*	Scotland			Potato
SCRI 193	*P. carotovorum*	Scotland			Potato

[a] All G-strains were obtained from a stem, except G-122 which was isolated from a daughter tuber.

Table 3 Biochemical identification and characteristics of Dutch and Israel *Dickeya* sp. potato strains used in the study

Test	Strain										
	G-87	G-115	G-118	G-120	G-121	G-122	IPO 2222	IPO 2225	IPO 981	IPO 982	IPO 2017
Growth at 39°C on NB	+w	+w	+w	+w	+w	+w	+w	+w	−	−	+
Growth at 41°C on NB	−	−	−	−	−	−	−	−	−	−	−
Raffinose	+	+	+	+	+	+	+	+	−	+	+
Melibiose	+	+	+	+	+	+	+	+	−	+	+
Mannitol	+	+	+	+	+	+	+	+	+	+	+
Inulin (from chicory)	−	−	−	−	−	−	−	−	−	−	−
Inulin (from dahlia)	−	−	−	−	−	−	−	−	−	−	−
D(−) tartrate	−	−	−	−	−	−	−	−	+	+	−
D(−) arabinose	+	+	+	+	+	+	+	+	−	−	+
Arginine dihydrolase	−	−	−	−	−	−	−	−	+	+	−
5-ketogluconate	−	−	−	−	−	−	−	−	−	−	−
B-gentobiose	+w	+w	+w	+w	+w	+w	+w	+w	−	−	+
(+)-L-Tartrate	−	−	−	−	−	−	−	−	−	−	−
Growth at 25°C (control)	+	+	+	+	+	+	+	+	+	+	+

+ Positive, − negative, +*w* weak growth or weak positive reaction.

with Pcc (SCRI 193) or with SDW. Each bacterial strain was inoculated into 20 plants of each cultivar, by each inoculation method. Plants were examined daily for symptom development for 14 days after inoculation. Isolations from inoculated plants were performed on CVP medium, and colony identification was confirmed by morphology, biochemical tests and PCR.

Results

Disease prevalence, collection and identification of strains as *Dickeya* sp.

In spring 2004, the disease was observed in several potato fields, only on cvs Mondial and Desirée. In spring 2005, a severe outbreak of the disease was observed in various cultivars (including Mondial, Desirée, Lady Crystal, Sapphire and Quincy) at different locations (Table 1). In addition to symptoms on the foliage, progeny tubers were also rotten in the soil. Disease incidence ranged from 5% to 30% (8.2% on average). In spring 2006, the disease was again observed at different locations in various cultivars (Mondial and Desirée being the major ones), with disease incidence that ranged from 2% to 30% (10% in average; Table 1). All samples collected from plants or tubers from the commercial plots (Table 1) were found to be positive to *Dickeya* sp. by ELISA or PCR tests. Only six randomly selected strains were purified and further characterised (Table 2).

Biochemical assays

The biochemical tests for production of phosphatase and indole, and the sensitivity to erythromycin agreed with those expected for *Dickeya* sp. (Hyman et al. 1998). The six potato strains from Israel were biochemically identical to strains recently isolated from Dutch seed potatoes (IPO 2222 and IPO 2225) and very similar to a Dutch strain from hyacinth (IPO 2017; Table 3). They grew weakly at 39°C but not at 41°C, they produced acids on raffinose, melibiose, mannitol, D(-)arabinose, and (weakly) B-gentobiose, but not on D(-)tartrate, 5-ketogluconate and (+)-

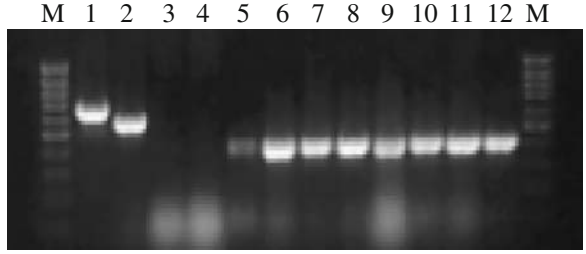

Fig. 1 Characterisation of strains with PCR-amplification, using specific primers ECAfr, EXPCCfr and ADE12. *1 Pectobacterium atrosepticum* (Pa) SCRI 1043 analysed with primers ECAfr; *2 P. carotovorum* subsp. *carotovorum* (Pcc) SCRI 193 analysed with primers EXPCCfr; *3–12* bacterial strains analysed with primers ADE12; *3* Pa SCRI 1043; *4* Pcc SCRI 193; *5 Dickeya* G87; *6 Dickeya* 1991; *7 Dickeya* G121; *8 Dickeya* G120; *9 Dickeya* G115; *10 Dickeya* G122; *11 Dickeya* G118; *12 Dickeya* G87; *M* 100-bp DNA ladder

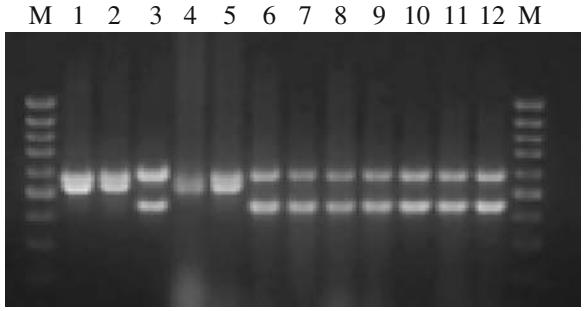

Fig. 2 Characterisation of strains with PCR-amplification, using universal primer L1G1. Bacterial DNA: *1 Pectobacterium atrosepticum* (Pa) SCRI 1043; *2 P. carotovorum* subsp. *carotovorum* (Pcc) SCRI 193; *3* Ech1991; Bacterial strains, : *4* Pa (SCRI 1043); *5* Pcc (SCRI 193); *6* Ech1991; *7 Dickeya* G121; *8 Dickeya* G120; *9 Dickeya* G115; *10 Dickeya* G122; *11 Dickeya* G118; *12 Dickeya* G87; *M* 100-bp DNA ladder

L-tartrate. No anaerobic hydrolysis of arginine or inulin assimilation was found. On the basis of these results, these strains were classified as biovar 3. Strains were clearly different from Dutch *D. dianthicola* strains IPO 981 and IPO 982, isolated in the past from potato.

Serological identification

ELISA results also confirmed the identity of presumed *Dickeya* strains. Antibodies reacted with all strains, as well as with the reference supplied with the kit, with mean absorbance values >10 times the mean values of the negative controls. No cross-reactions were observed with Pa and Pcc strains (data not shown).

Molecular identification

The expected 420 bp PCR-amplified fragment, corresponding to the conserved regions of the *Dickeya* sp. pectate lyase-encoding gene cluster (*pel*ADE; Nassar et al. 1996), was obtained with all strains tested (Fig. 1). Identical results were observed for the reference *Dickeya* sp. (Ech1991). As expected, no PCR products were obtained for Pa or Pcc. In an additional analysis, the expected 440 to 450 bp and 590 bp PCR-amplified fragments, corresponding to the conserved 16S-23S ITS (ITS-PCR) regions of *Dickeya* sp., were obtained for all strains tested (Fig. 2). Identical results were observed for the reference *Dickeya* sp. (Ech1991). PCR products obtained for Pa and Pcc were 540 and 575 bp PCR-amplified fragments, respectively (Toth et al. 2001).

Rep-PCR analysis followed by a cluster analysis was done on a selection of two biovar 3 strains from Israel (G87 and G122), was compared with two biovar 3 strains recently isolated from seed potatoes in the Netherlands (IPO 2222 and IPO 2225), two *D. dianthicola* strains (biovar 7) isolated from Dutch seed in the past (IPO 1991 and 982) and a Dutch biovar 3 strain from hyacinth (IPO 2017). All biovar 3 potato strains were identical, but could be distinguished from the hyacinth biovar 3 strain (Fig. 3). The *D. dianthicola* biovar 7 strains were similar to each other and differed from the biovar 3 strains. Fourteen strains were tested with biochemical assays and 16S rDNA sequencing (unpublished results). They were all identical. Only these four strains were

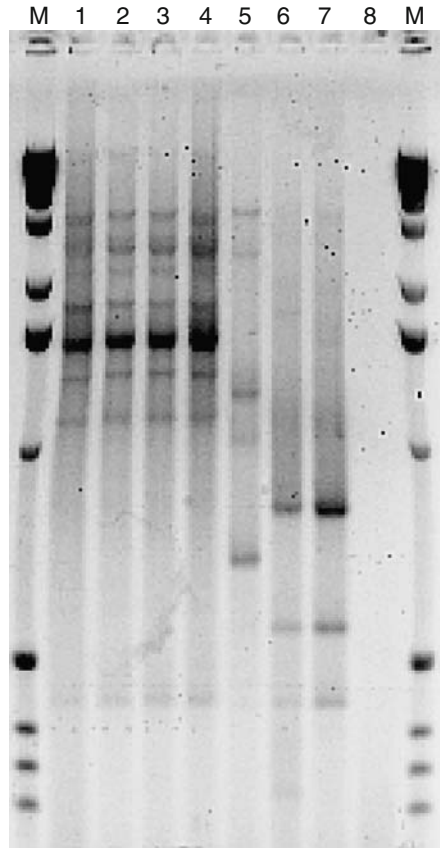

Fig. 3 Rep-PCR patterns of *Dickeya* strains. *M* Molecular markers, *1 Dickeya* sp. 2222 (potato, Netherlands), *2 Dickeya* sp. 2225 (potato Netherlands), *3 Dickeya* sp. G87 (PRI nr. 2187, potato, Israel), *4 Dickeya* sp. G122 (PRI nr. 2190, potato, Israel) *5 Dickeya* sp. 2017 (hyacinth, Netherlands), *6 D. dianthicola* 1991 (potato, Netherlands), *7 D. dianthicola* 982 (potato, Netherlands), *8* H_2O

analysed in REP-PCR. Although variation may exist within this group of biovar 3 strains, it is unlikely

Pathogenicity tests

Symptoms appeared 2 to 3 days after stem inoculation, and 7 to 10 days after soil inoculation. Variations in symptom severity were observed between replicate plants, with stem rotting sometimes appearing only at the inoculation point, or brown lesions developing at the injection site and spreading both upwards and downwards along the stems. Wilting of the leaves, followed by desiccation and eventually, complete collapse of stems and death of the inoculated plants was observed (Fig. 4). With plants subjected to soil inoculation, wilting of the top leaves was accompanied by brown discolouration of the vascular bundle at the stem base. Symptoms were observed first in cv. Nicola and later in cv. Mondial, but differences in severity of symptoms were negligible. The control plants inoculated with Pc or treated with SDW did not develop any symptoms with either method of inoculation.

When re-isolation from inoculated plants was performed (on CVP medium), typical pectolytic colonies were observed, whereas no cavity-forming colonies were obtained from the control plants. Purified single pectolytic colonies exhibited positive PCR (Fig. 5) and ELISA test results (data not shown).

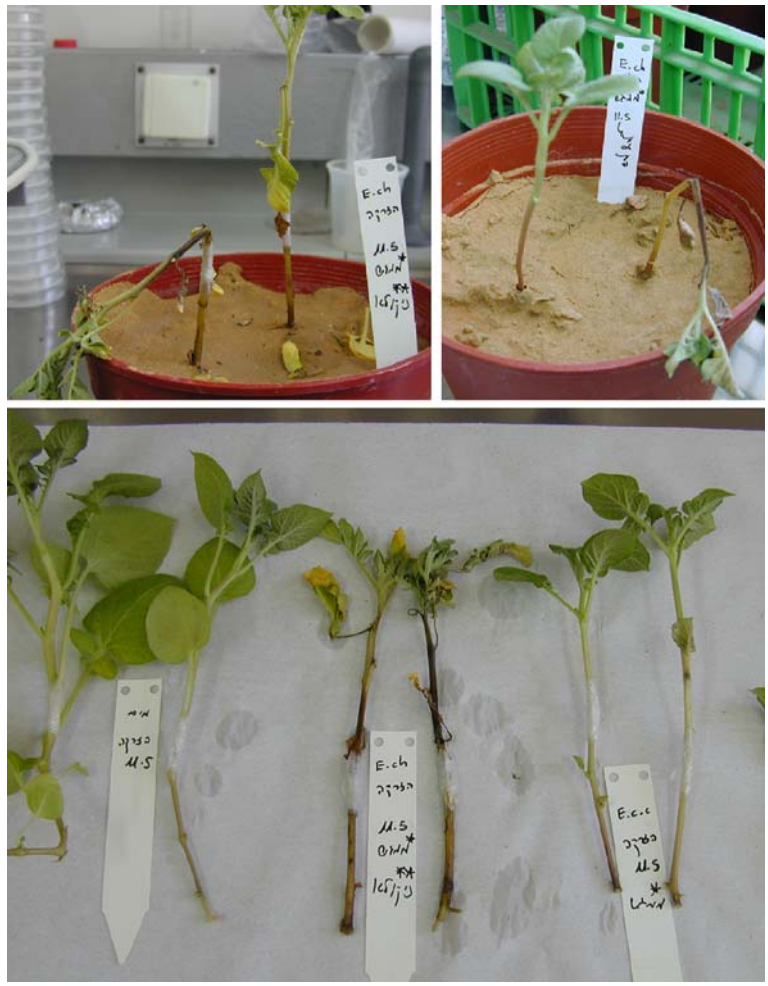

Fig. 4 Soft rot stem symptoms in potato cv. Nicola after inoculation with *Dickeya* sp. G-87 strain, *top left* plants inoculated by injection; *top right* soil inoculation; *bottom*: *left* control plants, *middle* plants inoculated with *Dickeya* sp., *right* plants inoculated with *P. carotovorum* subsp. *carotovorum* (Pcc) SCRI 193

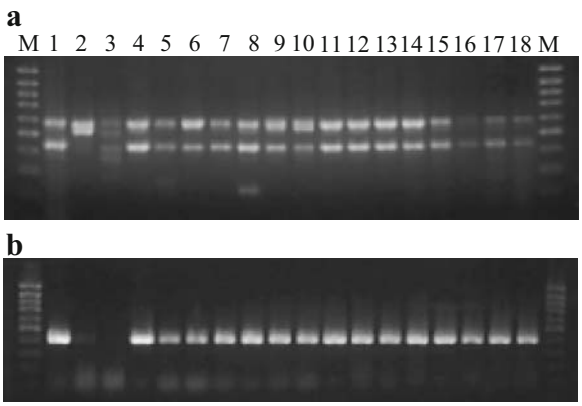

Fig. 5 Characterisation of strains recovered from plants inoculated with Ech (*Dickeya* G87), by PCR-amplification, using primers L1G1 (**a**), and primers ADE1 2 (**b**) *1* Ech1911; *2* Pcc (SCRI 193); *3* control plant injected with SDW; *4–8* plants (cv. Mondial) inoculated by soil drench): *4* upper and lower parts of stem from plant #1; *5* upper part of stem from plant #2; *6* lower part of stem from plant #2; *7* upper part of stem from plant #3; *8* lower part of stem from plant #3; *9–14* plants (cv. Mondial) injected with Ech: *9* upper part of stem from plant #4; *10* lower part of stem from plant #4; *11* upper part of stem from plant #5; *12* lower part of stem from plant #5; *13* upper part of stem from plant #6; *14* lower part of stem from plant #6; *15–18* plants (cv. Nicola) injected with Ech: *15* upper part of stem from plant #7; *16* lower part of stem from plant #7; *17* upper part of stem from plant #8; *18* lower part of stem from plant #8. *M* 100 bp DNA ladder

Discussion

Results from the rep-PCR and the biochemical assays showed that the strains isolated from blackleg-diseased plants in Israel were very similar, if not identical to strains isolated from Dutch seed potatoes, suggesting that the infection in Israel originated from the Dutch seed. The rep-PCR was able to distinguish biovar 3 potato strains from hyacinth strains and also two *D. dianthicola* biovar 7 potato strains from the Netherlands, indicating that the technique was sufficiently discriminative to distinguish between different clonal populations. This is the first time that the presence of biovar 3 strains in potato in the Netherlands is described. This biovar was only found previously in blackleg-diseased plants in high temperature regions, such as Australia and some parts of Peru (Cother 1980; De Lindo et al. 1978). So far all biovar 1 and 7 strains are grouped into *D. dianthicola* according to the publication of Samson et al. (2005) and our own results using genetic techniques to group these strains. However, for biovar 3 it is more complicated. According to Samson et al. (2005), all biovar 3 strains cluster into *D. zeae* and *D. dadantii*, but this new group does not fit in one of these genomic species and may be a new one (unpublished results); therefore, the new strains are referred to as *Dickeya* sp. biovar 3 strains.

Disease symptoms in Israel were associated with seed lots imported only from the Netherlands, although potatoes originating from France, Scotland or Germany were grown in the same fields using the same irrigation water (Tsror, unpublished data). Moreover, when domestic seed tubers (for the autumn) originating from a contaminated spring crop (cv. Sapphire) were planted, a wilt incidence of 10–15% was observed, whereas no disease was observed when seed tubers from non-contaminated fields were used (Tsror et al. 2006).

In the pathogenicity assay, the tested strain (G-87) was aggressive to potato, resulting in typical symptoms of wilting, followed by leaf desiccation with limited soft rot of the stem of inoculated plants at the injection points. These symptoms are in accordance with those observed in the field in Israel with natural infections, originating from seed tubers, confirming the first record of *Dickeya* sp. in Israel (Lumb et al. 1986), and with symptoms described for disease expression in the summer in Spain (Palacio-Bielsa et al. 2006). The pathogenicity results were in agreement with previous reports, demonstrating extensive stem-rot symptom expression associated with high relative humidity (RH) (100%) under the assay conditions (Palacio-Bielsa et al. 2006), whereas lower RH (80%) induced more leaf desiccation and less rotting (Lumb et al. 1986). The recent outbreak of *Dickeya* sp. in Israel was strongly associated with seed tubers imported from the Netherlands, reported also in the early 1980s (Lumb et al. 1986). In Spain, *Dickeya* sp. expression was also associated with seed potato cvs Edzina, Kondor and Agria that had been produced in the Netherlands (Palacio-Bielsa et al. 2006). Moreover, the previous outbreak of *Dickeya* sp. in Andalucía, Spain that occurred at high incidence (in 2002) was observed in potato crops obtained from seeds imported from the Netherlands in most cases (Andújar et al. 2004). These observations suggest that *Dickeya* sp. was introduced with latently infected seed tubers, similar to other seed-borne pathogens (Tsror et al. 1999). Moreover, according to Pérombelon (2002), latent infections by soft-rot bacteria of the genera *Pectobacterium* and *Dickeya* in

potato tubers are frequent and widespread. The *Dickeya* strains isolated from symptomatic plants in Israel were different from the *D. dianthicola* strains typically isolated in the Netherlands from blackleg-diseased plants. Only recently it was found that apart from *D. dianthicola* which is highly prevalent in the Netherlands, this biovar 3 *Dickeya* species is also latently present in Dutch seed. *Dickeya dianthicola* may prevail under temperate conditions, whereas, the undefined *Dickeya* species may cause symptoms at higher temperatures in tropical or subtropical environments.

In addition to the fact that *Dickeya* sp. may cause economic damage (yield reduction of 20–25% occurred when disease incidence was >15%) in Israel due to the prevailing favourable climatic conditions for *Dickeya* sp. there is great concern about the introduction, establishment and spread of *Dickeya* sp. to other potential hosts. This is why *Dickeya* sp. is still considered a quarantine pest in Israel, and considerable effort is currently being invested in developing a method for the detection of latent infections in imported seed tubers.

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