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Detection of soft rot *Erwinia* spp. on seed potatoes: conductimetry in comparison with dilution plating, PCR and serological assays

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Abstract

Automated conductance measurements in polypectate medium were used for the detection of pathogenic soft rot *Erwinia* spp. in potato peel extracts. The detection threshold for *Erwinia carotovora* subsp. *atroseptica* (*Eca*) in inoculated peel extracts was ca. 10^4 colony forming units (cfu) ml^{-1} when samples were considered positive on the basis of a response within 48 h at 20 °C. Detection of *E. chrysanthemi* (*Ech*) was less sensitive, only 10^5 cfu ml^{-1} peel extract were detected within 36 h at 25 °C. The linear correlation between detection times in conductimetry and inoculum levels of *Eca* and *Ech* in peel extracts was used for a quantitative estimation of *Eca* and *Ech* in naturally contaminated peel extracts. Samples giving a positive conductimetric response had to be confirmed with an enzyme-linked immunosorbent assay (ELISA) or polymerase chain reaction (PCR) for the presence of *Eca* and *Ech*, because *E. carotovora* subsp. *carotovora* (*Ecc*) also generated a conductance response. Conductimetry was sensitive and efficient for detection of contamination levels of *Eca* higher than 10^4 cfu ml^{-1} peel extract. For *Ech*, conductimetric detection was less sensitive and inefficient due to low contamination levels of *Ech* and the presence of high numbers of *Ecc* in many samples after enrichment, which interfered with the test. Immunofluorescence cell staining (IF) combined with enrichment and immunofluorescence colony staining (IFC) were suited to detect and quantify low numbers of *Eca* and *Ech* at less than 10^4 cfu ml^{-1} in peel extracts. However, since false positive and negative reactions in serology were observed, the use of PCR after enrichment, or in combination with IFC to confirm positive results, was required for accurate detection.

Introduction

Pectolytic erwinias are involved in soft rot diseases of various agricultural crops such as potato, sugar beet and chicory. *Erwinia* spp. associated with soft rot of potato have been studied extensively because of their economic importance. The different *Erwinia* spp. that are involved in potato diseases are *Erwinia carotovora* subsp. *atroseptica* (*Eca*), *Erwinia carotovora* subsp. *carotovora* (*Ecc*) and *Erwinia chrysanthemi* (*Ech*). They all can cause tuber soft rot. Under cool and moist conditions *Eca* is the main causal agent of blackleg, a blackening of the stem base of potato plants which originates from the mother tuber (Pérombelon and Kelman, 1987). *Ecc* mainly causes aerial stem rot, often due to injury, although under high field temperatures it has been reported to incite blackleg-like symptoms (Molina and Harrison, 1977). Under hot and humid (tropical) conditions *Ech* can also induce blackleg-like symptoms, but recently this bacterium has also been found in association with stem rot in cool temperate regions (De Vries, 1990). The symptoms of stem wet rot, caused by *Ech*, are often difficult to distinguish from typical blackleg symptoms of *Eca* in cool temperate regions where both pathogens occur (De Boer, 1994).

Stem rot diseases caused by *Eca* and *Ech* are seed tuber borne. Besides resistance breeding (see Düring et al., 1993), good cultural practices and the use of healthy seeds are the best strategies to control the disease (Pérombelon and Hyman, 1992). Bain et al. (1990) found a clear relationship between the number of *Eca* bacteria present on seed potatoes and the incidence of blackleg in the field. Moreover, the incidence

of blackleg late in the season was negatively correlated to tuber yield.

In temperate regions much attention has been paid to improve the quality and health of seed potatoes. Field inspection for blackleg failed to assure the status of this pathogen in seed lots, because post-harvest contamination and latent infections cannot be detected by visual observation. Therefore, laboratory testing with an enzyme-linked immunosorbent assay (ELISA) in addition to field inspection is currently used in The Netherlands for seed certification to control blackleg (De Boer et al., 1996).

The aim of laboratory testing is to detect 10^2-10^4 colony forming units (cfu) of *Eca* per tuber, which is the minimum amount of inoculum needed to incite blackleg symptoms under most field conditions (Bain et al., 1990). Techniques based on dilution plating, serology and DNA technology have been developed to detect *Eca* and *Ech* on potato tubers. Dilution plating on crystal violet pectate (CVP) medium (Pérombelon et al., 1987) lacks sensitivity due to the presence of large numbers of potato tuber-associated saprophytes and *Ecc*, and is both unreliable (Janse and Spit, 1989) and time-consuming. Immunofluorescence cell (IF) and colony staining (IFC) (Allan and Kelman, 1977; Van Vuurde and Roozen, 1990), although laborious, and ELISA, in combination with an enrichment step (Gorris et al., 1994), have good potential for routine application. However, serological techniques sometimes show false positive and negative reactions, due to cross-reacting saprophytes (Van der Wolf et al., 1994) and variation in serotypes (De Boer et al., 1987; Samson et al., 1987). Polymerase chain reaction (PCR) assays for *Eca* and *Ech* are rapid techniques with high specificity and sensitivity (De Boer and Ward, 1995; Smid et al., 1995). Nevertheless, the application of PCR for large-scale routine indexing of potato tuber seed lots is expensive and laborious.

Automated conductimetric assays have already shown their potential for rapid screening of food products for both pathogenic (Bolton, 1990) and non-pathogenic bacteria (Hardy et al., 1977; Cady et al., 1978) and might also be useful for routine indexing of seed potatoes for soft rot *Erwinia* spp. (Fraaije et al., 1996).

This paper describes the use of conductance measurements as a primary screening technique for quantifying the number of viable soft rot *Erwinia* spp. on potato tubers. Conductimetric assays were combined with ELISA, PCR and dilution plating to verify the presence of *Eca*, *Ech* and *Ecc* in potato peel extract samples after enrichment in polypectate medium. Results obtained with conductimetry were compared with dilution plating, IFC and IF combined with enrichment in order to determine the sensitivity, specificity and efficiency of the test.

Materials and methods

Bacterial strains

Two strains of *Eca*, 1061 and P644, and of *Ech*, P652 and P991, from the culture collection of the Research Institute for Plant Protection (IPO-DLO, Wageningen, The Netherlands) were used for inoculation of peel extracts. Bacteria were grown overnight at room-temperature on a rotary shaker at 250 rpm to ca. 10^9 cfu ml^{-1}. Dilutions of bacterial broth cultures were made in saline (0.85% (w/v) NaCl; pH 7) and potato peel extract for preparing pure culture dilutions and to inoculate potato peel extract.

Media

Nutrient agar (NA; Oxoid) and a growth-factors medium (Van Vuurde and Roozen, 1990) were used for maintaining bacterial cultures of *E. carotovora* and *E. chrysanthemi*, respectively. Tryptic Soy Broth (TSB, Difco) was used for growing overnight cultures. Double layer crystal violet pectate medium (DLCVP; Pérombelon and Burnett, 1991) without antibiotics was used for dilution plating. Minimal medium containing (g l^{-1}): $NaNO_3$, 1.50; $mgSO_4.7H_2O$, 0.30; sodium taurocholate (Difco), 0.2; NaCl, 2.30; K_2HPO_4, 4.0 and KH_2PO_4, 1.5, was supplemented with either 0.5% (w/v) L-asparagine monohydrate (AM) or 2.0% (w/v) sodium polypectate (HP Bulmer, Hereford, UK) (PM) for enrichment of *Erwinia* spp. in potato peel extracts. During enrichment of potato peel extracts in PM, the conductance change responses were recorded simultaneously.

Potato seed lots

In 1994, 122 potato tuber seed lots were tested within 4 weeks after harvest. The General Netherlands Inspection Service for Agricultural Seeds and Seed Potatoes (NAK) kindly provided 53 commercial seed lots, of which 4 (seed lots 1–4) were derived from fields without disease symptoms (category (cat.) A) and 49 (seed lots 5–53) from fields where blackleg symptoms were

observed sporadically (cat. B). We also harvested potatoes from healthy plants (cat. C; seed lots 54-61) and naturally *Erwinia*-infested plants (cat. D; seed lots 62–122) during the growing season from control field plots at the experimental farm of the NAK, Tollebeek, The Netherlands.

Preparation of samples

The peel from ten potatoes of every seed lot was extracted by a power-driven roller press (Pollähne, Wennigsen, Germany). Extracts were filtered through cheese cloth and left standing for at least 1 h to settle soil, starch and peel debris, before sampling from the top layer.

Enrichment in AM and IF

Tubes with 4.5 ml of AM were inoculated with 0.5 ml extract, and incubated without shaking at 25 °C for 22 h. Bacterial cells in enriched peel extracts (20 μl) were stained as described by Van Vuurde et al. (1983), using fluorescein isothiocyanate (FITC)-conjugated polyclonal antibodies (Pab) 9024/5C or 8898. Bacto-FA Rhodamine (Code 2340, Difco), diluted 1:100, was used as counterstain. The working dilutions of Pab 9024/5C, directed against lipopolysaccharide (LPS) of *Ech*, and Pab 8898, directed against LPS of *Eca*, were 1:600 and 1:1000, respectively. Stained cells fixed on multitest slides (diam. 8 mm) were counted in at least 25 fields under a UV microscope (field coefficient 18, objective magnification 63, internal magnification 1.25, ocular magnification 10), which corresponds to ca. 1.0 μl extract.

Enrichment in PM and conductance measurements

A Malthus 2000 series analyzer (Malthus Instruments Ltd, Crawley, UK) was used for recording conductance change responses of potato peel extracts during enrichment in PM. Reusable 8 ml tubes with 2 ml of PM were inoculated with peel extract and filled with distilled water to a final volume of 3 ml. Only small volumes of 20 and 50 μl peel extract were tested at 25 and 20 °C, respectively, in order to reduce the temperature-dependent background conductance signal of peel extract itself (Fraaije et al., 1996) to a value below 15 μS. Conductance changes were recorded in duplicate at 18 min intervals for at least 48 h. To determine the detection times in conductimetry for *Eca* and *Ech* in peel extracts, the response was considered to be positive when the conductance change of peel extracts in PM exceeded 25 μS. After 48 h enrichment in PM, samples were used directly for dilution plating or transferred to Eppendorf tubes and stored at −20 °C until ELISA and PCR were performed.

Dilution plating

Dilutions of potato peel extract, before and after enrichment in AM or PM, were plated on DLCVP plates. Suspected *Erwinia* spp. colonies, which formed typical pits on DLCVP after 48 to 96 h incubation at 25 °C, were isolated and identified by biochemical, serological and fatty acid analyses as described previously (Fraaije et al., 1996).

IFC

IFC of 20 μl samples of diluted potato peel extracts was performed in 24-well tissue culture plates, with each well containing 300 μl molten PT medium (Burr and Schroth, 1977) with polygalacturonate, according to the procedure of Van Vuurde and Van der Wolf (1995). After 48 h of incubation at 27 °C, target colonies were stained with FITC-conjugated Pab 8898 or 8174. The working dilutions of Pab 8898, directed against LPS of *Eca*, and Pab 8174, directed against LPS of *Ech*, were both 1:100. Stained colonies in the agar were counted under an UV microscope at a magnification of 20 times. Fluorescent colonies suspected of being *Eca* were further tested by PCR.

ELISA

ELISA was done according to an indirect antigen coated plate procedure. Polystyrene plates were coated with 100 μl sample and incubated overnight at 4 °C. After 2 washings with tap water, wells were blocked for 1 h at 37 °C with PBST (0.1% Tween-20 in 0.01 mol l^{-1} phosphate buffered saline, pH 7.2) containing 5% (w/v) skim milk powder. After washing, 1:2000 diluted Pab 8898 (in PBST with 0.1% bovine serum albumin (BSA)) or 1:0.25 similarly diluted Mab 27D10 exudate, a monoclonal antibody directed against LPS of *Ech*, was added to the wells and the plate was incubated for 2 h at 4 °C. After washing, the plate was incubated for 1 h at 4 °C with a 1:1000 dilution (in PBST with 0.1% BSA) of goat anti-rabbit or anti-mouse immunoglobulins, conjugated with alkaline phosphatase. After 4 washings, the substrate, 0.75 mg ml^{-1} of *p*-nitrophenyl phosphate in substrate buffer

(10% (v/v) diethanolamine, pH 9.8), was added. The absorbance was measured with an automatic reader at 405 nm after 1 h (A_{405}-values). A_{405}-values greater than twice the mean of negative control values (clean peel extract) were considered to be positive.

PCR

PCR was used to verify positive results for *Eca* in IFC and conductimetric assays. Positive colonies in IFC were punched from the agar, suspended in 20 μl purified water, boiled for 10 min, and tested directly in the PCR reaction. DNA was extracted from 40 μl of conductimetric samples enriched in PM. Five μl 14% (w/v) SDS and 5 μl 100 mm EDTA solution was added to each sample and heated for 1 h at 55 °C. Twenty-five μl ammonium acetate (7.5 m) was mixed with heat-treated samples which were then centrifuged at 14,000 g for 10 min. DNA was precipitated by adding 1 volume of isopropanol and washed with 70% (v/v) ethanol. Final pellets were dried at 55 °C for 15 min, dissolved in 50 μl purified water, and heated to 55 °C prior to PCR. The PCR assays, using *Taq* DNA polymerase (BioCan Scientific, Missisauga, Canada) and the ECA1f and ECA1r primers, were performed with 1 μl sample in 20 μl reaction mixture as described previously by De Boer and Ward (1995), except that 0.2% (w/v) skim milk powder was added to the reaction mixture (De Boer et al., 1995). The PCR conditions with an Ericomp Easy Cycler (San Diego, US) were 95 °C for 4 min, followed by 40 cycles at 94 °C for 45 s, 62 °C for 45 s and 72 °C for 1 min. The PCR was terminated with a DNA extension at 72 °C for 5 min. PCR products were analyzed on 2% (w/v) agarose gels, containing ethidium bromide, by electrophoresis of 5 μl sample at 5 V cm^{-1} for 1 h. The presence of a 690-bp DNA fragment confirmed the presence of *Eca*.

Results

Conductimetric detection of Eca *and* Ech

A clear positive relationship occurred between detection time (Td) in conductimetry and the concentration of *Erwinia* spp. inoculated into potato peel extracts (Figures 1a, c). Within 48 h, detection time in PM at 20 °C (Td_{20}) was linearly correlated ($r = -0.94$) with *Eca* concentrations higher than log 4.0 cfu ml^{-1} potato peel extract (Figure 1b). *Eca* detection was less sensitive at 25 °C in comparison with 20 °C, probably due to poor competition with other micro-organisms present in potato peel extracts, which could be isolated in higher numbers after enrichment at 25 °C. *Ech* was detected somewhat earlier at 25 than 20 °C, probably because the positive effect of the higher growth rate of *Ech* at 25 °C (results not shown) was almost counteracted by the higher competitive ability at 20 °C. The detection threshold for *Ech*, i.e. the lowest concentration of bacterial cells that gave a positive response in the assay, was 10 times higher than that for *Eca* after 48 h incubation. For *Ech*, a high linear correlation coefficient ($r = -0.96$) between detection time in PM at 25 °C and inoculum concentrations higher than log 5.0 cfu ml^{-1} was obtained (Figure 1d).

Comparison of conductimetry with ELISA and PCR

Results of conductance measurements were verified with PCR and ELISA. After 48 h enrichment in PM at 20 °C, initial inoculum concentrations of 10^2, 10^3–10^4 and 10^4 cfu of *Eca* ml^{-1} potato peel extract were detected in PCR, ELISA and conductimetry, respectively (Table 1). The detection thresholds for *Ech* were 10^5 cfu ml^{-1} for both ELISA and conductimetry after 36 h enrichment in PM at 25 °C, whereas a reliable PCR assay for *Ech* was not available at the time (Table 1). Conductimetric detection of both *Eca* and *Ech* occurred when cell concentrations reached 10^7–10^8 cells ml^{-1} during enrichment. As the detection threshold of PCR for pure cultures of *Eca* was 10^4 cells ml^{-1} (results not shown) and 10^6–10^7 *Eca* or *Ech* cells ml^{-1} were detected in ELISA (results not shown), both techniques could be used for confirmation. Indeed, all samples positive in conductimetry were confirmed by both techniques (Table 1).

Screening of peel extracts naturally infected with Erwiniae

To determine the efficiency of the conductance measurements, all 122 seed lots were also tested with ELISA for the presence of *Eca* and *Ech*. All samples that were positive in ELISA or conductimetry were further tested by dilution plating and PCR (Table 2).

Nineteen and 32 seed lots were positive for *Eca* in ELISA and conductimetry, respectively. For 15 of the 32 positive conductimetric samples the presence of *Eca* was confirmed by both ELISA and PCR. For 3 of the 4 samples (seed lots 70, 95 and 111) which were positive in ELISA and negative in conductimetry, the inconsistency between ELISA and conductimetry

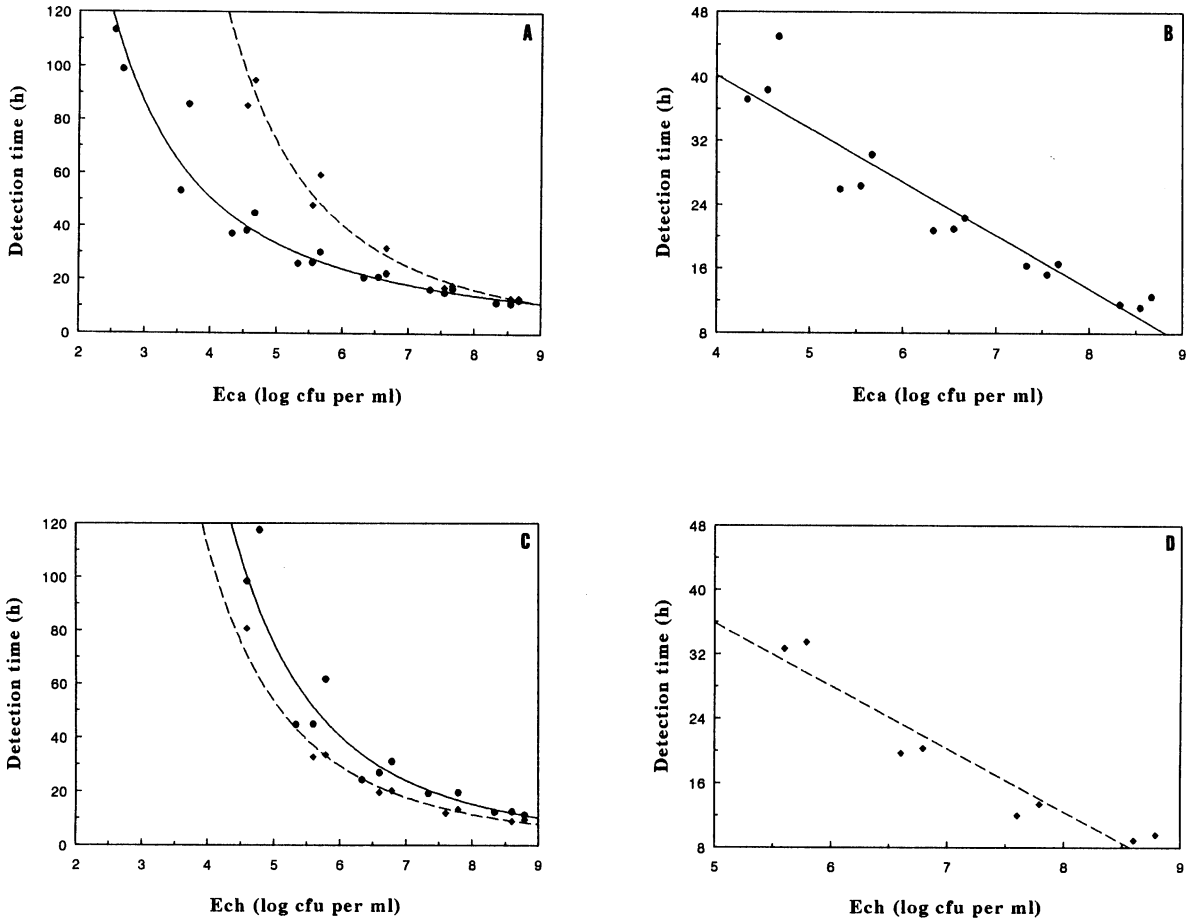

Figure 1. The relationship between detection times in conductimetry and concentration of *Erwinia* spp. in inoculated potato peel extracts at 20 °C (-●-) and 25 °C (-◆-). (A) Conductimetric detection of *Eca* in inoculated peel extracts during enrichment in polypectate medium at 20 and 25 °C, (B) Linear relationship between detection time and *Eca* concen trations $>$ log 4.0 cfu ml^{-1}; y = −6.9x + 66.9, (C) Conductimetric detection of Ech in inoculated peel extracts during enrichment in polypectate medium at 20 and 25 °C, (D) Linear relationship between detection time and *Ech* concen trations $>$ log 5.0 cfu ml^{-1}; y = −7.8x + 74.9.

results could be explained by the higher sensitivity of ELISA, as these samples were also found positive in PCR and dilution plating. The positive ELISA result of seed lot 90, which was negative in all other tests, was probably caused by dead cells or a false positive reaction in serology. The negative ELISA, and positive PCR and conductimetric results of seed lots 21 and 31 can be explained by mixed *Eca* and *Ecc* populations, which were present in these samples. The conductance signals were probably caused by high numbers of *Ecc* ($> 10^7$ cells ml^{-1}) during enrichment, but low numbers of *Eca* ($< 10^6$ cells ml^{-1}), not detectable by ELISA and conductimetry, could still be detected by PCR. The positive conductance signals of the 15 remaining seed lots, which were negative in both ELISA and PCR, were probably due to high numbers of *Ecc*, which were isolated before or after enrichment from all these samples (Table 2).

Respectively, 2 and 40 of the 122 seed lots tested were found positive for *Ech* in ELISA and conductimetry. The positive ELISA results of seed lots 27 and 93 were probably due to dead cells or false positive reactions since *Ech* could not be isolated and both samples were negative in IFC (Tables 2 and 3). Furthermore, the conductance response of most of the 40 positive conductimetric samples were probably generated by *Ecc* and *Eca*, which could be isolated in high numbers after enrichment (Table 2).

The results of the conductimetric assays at 20 and 25 °C indicated that the higher temperature favoured

Table 1. Results of conductimetry, ELISA and PCR for detection of *Eca* and *Ech* in inoculated potato peel extracts after enrichment in polypectate medium (PM) at 20 and 25 °C

Concentration of cells add to peel extract (cfu ml^{-1})	Detection of *Eca*			Detection of *Ech*	
	Td20[1]	ELISA	PCR	Td25[2]	ELISA
0	−	−	−	−	−
10	−	−	−	−	−
10^2	−	−	+	−	−
10^3	−	+/−	+	−	−
10^4	+	+	+	−	−
10^5	+	+	+	+	+
10^6	+	+	+	+	+
10^7	+	+	+	+	+
10^8	+	+	+	+	+

+, positive reaction; −, negative reaction; +/−, variable reaction.
[1] Td20, detection time (h) of peel extract in PM at 20 °C, all samples detected within 48 h were considered to be positive for presence of *Eca*.
[2] Td25, detection time (h) of peel extract in PM at 25 °C, all samples detected within 36 h were considered to be positive for presence of *Ech*.

the growth of *Ecc* in the peel extracts, while *Eca* was enriched better at the lower temperature. *Eca* was generally detected slightly faster at 20 °C, whereas *Ecc* was detected about twice as fast at 25 °C (Table 2 and Figure 2).

Quantitative estimation of Eca *and* Ech *in potato peel extracts*

To evaluate the value of conductimetry, a quantitative comparison with immunofluorescence techniques and dilution plating was made. For all positive seed lots, the contamination level with *Eca* and *Ech* in peel extracts before or after enrichment was estimated by conductimetry, dilution plating and immunofluorescence (Table 3).

IFC-PCR for *Eca* was positive for 23 of 122 potato seed lots tested, of which 18 were positive after enrichment in PCR (Table 2) and 15 in conductimetry combined with PCR (Table 3). IFC-PCR was more sensitive than dilution plating and conductimetry, although for some seed lots (98 and 111) the contamination level of *Eca* was underestimated. False negative reactions in IFC-PCR were found for 2 seed lots (31 and 97), which were positive with at least 3 other techniques and from which *Eca* was isolated. The negative IFC result for seed lot 31 could be ascribed to the presence of an unusual *Eca* serotype, which reacted only weakly with Pab 8898, directed against LPS of serogroup I. All *Eca* strains tested in The Netherlands thus far, have been placed in serogroup I (M. Appels, pers. comm.). Twenty-five peel extracts enriched in AM had $>10^4$ cells ml^{-1} of *Eca* in IF. Of these, 18 lots were also positive in IFC-PCR, 8 in dilution plating, 20 in PCR after enrichment, 18 in both ELISA and PCR after enrichment (Table 2, 3), and 17 in conductimetry combined with PCR. Apparent false positive IF-reactions were only found for seed lots 16, 17, 19 and 22.

For *Ech*, 16 potato seed lots were found positive by one of the five techniques tested, whereas only 10 seed lots were positive with 2 techniques and no seed lots were positive with all techniques tested. Seven seed lots were positive in IFC or dilution plating and contained at least 10^4 cfu ml^{-1}, but in ELISA none of these 7 lots were positive, while only 5 were positive in conductimetry. The conductimetric response of these 5 lots was probably caused by *Ecc* or *Eca*, because they were negative in ELISA for *Ech*. Of the 10 seed lots found positive with 2 techniques, 8 were positive in IFC and 7 in IF. Unfortunately, since *Ech* was only isolated from 2 seed lots and serological tests were inconsistent, another confirmation test, like PCR, was not available to verify results obtained in conductimetry and serology.

Discussion

The detection thresholds of the conductimetric assays for *Eca* and *Ech* in potato peel extract were temperature-dependent. High linear correlations

Table 2. Results of naturally infected potato seed lots positive for *Eca* or *Ech* with conductimetry or ELISA after enrichment in polypectate medium (PM), using PCR and dilution plating to verify results of enriched samples

Seed lots	Detection of *Eca*			Detection of *Ech*		Isolation[3]	
	Td20[1]	ELISA	PCR	Td25[2]	ELISA	Direct	After enrichment
1	28.8	–	–	15.9	–	–	*Ecc*
2	–	–	–	30.6	–	–	nd
4	–	–	–	26.9	–	–	nd
6	–	–	–	34.5	–	–	nd
7	–	–	–	34.5	–	–	*Ecc*
8	–	–	–	30.5	–	–	nd
12	25.1	–	–	14.1	–	*Ecc*	*Ecc*
13	42.2	–	–	23.1	–	*Ecc*	*Ecc*
14	–	–	–	34.4	–	–	nd
15	20.4	–	–	11.7	–	*Ecc*	*Ecc*
16	–	–	–	29.3	–	–	nd
17	–	–	–	31.7	–	–	nd
18	45.0	–	–	27.8	–	–	nd
19	–	–	–	17.7	–	*Ecc*	nd
20	12.0	–	–	6.9	–	–	nd
21	35.1	–	+	22.4	–	*Eca*	*Ecc*
22	–	–	–	30.8	–	–	nd
25	–	–	–	33.9	–	–	nd
27	–	–	–	17.1	+	–	nd
28	46.2	–	–	–	–	*Ecc*	nd
30	–	–	–	34.5	–	–	nd
31	36.3	–	+	16.5	–	*Eca/Ecc*	*Ecc*
33	47.1	–	–	29.1	–	–	nd
38	24.9	–	–	11.9	–	*Ech*	*Ecc*
45	13.4	–	–	8.4	–	*Ech*	*Ecc*
46	42.7	–	–	29.3	–	–	*Ecc*
48	–	–	–	30.8	–	–	*Ecc*
49	39.3	–	–	23.9	–	–	*Ecc*
50	34.1	–	–	25.8	–	*Ecc*	*Ecc*
51	–	–	–	27.0	–	*Ecc*	*Ecc*
52	34.5	–	–	22.2	–	*Ecc*	*Ecc*
53	44.4	+	+	–	–	*Eca*	*Eca*
66	38.6	–	–	24.9	–	–	*Ecc*
69	–	–	–	31.2	–	–	*Ecc*
70	–	+	+	–	–	—	*Ecc/Eca*
88	–	–	–	21.6	–	–	*Ecc*
89	37.8	+	+	–	–	–	*Eca*
90	–	+	–	–	–	–	–
91	25.8	+	+	26.6	–	*Eca*	*Eca*
93	37.5	+	+	36.0	+	–	*Eca*
94	44.1	+	+	–	–	–	*Eca*
95	–	+	+	29.4	–	–	*Eca*
96	33.8	+	+	–	–	–	*Eca*
97	45.0	+	+	–	–	–	*Eca*
98	14.6	+	+	13.8	–	*Eca*	*Eca*
99	39.9	+	+	–	–	–	*Eca*
100	41.4	+	+	–	–	–	*Eca*

Table 2. Continued

Seed lots	Detection of *Eca*			Detection of *Ech*		Isolation[3]	
	Td20[1]	ELISA	PCR	Td25[2]	ELISA	Direct	After enrichment
101	39.9	+	+	–	–	–	*Eca*
102	32.6	+	+	33.8	–	*Eca*	*Eca*
103	31.8	+	+	30.8	–	*Eca*	*Eca*
104	36.9	+	+	35.4	–	–	*Eca*
105	41.9	+	+	–	–	–	*Eca*
111	–	+	+	–	–	*Eca*	*Eca*

+, positive reaction in ELISA and PCR; −, negative reaction in ELISA and PCR.
[1] Td20, detection time (h) of peel extract in PM at 20 °C, all samples with Td20 <48 h were considered to be positive in conductimetry.
[2] Td25, detection time (h) of peel extract in PM at 25 °C, all samples with Td25 <36 h were considered to be positive in conductimetry.
[3] Isolation of *Erwinia* spp. after dilution plating directly and after enrichment of peel extracts; the detection thresholds of dilution plating performed directly with peel extracts and after enrichment were higher than 10^4 and 10^6 cfu ml^{-1}, respectively; –, *Erwinia* spp. were not isolated; nd, dilution plating was not done.

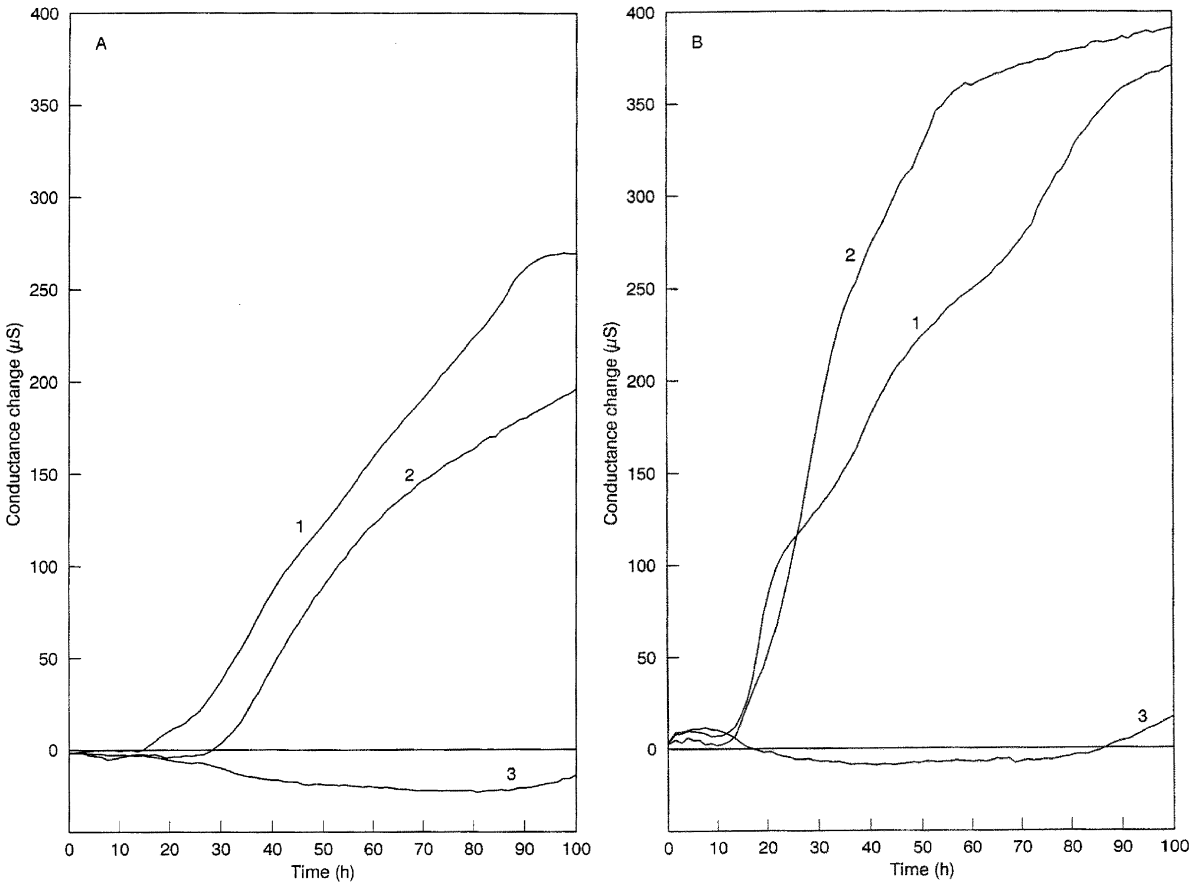

Figure 2. Conductance responses of potato peel extracts in polypectate medium: (A) peel extracts at 20 °C; (B) peel extracts at 25 °C; 1, peel extract of seed lot 1, contaminated with Ecc; 2, peel extract of seed lot 31, contaminated with Ecc and Eca; 3, peel extract of seed lot 37, no *Erwinia* spp. isolated.

Table 3. Quantitative estimation of *Eca* and *Ech* in peel extracts of naturally infected potato seed lots. Comparison of conductimetry with IFC and dilution plating, performed directly, and with IF, PCR and ELISA, performed after enrichment

Concentration of *Eca* (log cells ml^{-1})	Positive potato seed lots				
	Without enrichment[1]		Conductimetry +PCR[4]	After enrichment[2]	
	IFC-PCR[3]	Plating		IF	PCR-ELISA[5]
<4.0	7,13,14,24 62,70,89,93 94,95,96,99 100,104,105 111				
4.0–6.0	21,53,91,98 101,102,103	21,31,53,91 102,103,111	21,31,53,89 93,94,96,97 99,100,101 102,103,104 105	16,17,19,22 31,33	21,31
>6.0		98	91,98	21,53,70,89 91,93,94,95 96,97,98,99 100,101,102 103,104,105 111	53,70,89,91 93,94,95,96 97,98,99 100,101,102 103,104,105 111

Concentration of *Ech* (log cells ml^{-1})	Positive potato seed lots				
	Without enrichment[1]		Conductimetry +ELISA[6]	After enrichment[2]	
	IFC	Plating		IF	ELISA
<4.0	6,8,12,14 16,20,23,30 33,34,35,47 65,86,119				
4.0–6.0	21,25,38,50 51,82	38,42	93	8,23,25,26 30,33,36,65 119	
>6.0			27		27,93

[1] IFC and plating on DLCVP were done directly with dilutions of peel extracts.
[2] For doing IF, peel extracts were enriched in AM for 22 h at 25 °C; ELISA and PCR were done after enrichment of peel extracts in PM for 48 h at 20 °C or 36 h at 25 °C for the detection of *Eca* and *Ech*, respectively.
[3] IFC-PCR for detection of *Eca*, PCR was used for confirmation of positive colonies in IFC.
[4] Conductimetry-PCR, all samples with Td20 <26 h were suspected to be contaminated with >10^6 cfu ml^{-1}, while samples with a Td20 between 26 and 48 h were suspected to be contaminated with 10^4 to 10^6 cfu ml^{-1}, PCR was used for confirmation of the presence of *Eca* in the suspected samples.
[5] PCR-ELISA, samples positive in PCR were considered to contain at least 10^4 cells ml^{-1}, ELISA was performed to determine which samples yielded more than 10^6 cell ml^{-1}.
[6] Conductimetry-ELISA, all samples with Td25 <28 h were suspected to be contaminated with >10^6 cells ml^{-1}, while samples with a Td25 between 28 and 36 h were suspected to yield at least >10^5 cells ml^{-1}, ELISA was used for confirmation of the presence of *Ech* in the suspected samples.

between detection times in conductimetry and cell concentrations in inoculated peel extract were obtained for *Eca* and *Ech*. By using calibration curves, it was possible to estimate the tuber-borne contamination levels of *Eca* and *Ech* in potato tuber seed lots. *Eca* in inoculated peel extracts was detected in 48 h at 20 °C with a detection threshold of 10^4 cfu ml^{-1}. *Ech* was detected in 36 h at 25 °C with a detection threshold of 10^5 cfu ml^{-1}. However, when seed lots naturally contaminated with *Erwinia* spp. were tested, *Ecc* was also able to generate conductance responses in PM, particularly at 25 °C, making the calibration curves less reliable. Therefore, the presence of *Eca* and *Ech* in positive conductimetric samples had to be confirmed with ELISA or PCR.

It was difficult to determine the efficiency and sensitivity of conductimetry for *Ech*, as *Ech* was only isolated from 2 of 122 seed lots tested and there were discrepancies among the serological test results. The contradictory results in the different serological assays may partly be caused by non-specific binding or cross-reactions of *Pseudomonas* spp. with the *Ech* antisera, as reported by Van der Wolf et al. (1993). High numbers of *Pseudomonas* spp. were especially present after enrichment in AM at 25 °C (results not shown). Furthermore, many samples were detected in conductimetry due to the presence of high numbers of *E. carotovora* after enrichment, and a PCR assay with high specificity was not available to confirm the results obtained in serology and conductimetry. However, in a later test series with other samples, a nested PCR procedure to confirm IFC-positive colonies showed promising results, as the presence of *Ech* could be confirmed in ca. 95% of the samples tested (J.M. Van der Wolf, pers. comm.).

About 20% of the 122 seed lots was found positive for *Eca* by direct IFC combined with PCR or by PCR after enrichment. In conductimetry 26% of the seed lots tested was found suspected to be contaminated with *Eca*. After verification of the conductimetric-positive samples, 53% of these samples was found positive for *Eca*. Most of the conductance responses of the other samples, if not all, was caused by *Ecc*. Of the seed lots positive in IFC-PCR or PCR after enrichment, 68% was also found positive in conductimetry combined with PCR. The remaining samples were probably not detected due to the low contamination levels of *Eca* less than 10^4 cfu ml^{-1} peel extract. Conductimetry was less laborious and more sensitive than dilution plating for the detection of *Eca* in naturally contaminated peel extracts, because more samples were found positive (Table 3). Direct IFC, performed with crude peel extracts, and IF and PCR, performed after enrichment, were adequately sensitive for quantifying and detecting low numbers ($< 10^4$ cells ml^{-1}) of *Eca* in peel extracts.

Our results suggest that automated conductance measurements can already be used for an efficient screening of seed potatoes for *Eca*. Samples negative in conductimetry can be certified without further testing, because the contamination level of *Eca* will be less than 10^4 cfu ml^{-1}. The conductimetric detection of *Ech* was less sensitive and inefficient, because too many samples needed further testing.

Different approaches are possible to improve the sensitivity and specificity of the conductimetric assays for both *Eca* and *Ech*. Other procedures to prepare samples, such as immunomagnetic separation (Parmar et al., 1992), may be used to reduce the number of *Ecc* and other non-target organisms in test samples. Furthermore, to restrict the growth of *Ecc* and other non-target organisms the use of a selective pre-enrichment medium, commonly used for conductimetric detection of food-borne bacteria such as *Salmonella* (Pless et al., 1994) and *Listeria* spp. (Capell et al., 1995), might be considered.

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