PCR Detection of *Erwinia carotovora* subsp. *atroseptica* Associated with Potato Tissue

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ABSTRACT

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The nucleotide sequence for a DNA hybridization probe specific for *Erwinia carotovora* subsp. *atroseptica* was determined, and primers were selected for detection of the blackleg pathogen using the polymerase chain reaction (PCR). Primers ECA1f (5′-CGGCATCATAAAAACACG-3′) and ECA2r (5′-GCACACTTCATCCAGCGA-3′) specifically amplified a 690-bp DNA fragment of all *E. carotovora* subsp. *atroseptica* strains tested but not strains of other *E. carotovora* subspecies isolated from various hosts and geographic regions or other plant- and soil-associated bacteria. Visualization of the *E. carotovora* subsp. *atroseptica*-specific PCR product on ethidium bromide-stained agarose gels required a minimum of 250 to 500 CFU per ml. A total of 170 samples of potato stem and tuber tissue was tested by PCR and compared with reactions in enzyme-linked immunosorbent assay (ELISA) with a monoclonal antibody specific for the lipopolysaccharide of *E. carotovora* subsp. *atroseptica*. Although 50.6% of these samples were positive in PCR compared to 46.5% in ELISA, some of the ELISA-positive samples were negative in PCR. When *E. carotovora* subsp. *atroseptica* DNA was added to these samples, amplified products were obtained in all but two samples after repeating the PCR, indicating that in most cases the failure to obtain PCR amplification for some of the ELISA-positive samples was not due to the presence of inhibitors.

Erwinia carotovora subsp. *atroseptica*, which causes blackleg of potato, closely resembles *E. carotovora* subsp. *carotovora* in many physiological and biochemical characteristics and in production of pectolytic enzymes. In contrast to *E. carotovora* subsp. *atroseptica*, *E. carotovora* subsp. *carotovora* is ubiquitous, surviving in surface water and persisting in various agricultural and nonagricultural niches. Although *E. carotovora* subsp. *atroseptica* also has been isolated from sources other than potatoes, it is found far less frequently than *E. carotovora* subsp. *carotovora* (9,14); some of these isolates differ in some physiological and biochemical characteristics from typical blackleg-causing strains, and their role in the epidemiology of potato disease is not well understood (G. D. Franc, *personal communication*).

E. carotovora subsp. *atroseptica* contaminates potato tuber surfaces (12,17), and the incidence of blackleg has been significantly reduced in some potato-growing regions by not using tubers as seed to initiate production cycles (4). Occurrence of blackleg in potatoes derived from nontuber sources, such as micropropagated plantlets and stem cuttings (7; S. H. De Boer, *unpublished data*), suggests that there are sources of inoculum other than potato seed. It is not known whether the pathogen spreads from nearby potato fields or from other niches in the environment. However, these inoculum sources affect the potential success of strategies to avoid introduction of the pathogen to *Erwinia*-free plants.

The survival of *E. carotovora* subsp. *atroseptica* outside the potato crop is poorly understood, in part, because classic detection methods based on isolation are inadequate for determining the persistence of low bacterial numbers in the environment. With the advent of DNA-based procedures, such as those utilizing the polymerase chain reaction (PCR), a much higher degree of sensitivity can be attained. The use of PCR in ecological studies already has indicated that the composition of microbial communities may be quite different from those observed by isolating culturable bacteria (19). Although PCR has been applied for detection of some phytopathogenic prokaryotes, it has not yet been used for the soft rot Erwinia (10,15,16).

DNA hybridization probes specific for *E. carotovora* subsp. *atroseptica* were designed by Darrasse et al. (2) and ourselves (20), but a useful PCR procedure to enhance sensitivity of detection has not yet been developed. Although Darrasse et al. (3) designed a PCR test based on pectate lyase-encoding *pel* gene sequences, it only permits *E. carotovora* subsp. *atroseptica* to be identified by analysis of restriction fragment length polymorphisms (RFLP) of the amplified product. In this paper, we report the development of a PCR test in which only *E. carotovora* subsp. *atroseptica* DNA is amplified. We report on the test's specificity and sensitivity and compare it with an enzyme-linked, immunosorbent assay (ELISA) for detection of *E. carotovora* subsp. *atroseptica* associated with potato stems and tubers.

MATERIALS AND METHODS

Bacterial strains and culture conditions. Bacterial cultures used to test specificity of PCR amplification included 37 strains of *E. carotovora* subsp. *atroseptica* representing four serogroups, 13 untyped *E. carotovora* subsp. *atroseptica* strains, 39 *E. carotovora* subsp. *carotovora* strains representing 36 serogroups, and 5 untyped *E. carotovora* subsp. *carotovora* strains (Table 1). In addition, 18 strains of other *E. carotovora* subspecies or undetermined subspecies and 12 strains of bacteria representing other species and genera often associated with plants and soil were tested. Bacteria were stored at –80°C on Protect beads (Technical Service Consultants, Ltd., Heywood, England) and grown on nutrient agar.

Selection of primers. The nucleotide sequence of the *Erwinia carotovora* subsp. *atroseptica*-specific DNA probe (20) was determined using T3 and T7 sequencing primers and an Applied Bio-

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systems (Foster City, CA) automatic sequencer (model 373A) by the Nucleic Acid-Protein Service Unit, Biotechnology Laboratory, University of British Columbia (NAPS), Vancouver, Canada. Two oligonucleotides, each 18 bp in length and located near the 3′ and 5′ end of the probe, were selected for PCR amplification of a 690-bp fragment. Primers ECA1f (5′-CGGCATCATAAAAACACG-3′) and ECA2r (5′-GCACACTTCATCCAGCGA-3′) were synthesized on an Applied Biosystems DNA synthesizer (model 391) by NAPS.

DNA extraction procedure. Bacteria from 1 ml of cell suspension or plant extraction fluid (described below) were pelleted in an Eppendorf tube by centrifugation at 14,000 × *g* for 20 min and resuspended in 100 µl of Tris-EDTA buffer (pH 8.0) containing 1% (wt/vol) sodium-dodecyl sulfate. Samples from pure cultures

TABLE 1. Strains of bacteria tested for reaction in a polymerase chain reaction (PCR) test with primers specific for *Erwinia carotovora* subsp. *atroseptica*

Species/subspecies	Strain/ serogroup	Host	Geographic origin[a]	PCR
E. c. atroseptica	1 XVIII	Potato	BC	+
E. c. atroseptica	3 I	Potato	BC	+
E. c. atroseptica	6 XVIII	Potato	BC	+
E. c. atroseptica	15 I	Potato	BC	+
E. c. atroseptica	17 I	Potato	BC	+
E. c. atroseptica	19 I	Potato	BC	+
E. c. atroseptica	20 I	Potato	BC	+
E. c. atroseptica	30 I	NK[b]	AZ	+
E. c. atroseptica	31 I	Potato	WI	+
E. c. atroseptica	32 XVIII	Potato	WI	+
E. c. atroseptica	34 XX	Potato	WI	+
E. c. atroseptica	37 XVIII	Potato	WI	+
E. c. atroseptica	39 XVIII	Potato	WI	+
E. c. atroseptica	40 XVIII	Potato	WI	+
E. c. atroseptica	41 I	Potato	WI	+
E. c. atroseptica	42 I	Potato	WI	+
E. c. atroseptica	46 I	Potato	WI	+
E. c. atroseptica	98 I	Potato	WI	+
E. c. atroseptica	125 I	Potato	WI	+
E. c. atroseptica	180	Potato	Netherlands	+
E. c. atroseptica	191 I	Potato	BC	+
E. c. atroseptica	195 I	Potato	BC	+
E. c. atroseptica	196 XX	Potato	BC	+
E. c. atroseptica	198 XXII	Potato	BC	+
E. c. atroseptica	199 XX	Potato	Scotland	+
E. c. atroseptica	203 XX	Potato	BC	+
E. c. atroseptica	205 XXII	Potato	BC	+
E. c. atroseptica	266 XX	Potato	Scotland	+
E. c. atroseptica	296 XX	Potato	Scotland	+
E. c. atroseptica	417 I	Potato	Scotland	+
E. c. atroseptica	418	Potato	Scotland	+
E. c. atroseptica	419 XXII	Potato	Scotland	+
E. c. atroseptica	420 XXII	Potato	Scotland	+
E. c. atroseptica	438 XXII	Potato	BC	+
E. c. atroseptica	547 I	Potato	Netherlands	+
E. c. atroseptica	553 XXII	Potato	Sweden	+
E. c. atroseptica	556 XX	Potato	Sweden	+
E. c. atroseptica	557	Potato	Finland	+
E. c. atroseptica	558	Potato	Finland	+
E. c. atroseptica	559	Potato	Finland	+
E. c. atroseptica	561 XVIII	Potato	WI	+
E. c. atroseptica	565 XXII	Potato	NK	+
E. c. atroseptica	583	Potato	Scotland	+
E. c. atroseptica	587	Potato	Scotland	+
E. c. atroseptica	588	Potato	Scotland	+
E. c. atroseptica	591	Potato	Scotland	+
E. c. atroseptica	593	Potato	Scotland	+
E. c. atroseptica	594	Potato	Scotland	+
E. c. atroseptica	595	Potato	Scotland	+
E. c. atroseptica	596	Potato	Scotland	+
E. c. carotovora	21 II	Potato	Netherlands	–
E. c. carotovora	23 XV	Potato	Netherlands	–
E. c. carotovora	26 V	Potato	Netherlands	–
E. c. carotovora	59 XIII	Potato	Netherlands	–
E. c. carotovora	61 X	Potato	Netherlands	–
E. c. carotovora	62 VIII	Potato	Netherlands	–
E. c. carotovora	63 IX	Potato	Netherlands	–
E. c. carotovora	65 XIV	Potato	Netherlands	–
E. c. carotovora	67 XII	Potato	WI	–
E. c. carotovora	68 VII	Potato	Netherlands	–
E. c. carotovora	69 III	Iris	Netherlands	–
E. c. carotovora	71 III	Potato	Netherlands	–
E. c. carotovora	72 VI	Chicory	Netherlands	–
E. c. carotovora	92 XVII	Potato	BC	–
E. c. carotovora	94 XVI	Potato	BC	–
E. c. carotovora	189 VI	Potato	WI	–
E. c. carotovora	190 IV	Potato	WI	–
E. c. carotovora	193 XI	Potato	BC	–
E. c. carotovora	194 XIX	Potato	BC	–
E. c. carotovora	197 XXI	Potato	BC	–
E. c. carotovora	207 XXIII	Potato	BC	–
E. c. carotovora	208 XXIV	Potato	BC	–
E. c. carotovora	209 XXX	Potato	BC	–
E. c. carotovora	210 XXV	Potato	OR	–
E. c. carotovora	211 XXVI	Potato	CT	–
E. c. carotovora	360 XXVII	Potato	OR	–
E. c. carotovora	365 XXVIII	Potato	OR	–
E. c. carotovora	372	Hibiscus	BC	–
E. c. carotovora	376	Broccoli	BC	–
E. c. carotovora	380 XXIX	Potato	OR	–
E. c. carotovora	400 XVIII	Potato	Scotland	–
E. c. carotovora	441	Sunflower	Mexico	–
E. c. carotovora	462	Turnip	BC	–
E. c. carotovora	474 XXXIII	Potato	OR	–
E. c. carotovora	475 XXXIV	Potato	OR	–
E. c. carotovora	476 XXXII	Potato	OR	–
E. c. carotovora	477 XXXI	Potato	OR	–
E. c. carotovora	497 XXXVI	Potato	OR	–
E. c. carotovora	498 XXXVII	Potato	OR	–
E. c. carotovora	499 XXXV	Potato	OR	–
E. c. carotovora	500 XXXVIII	Potato	OR	–
E. c. carotovora	517 XL	Potato	OR	–
E. c. carotovora	518 XXXIX	Potato	OR	–
E. c. carotovora	539	Artichoke	CA	–
E. c. betavasculorum	29	Sugar beet	CO	–
E. c. betavasculorum	545	Sugar beet	CO	–
E. c. odorifera	CFBP 1878	Chicory	France	–
E. c. odorifera	CFBP 1880	Chicory	France	–
E. c. odorifera	CFBP 1893	Chicory	France	–
E. c. odorifera	CFBP 1645-1	Chicory	France	–
E. c. wasabiae	SR 91	Horseradish	Japan	–
E. c. wasabiae	SR 92	Horseradish	Japan	–
E. c. wasabiae	SR 93	Horseradish	Japan	–
E. c. wasabiae	SR 94	Horseradish	Japan	–
E. carotovora	43	Tomato	Scotland	–
E. carotovora	44	Tomato	Scotland	–
E. carotovora	45	Tomato	FL	–
E. carotovora	53	Cauliflower	England	–
E. carotovora	57 XXIX	Cauliflower	England	–
E. carotovora	439 IX	Carrot	Mexico	–
E. carotovora	552	Potato	Switzerland	–
E. carotovora	560	Lettuce	Netherlands	–
E. chrysanthemi	340	Potato	Peru	–
E. herbicola	LMG 2565	Cereal	Canada	–
Serratia plymuthica	LMG 6823	Chicory	Belgium	–
Pseudomonas aureofaciens	LMG 1245	Clay	Netherlands	–
P. corrugata	LMG 2172	Tomato	UK	–
P. marginalis	LMG 2210	Chicory	Belgium	–
P. solanacearum	IPO 267	Potato	NK	–
Arthrobacter globiformis	LMG 3820	NK	NK	–
A. histinolovorans	LMG 3822	Soil	NK	–
A. protophormiae	ATCC 17775	Soil	NK	–
Unidentified enteric	6A	Potato	NB	–
Unidentified enteric	7G	Potato	NB	–

[a] BC = British Columbia; AZ = Arizona; WI = Wisconsin; OR = Oregon; CT = Connecticut; CA = California; CO = Colorado; FL = Florida; UK = United Kingdom; and NB = New Brunswick.

[b] Not known.

were heated to 50°C for 3 h and did not require enzymatic digestion. Plant samples were treated with Proteinase K (British Drug House, Vancouver, BC, Canada) at 10 µg/ml at 50°C for 3 h. One-half volume of 7.5 M ammonium acetate was mixed with heat-treated samples that then were centrifuged at 14,000 × *g* for 10 min in an Eppendorf centrifuge or in 96-tube blocks (Beckman Instruments, Inc., Mississauga, ON, Canada) for 1 h at 3,000 × *g* in a plate rotor. DNA was precipitated from the supernatant fractions by adding 1 volume of isopropanol and pelleted by centrifugation. Pellets were washed with 70% ethanol, dried at 58°C for 10 min, dissolved in 50 µl of purified water, and heated to 50 to 55°C prior to PCR.

PCR amplification. PCR was usually performed using an Ericomp Easy Cycler (San Diego, CA), but some amplification reactions were compared in a Perkin-Elmer thermal cycler 480 (Norwalk, CT). Individual PCR reactions (20 µl) were run for 40 cycles and contained 0.5 µM each primer; 100 µM each of the deoxynucleotides dATP, dCTP, dGTP, and dTTP; 2.0 mM Mg^{++}; and 0.5 units of *Taq* DNA polymerase (BioCan Scientific, Mississauga, ON, Canada) in the buffer provided by the manufacturer to which 1 µl of DNA template extracted from pure cultures or plant samples was added.

Template DNA was denatured for 5 min at 95°C in the first PCR cycle and for 30 s at 94°C in subsequent cycles. Annealing was at 62°C for 45 s, and DNA extension at 72°C for 45 s. An 8-min extension period was added after the final cycle. Aliquots (5 µl) of PCR products were analyzed on agarose gels containing ethidium bromide elecrophoresed at 5 V/cm for 1 h. A standard 100-bp DNA ladder (Gibco/BRL, Burlington, ON, Canada) was included on each gel.

Plant samples. To compare PCR detection with ELISA, symptomatic and asymptomatic potato stem and tuber samples were collected from field plots planted with *E. carotovora* subsp. *atroseptica*-inoculated seed tubers and from commercial potato farms. Stems were sampled by cutting 1-cm segments from the base of the stem or at soil level. Seed tubers were sampled by removing a portion of intact tissue near growing stems, and progeny tubers were sampled either by removing a 0.5 g of core from the stolon attachment site or a 5-cm-long strip of periderm with a vegetable peeler.

Individual stem and tuber samples were crushed and shaken overnight at 23°C in 1 ml of sterile distilled water in resealable plastic bags. The extract from each sample was tested in duplicate by ELISA, and a 1-ml aliquot was frozen at –20°C for later DNA extraction. To determine whether the presence of PCR inhibitors was a problem in PCR-negative samples, *E. carotovora* subsp. *atroseptica* DNA was added at 10 ng/µl to negative DNA samples that were tested again by PCR.

ELISA. The ELISA protocol used in this study was an indirect, double-antibody sandwich procedure using monoclonal antibody (MAb) 4F6 as described previously (5), except that 50 µl of sample buffer (2% polyvinylpyrolidone, 0.2% skim milk powder, and 0.05% Tween in 0.01 phosphate buffered saline, pH 7.2) was pipetted into each well prior to addition of 50 µl of plant extract fluid. Absorbance values greater than 3× the mean of negative control values were considered positive.

Sensitivity of PCR. To determine the minimum number of cells that could be detected by PCR, a pure culture of *E. carotovora* subsp. *atroseptica* strain 31 was diluted serially in 10-fold increments in distilled water or plant extract fluid. Concentration of *E. carotovora* subsp. *atroseptica* was determined by a standard plate count procedure on nutrient agar. DNA was extracted from eight dilutions and subjected to PCR. The experiment was repeated three times each for distilled water, stem fluid, and tuber fluid.

RESULTS

Specificity of PCR. All strains of *E. carotovora* subsp. *atroseptica* yielded a 690-bp DNA fragment in PCR with the ECA1f and ECA2r primers (Fig. 1). Of the 112 *E. carotovora* strains from various hosts and geographic regions that were tested, only the *E. carotovora* subsp. *atroseptica* strains yielded an amplification product (Table 1). Strains of the *E. carotovora* subsp. *carotovora, betavasculorum, odorifera*, and *wasabiae* and unknown subspecies of *E. carotovora* from nonpotato hosts that were similar to *E. carotovora* subsp. *atroseptica* in some of their biochemical reactions (such as production of acid from α-methyl glucoside and reducing substances from sucrose) were all negative in PCR. Similarly, strains of other *Erwinia* spp. and other genera of bacteria listed in Table 1 did not yield PCR products.

Sensitivity of PCR. A 690-bp amplification product was obtained from aqueous suspensions of pure culture as low as 332 ± 77 CFU per ml (Fig. 2). This was equivalent to detecting DNA from 5 to 10 CFU per PCR reaction. The minimum concentration detected was 521 ± 75 and 239 ± 4 CFU per ml for pure cultures added to stem and tuber extracts, respectively, prior to DNA extraction.

PCR and ELISA of potato samples. The 690-bp PCR product was obtained with extracts from all stems with blackleg-like symptoms (Table 2). Extracts from all symptomatic stems also tested positive in ELISA, with absorbance values ranging from 1.2 to 1.8 compared to healthy potato controls that gave readings of 0.030 to 0.045. Two of eight asymptomatic stem samples that were ELISA positive tested negative in PCR, whereas 21% of ELISA-negative, asymptomatic stem samples were positive in PCR (Table 2). Progeny tubers with blackleg symptoms also tested positive by both PCR and ELISA, as did some extracts from

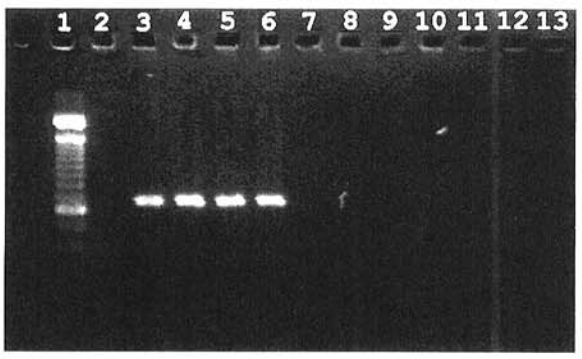

Fig. 1. Ethidium bromide-stained agarose gel of polymerase chain reaction products generated from *Erwinia carotovora* subsp. *atroseptica* (Eca) and *E. carotovora* subsp. *carotovora* (Ecc) strains with primers ECA1f and ECA2r. Lane 1, DNA ladder; lane 3, Eca 31; lane 4, Eca 6; lane 5, Eca 196; lane 6, Eca 198; lane 7, Ecc 71; lane 8, Ecc 190; lane 9, Ecc 26; lane 10, Ecc 189; and lane 11, Ecc 68. Lanes 2, 12, and 13 are empty.

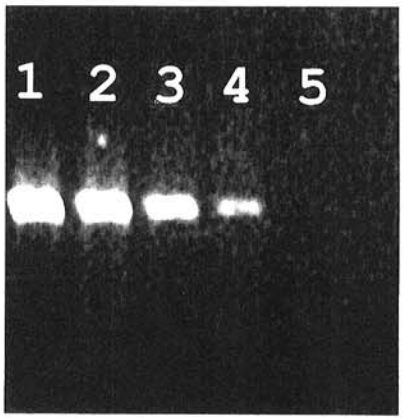

Fig. 2. Ethidium bromide-stained agarose gel of polymerase chain reaction products obtained with extracts of potato stems to which cells of *Erwinia carotovora* subsp. *atroseptica* had been added at 9×10^4 (lane 1), 9×10^3 (lane 2), 9×10^2 (lane 3), 4.5×10^2 (lane 4), and 2.25×10^2 CFU per ml (lane 5).

asymptomatic tubers of blackleg-infected plants. Overall 91% of asymptomatic ELISA-positive tuber samples tested positive in PCR, and 12% of ELISA-negative tuber samples tested positive in PCR (Table 2).

The seed tubers, which were sampled 90 days after planting, all had been inoculated with *E. carotovora* subsp. *atroseptica* prior to planting and had produced symptomless plants. Of the seed tuber samples, 20% tested positive in PCR, but 30% tested positive in ELISA; only two (10%) seed pieces tested positive in both tests (Table 2).

All but two of 84 PCR-negative field samples to which purified *E. carotovora* subsp. *atroseptica* DNA was subsequently added yielded the 690-bp PCR product after repeating the PCR test. These two tuber samples apparently contained compounds inhibitory to PCR.

DISCUSSION

DNA from strains isolated from potato and previously identified as *E. carotovora* subsp. *atroseptica* on the basis of physiological and serological reactions was specifically amplified by PCR using the primers selected in this study. DNA extracted from field-grown plants with blackleg disease also was amplified, and, along with the negative results for the other strains tested, provided further confirmation that the ECA1f and ECA2r primers were highly specific for the blackleg pathogen. As expected, this specificity closely paralleled the specificity of the DNA probe (20) from which the primers were selected. DNA from strain 57, an *E. carotovora* serogroup XXIX isolate from cauliflower that hybridized weakly with the *E. carotovora* subsp. *atroseptica* probe, was not amplified by PCR using the selected primers.

The specificity of our PCR test contrasts with the PCR test based on *pel* gene nucleotide sequences (3). The latter primers amplified all *E. carotovora* strains except those identified as *E. carotovora* subsp. *betavasculorum*, and RFLP analysis was required to identify *E. carotovora* subsp. *atroseptica* strains. Interestingly, RFLP analysis of the product amplified by the *pel* primers divided *E. carotovora* subsp. *atroseptica* into two groups (3). Differences in the electrophoretic mobility of PCR products, which also would have suggested heterogeneity among *E. carotovora* subsp. *atroseptica* strains, were not observed in our work.

Our DNA extraction procedure, which avoided use of organic solvents, was designed to process a large number of field samples and was similar to that described by Flemming et al. (6). The DNA extract was sufficient for PCR amplification and, with few exceptions, did not contain compounds inhibitory to the reaction. Furthermore, sensitivity of detection by PCR in plant extraction fluids was similar to that for pure cultures (Table 2), which also indicated that residual plant components were not inhibitory. The source of PCR inhibitory substances in the two samples was not determined.

TABLE 2. Comparison of enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR) for detection of *Erwinia carotovora* subsp. *atroseptica* in potato tissues

Potato tissue	No. of samples tested	ELISA	No. of samples positive by PCR
Stems			
Symptomatic	25	+	25
Asymptomatic	8	+	6
	52	–	11
Seed tubers	6	+	2
	14	–	2
Progeny tubers			
Symptomatic	5	+	5
Asymptomatic	35	+	32
	25	–	3

Although the PCR reaction, which detected as few as 3×10^2 CFU per ml, was considerably more sensitive than ELISA, which requires about 10^5 CFU per ml (8,13), some of the ELISA-positive samples were negative in PCR. More than half of the samples from decaying seed tubers and a few stem and tuber samples tested positive in ELISA but negative in PCR (Table 2). Perhaps these positive ELISA tests resulted from serological cross-reactions, although the only cross-reactions with MAb 4F6 that have been detected are those with a *Janthinobacterium* sp. and a *Comamonas*-like bacterium in the Netherlands (J. van der Wolf and J. van Vuurde, *personal communication*). Alternatively, the conflicting results could be due to differences in the persistence of the target molecules in the environment.

ELISA was specific for *E. carotovora* subsp. *atroseptica* lipopolysaccharide (5), and this antigen may be considerably more stable than DNA after the death of bacterial cells, accounting for the positive ELISA results for samples that were not amplified by PCR. Recently, van Vuurde and de Vries (18) showed that *E. carotovora* subsp. *atroseptica* remain viable for only short periods after inoculation onto potato tubers. Cells do not need to be viable to obtain successful PCR amplification, but intact target nucleic acid sequences are required. In the environment, nucleic acids of nonviable cells probably are degraded quickly, as demonstrated for boiled *Escherichia coli* cells added to natural pond water (11). In this case, 2.7×10^7 boiled cells per ml could no longer be detected by PCR after 3 weeks. Similarly, intact cells of *Legionella pneumophila* killed by exposure to biocide also could not be detected by PCR, whereas unculturable viable cells were detected (1). Perhaps the *E. carotovora* subsp. *atroseptica* cells do not survive competition with secondary saprophytes very well. This hypothesis is supported by the observation that generally only a few intact bacterial cells are found in badly decayed blackleg-infected tissue by immunofluorescence staining with the same MAbs (S. H. De Boer and L. J. Ward, *unpublished data*). Background staining in such preparations, however, is usually high, which suggests that the soluble lipopolysaccharide antigen persists longer than intact cells.

PCR detection of *E. carotovora* subsp. *atroseptica* in ELISA-negative samples exemplifies the greater sensitivity of PCR compared to ELISA for detecting low populations of bacteria. However, our results also suggest that the persistence of target molecules may differ in the environment, which is an important consideration when detection is not based on isolation procedures. Nevertheless, PCR is expected to serve as an important additional tool for elucidating the spread and survival of the blackleg pathogen in relation to the potato crop.

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