Specific Detection of *Erwinia carotovora* subsp. *atroseptica* with a Digoxigenin-labeled DNA Probe

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ABSTRACT

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A DNA probe specific for all four serogroups of *Erwinia carotovora* subsp. *atroseptica* was selected from an *Eco*RI digest of a cloned library constructed with the combined genomic DNA from strains 6, 31, 196, and 198, which each represented one of the serogroups of *E. c. atroseptica*. Specificity of the probe was initially tested against a panel of 40 *E. carotovora* strains representing serogroups I through XL. Subsequently hybridization of the probe, labeled with digoxigenin, was tested against an additional 82 strains of *E. carotovora*. The probe hybridized to all strains previously identified as *E. c. atroseptica* on the basis of biochemical and physiological tests, and it hybridized weakly with only one strain that was not a typical *E. c. atroseptica* strain. This strain had been isolated from cauliflower in the United Kingdom. The probe hybridized with partially purified DNA extracted from enrichments of potato periderm to which as few as 10 colony-forming units per milliliter were added prior to incubation for 48 h. The hybridization reactions to enrichment preparations were quantified by reading gray values from video images of the chemilumograms.

Erwinia carotovora subsp. *atroseptica* and *E. c. carotovora* both cause decay of potato stems and tubers. *E. c. atroseptica* is usually associated with the blackleg disease of potato stems, in which the infection originates from the seed piece, whereas stem decay initiated by *E. c. carotovora* normally begins at a wound site in the aboveground portion of the plant. *E. c. carotovora* infections usually do not develop the black pigmentation typical of *E. c. atroseptica* infections, although under conditions of high field temperatures *E. c. carotovora* has been reported to cause blackleg-like symptoms (12).

Potato tubers probably are not the only inoculum source for *E. c. atroseptica*, but they are an important source that directly affects the amount of disease that develops in a crop. However, the incidence of blackleg in a crop is often not a good indicator of progeny tuber contamination, because the bacteria spread in the root zone and contaminate developing tubers even in the absence of disease symptoms. Indexing of the tubers themselves is required to determine the inoculum load they carry. To detect pectolytic erwinias on potato tubers, bacteria are isolated on selective media and then identified to subspecies (14).

E. c. atroseptica strains can be differentiated from *E. c. carotovora* on the basis of pathogenicity and biochemical-physiological characteristics (7), cellular fatty acid composition (5), and electrophoretic mobility of pectic lyase in sodium dodecyl sulfate (SDS) polyacrylamide gels (19). Serologically, *E. c. atroseptica* is far more homogenous than *E. c. carotovora*. Most *E. c. atroseptica* strains isolated from potato in Canada (3), the United States (2), Australia (13), the Netherlands (6), and Sweden (1) are serogroup I. However, additional serogroups do occur and have been detected on potato in geographic areas in which a significant number of strains have been tested (6). For example, in British Columbia three other serogroups (XVIII, XX, and XXII) occur at low (<4%) frequencies (3). Finland appears to be an exception in that only 55% of the *E. c. atroseptica* strains isolated there were serogroup I (6). It has been impractical to use serological procedures for detection of pectolytic erwinias on potato because of their serological heterogeneity.

With DNA probe technology, the presence of a specific bacterial pathogen can be detected in potato tuber extracts without having to isolate the pathogen. Previously we reported development of a probe specific for all *E. carotovora* strains including both *E. c. atroseptica* and *E. c. carotovora* (20). We have now isolated a fragment of *E. c. atroseptica* DNA that can be used as a specific probe for *E. c. atroseptica* alone; its specificity was tested using a nonradioactive labeling and detection system.

MATERIALS AND METHODS

Probe selection. Genomic DNA from *E. c. atroseptica* strains 31 (serogroup I), 6 (serogroup XVIII), 196 (serogroup XX), and 198 (serogroup XXII) were mixed and partially digested with *Sau*3AI and then cloned into the *Bam*HI site of the Bluescribe (Vector Cloning Systems, San Diego, CA) vector and propagated in *Escherichia coli* strain DH5α as described previously (20). The entire library of clones was grown in 100 ml of Luria-Bertani medium (10) plus ampicillin (100 μg/ml) at 37 C to approximately 10^8 colony-forming units (cfu)/ml. A 1.0-ml aliquot was removed and frozen in 25% glycerol for future use. Plasmid DNA was purified from the remaining culture by alkaline lysis, cleaved separately with *Bam*HI and *Eco*RI (Bethesda Research Laboratories, Burlington, ON), and analyzed by agarose gel electrophoresis. Fragments in clearly resolved bands smaller than vector DNA were gel purified using Geneclean (Bio/Can Scientific Inc., Mississauga, ON). Purified fragments were random primed and labeled with α^{32}P-dATP using a commercially available kit (Bethesda Research Laboratories), and separately labeled with digoxigenin using the Boehringer Mannheim (Laval, PQ) DNA-labeling kit.

Labeled DNA was used to probe *E. c. atroseptica*, *E. c. carotovora*, and *E. coli* alkaline-fixed in spots on strips of Zeta-Probe GT membrane (Bio-Rad Laboratories, Mississauga, ON). Membranes were prehybridized for 4–6 h with denatured salmon sperm DNA (50 μg/ml) in 5× SSC (1× SSC is 0.15 M NaCl, 0.015 M trisodium citrate), 1% Blotto, 1× Denhardt's solution, 1% SDS, and 50% formamide and then hybridized overnight with probe in prehybridization mixture at 45 C (8). For hybridizations, radiolabeled probe concentrations were adjusted to 3–5 ng/ml, and autoradiograms (using two intensifying screens at −80 C) were developed after 4- and 16-h exposures. Fragments that preferentially hybridized with *E. c. atroseptica* strains were selected as possible sources of specific probes. Selected fragments were subcloned into Bluescribe, and subsequent probe DNA was gen-

erated from single-colony sources.

Colony hybridization. Initial screening of probe specificity was done with a panel of four *E. c. atroseptica* and 36 *E. c. carotovora* strains, each representing one of 40 different serogroups. A strain of *E. chrysanthemi* and of *E. coli* were included as controls. Subsequently, the selected probe was tested against an additional 82 strains of pectolytic erwinias from the bacterial collection at the Vancouver Research Station. Each strain was tested at least three times. Bacterial colonies to be tested were picked from agar medium and resuspended in individual wells of a sterile 96-well microtiter plate containing 150 μl of phosphate-buffered saline. Bacteria were transferred to Zeta-Probe membrane under vacuum using a dot blot manifold, sequentially treated with NaOH and proteinase K, and baked under vacuum at 80 C for 2 h. Filters were prehybridized at 45 C in standard prehybridization solution containing formamide and 50 μg/ml sonicated salmon sperm DNA.

Denatured radio- or digoxigenin-labeled probe was added to prehybridization solutions at 3–5 ng/ml and incubated overnight at 45 C. Filters were sequentially washed for 15 min each in 2×, 0.5×, and 0.1× SSC, all with 1% SDS at 68 C. Hybridization of radiolabeled probe was detected as above. Digoxigenin-labeled probe was detected with minor modifications of the manufacturer's instructions (Boehringer Mannheim). Filters were blocked for 2 h at 60 C in 5% Blotto (10% skim milk powder, 0.2% sodium azide) in 0.1 M Tris-buffered saline, pH 7.5. The anti-digoxigenin antibody-alkaline phosphatase conjugate was diluted in blocking solution and added to the filters at room temperature for 45 min. Excess conjugate was removed with three 15-min washes in Tris-buffered saline containing 0.2% Blotto and 0.05% Brij 35 (Pierce, Rockford, IL). Bound enzyme was detected with 3-(2′-spiroadamantane)-4-methoxy-4-(3″-phosphoryloxy)-phenyl-1,2-dioxetane (AMPPD, Boehringer Mannheim), and resulting chemiluminescence was recorded in 5- to 60-min exposures on XOMAT-K X-ray film (Eastman Kodak Co., Rochester, NY), or from 1 h to overnight exposures on Kodak 4141 film with the emulsion side facing the filter.

Enzyme-linked immunosorbent assay. The series of pectolytic *Erwinia* strains tested for reactivity with the DNA probe were also screened against monoclonal antibody 4F6 by indirect sandwich enzyme-linked immunoassay as described (4).

Southern analysis. Following electrophoresis in a 1% agarose gel with Tris-borate buffer (0.089 M Tris, 0.89 M boric acid, 0.002 M EDTA), DNA fragments were transferred to Zeta-Probe membrane by vacuum blotting for 1 h in 0.4 M NaOH. Membranes were baked, prehybridized, and hybridized as above using digoxigenin-labeled probes.

Enrichments. Certified seed potato tubers (cv. Red Pontiac) were washed with tap water, and 1–2 g of periderm was removed and added to 5 ml of enrichment broth (11). Pure cultures of *E. c. atroseptica* strains 31, 6, 196, and 198, representing serogroups I, XVIII, XX, and XXII, were added separately to periderm enrichments. For each strain, a series of 10-fold dilutions was added to separate enrichment tubes to provide final densities of 10^4 to <1 cfu/ml. Control enrichments were either uninoculated or inoculated with *E. coli* or *E. chrysanthemi*. Enrichments were incubated aerobically without shaking at 23 C and sampled at 48 and 72 h. A 500-μl sample from each enrichment was digested with proteinase K at a final concentration of 10 μg/ml in 10 mM Tris HCl/1 mM EDTA, pH 7.4 (TE) with 1% SDS from 4 h to overnight at 50 C. Cellular debris was precipitated by the addition of one half volume of 7.5 M ammonium acetate followed by brief mixing and centrifugation at 16,000 *g* for 5 min. The supernatant was transferred to a fresh tube and precipitated with three volumes of 95% ethanol at −20 C for 2–3 h. The precipitate was collected by centrifugation, dried under vacuum, and dissolved in 250 μl of TE. Aliquots (50 μl) mixed with equal volumes of 1 M NaOH were applied to a Zeta-Probe membrane under vacuum in a 96-well manifold. Filters were baked at 120 C for 30 min, rinsed in TE, prehybridized for 4–6 h, and then probed with digoxigenin-labeled probe as described above. Reactions were quantified as gray values from video images of chemilumograms captured with a monochrome CCD (charge coupled device) camera (Cohu Inc., San Diego, CA) using an image analysis program (Vidas, Carl Zeiss Canada, Don Mills, ON). Values were transformed to a scale of 0–100 units on the basis of background reaction (0 units) and mean of positive controls (100 units). Standard errors were calculated on four replications of each sample. The experiment was repeated twice with all four strains.

RESULTS AND DISCUSSION

Size selection of DNA fragments was used in our study to simplify isolation of specific probes. The procedure involved simple fractionation of total cloned DNA by gel electrophoresis after endonuclease digestion. Depending on the sequence of the

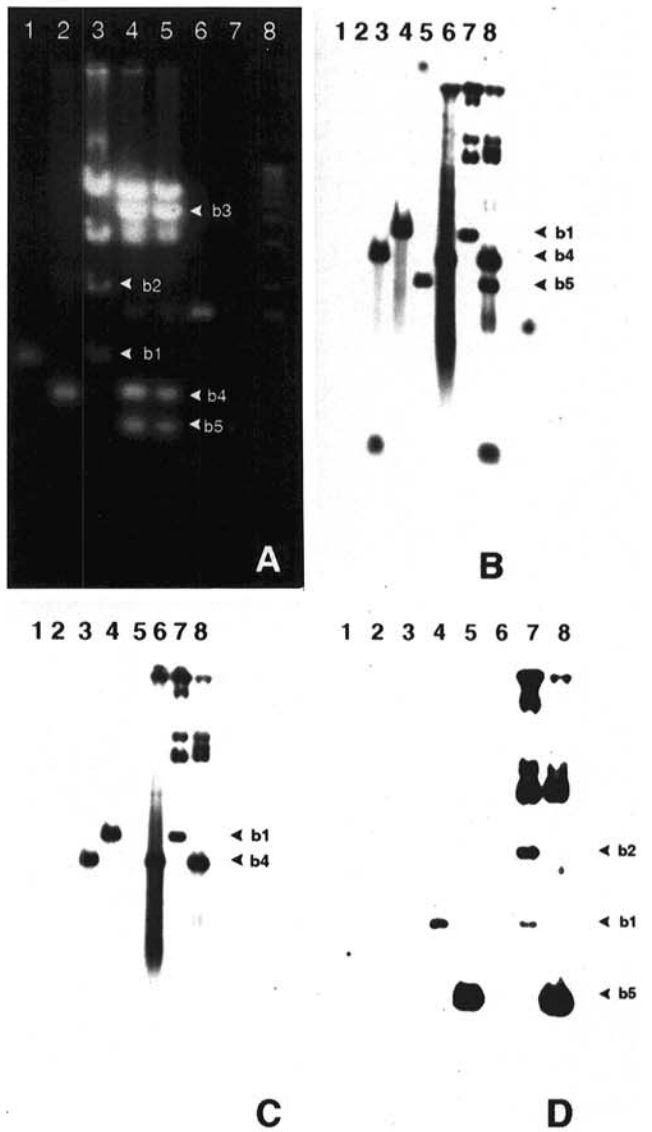

Fig. 1. A, Ethidium bromide-stained agarose gel and **B–D,** Southern blots of electrophoresed restriction digests of a cloned *Erwinia carotovora* subsp. *atroseptica* genomic library and gel-purified fragments. Bands labeled b1–b5 designate bands 1–5. **A,** agarose gel: lane 1, gel purified band 1; lane 2, gel purified band 1 cut with *Eco*RI; lane 3, genomic library cut with *Bam*HI; lanes 4 and 5, genomic library cut with *Eco*RI; lane 6, *E. carotovora*-specific probe B; lane 7, gel purified band 5; lane 8, 1 kb DNA ladder. **B–D,** Southern blots probed with digoxigenin-labeled bands 1, 4, and 5, respectively: lane 1, 1-kb DNA ladder; lane 2, *E. carotovora*-specific probe B; lane 3, gel-purified band 1 digested with *Eco*RI; lane 4, gel-purified band 1; lane 5, gel-purified band 5; lane 6, gel-purified band 4; lane 7, cloned *E. c. atroseptica* genomic library digested with *Bam*HI; lane 8, cloned *E. c. atroseptica* genomic library digested with *Eco*RI.

*Sau*3A-generated fragments from which the original library was constructed, the *Bam*HI cloning site in the Bluescribe vector may or may not be regenerated after ligation. The *Eco*RI site, however, should still exist as part of the multiple cloning site. The plasmid DNA from the library of clones that cut with *Bam*HI or *Eco*RI must have contained at least two sites for each enzyme in order to generate a subset of cloned fragments. One restriction site for each enzyme may have been in the multiple cloning site, but the other sites would have been in the insert. Regardless, fragments produced by these enzymes that were smaller than vector DNA contained minimum vector sequences.

Agarose gel electrophoresis of the *Bam*HI digest of the *E. c. atroseptica* genomic library revealed several bands, two of which were of lower molecular weight than vector DNA (band 1 at 1.2 kb and band 2 at 2.0 kb, lane 3, Fig. 1A). Similarly, the *Eco*RI digest also yielded two bands that were smaller than vector DNA (band 4 at 1.0 kb and band 5 at 0.8 kb, lanes 4 and 5, Fig. 1A). For comparison, a band from the *Eco*RI digest that was larger than vector DNA was also chosen for testing (band 3, lanes 4 and 5, Fig. 1A).

To determine whether the DNA in bands 1–5 hybridized selectively with *E. c. atroseptica*, gel-purified, radiolabeled DNA from each of the bands was hybridized to *E. chrysanthemi, E. coli*, and the panel of 40 *E. carotovora* strains representing different serogroups. Bands 1, 2, and 4 hybridized to some strains of *E. c. carotovora* and *E. c. atroseptica*, whereas band 3 hybridized to all the *E. carotovora* strains in the panel (Table 1). Bands 2 and 3 also hybridized to *E. chrysanthemi* and *E. coli*. The 0.8-kb *Eco*RI fragment (band 5) hybridized exclusively with strains 31, 6, 196, and 198, which represented the four *E. c. atroseptica* serogroups in the panel.

To determine whether the selected bands cross-hybridized, labeled DNA from bands 1, 4, and 5 was used for Southern analysis (Fig. 1B–D). Bands 2 and 3 were omitted because they hybridized with *E. coli*. Band 1 hybridized strongly with bands 4 and 5, but bands 4 and 5 did not cross-hybridize (Fig. 1B and C, respectively). To confirm the lack of cross-hybridization between bands 4 and 5, band 4 was overloaded in the gel (>200 ng/well) (lane 6, Fig. 1B–D). This resulted in some smearing, probably due to multimers and degradation of band 4 when probed with cross-hybridizing DNA. When band 1 was digested with *Eco*RI, a 1-kb fragment was generated that was indistinguishable from band 4 (lane 2, Fig 1A; lane 3, Fig. 1B and 1C). Band 5 was distinct and specific for *E. c. atroseptica* strains but cross-hybridized weakly with bands 1 and 2 (lane 7, Fig. 1D). These results suggest that bands 1, 2, 4, and 5 represent a related series of fragments and that bands 1 and 4 are derived from an overlapping sequence. In all three Southern blots, weak cross-hybridizations were observed with large molecular weight fragments in the digested genomic library and probably represent incomplete digestion of DNA sequences containing the labeled fragments. None of the labeled bands hybridized with the *E. carotovora* probe we developed earlier (lane 2, Fig. 1B–D) (20).

The specificity of band 5 for *E. c. atroseptica* was evident from

TABLE 1. Strains of *Erwinia carotovora* subsp. *atroseptica* and *E. c. carotovora* representing 40 serogroups and their hybridization with gel-purified DNA bands

Serogroup	Subspecies	Strain	Source	DNA fragments				
				1	2	3	4	5
I	*atroseptica*	31	A. Kelman SR8	+	+	+	+	+
II	*carotovora*	21	H. P. Maas Geesteranus 139	−	+	+	−	−
III	*carotovora*	71	H. P. Maas Geesteranus 226	+	+	+	+	−
IV	*carotovora*	190	A. Kelman SR204	+	+	+	+	−
V	*carotovora*	26	H. P. Maas Geesteranus 200	−	+	+	−	−
VI	*carotovora*	189	A. Kelman SR123a	+	−	+	+	−
VII	*carotovora*	68	A. Kelman SR165	+	−	+	+	−
VIII	*carotovora*	62	H. P. Maas Geesteranus 195	+	+	+	+	−
IX	*carotovora*	63	H. P. Maas Geesteranus 202	+	+	+	−	−
X	*carotovora*	61	H. P. Maas Geesteranus 222	+	+	+	−	−
XI	*carotovora*	193	R. J. Copeman E193	+	+	+	+	−
XII	*carotovora*	67	A. Kelman S162	−	+	+	−	−
XIII	*carotovora*	59	H. P. Maas Geesteranus 257	−	−	+	−	−
XIV	*carotovora*	65	H. P. Maas Geesteranus 196	−	+	+	−	−
XV	*carotovora*	23	H. P. Maas Geesteranus 162	+	+	+	+	−
XVI	*carotovora*	94	R. J. Copeman E315	−	−	+	−	−
XVII	*carotovora*	92	R. J. Copeman E6	+	−	+	+	−
XVIII	*atroseptica*	6	R. J. Copeman E17	+	+	+	+	+
XIX	*carotovora*	194	R. J. Copeman E103	+	−	+	−	−
XX	*atroseptica*	196	S. H. De Boer 196	+	+	+	+	+
XXI	*carotovora*	197	R. J. Copeman E295	+	−	+	−	−
XXII	*atroseptica*	198	R. J. Copeman E555	+	+	+	+	+
XXIII	*carotovora*	207	R. J. Copeman E125	−	±	+	−	−
XXIV	*carotovora*	208	E. J. Copeman E254	+	−	+	+	−
XXV	*carotovora*	210	M. L. Powelson 109-1	−	−	+	−	−
XXVI	*carotovora*	211	A. Kelman SR206	+	+	+	+	−
XXVII	*carotovora*	360	M. L. Powelson 86-5-8	+	+	+	+	−
XXVIII	*carotovora*	365	M. L. Powelson 78-24-7	−	±	+	−	−
XXIX	*carotovora*	380	M. L. Powelson 79-42-8	+	−	+	+	−
XXX	*carotovora*	209	S. H. De Boer 209	+	−	+	+	−
XXXI	*carotovora*	477	M. L. Powelson cc305	−	−	+	−	−
XXXII	*carotovora*	476	M. L. Powelson cc506	−	−	+	−	−
XXXIII	*carotovora*	474	M. L. Powelson cc306	−	−	+	−	−
XXXIV	*carotovora*	475	M. L. Powelson cc504	−	−	+	+	−
XXXV	*carotovora*	499	M. L. Powelson cc301	−	−	+	−	−
XXXVI	*carotovora*	497	M. L. Powelson cc108	−	−	+	−	−
XXXVII	*carotovora*	498	M. L. Powelson cc303	−	−	+	−	−
XXXVIII	*carotovora*	500	M. L. Powelson cc110	−	−	+	−	−
XXXIX	*carotovora*	518	M. L. Powelson cc105	+	−	+	+	−
XL	*carotovora*	517	M. L. Powelson 103	−	−	+	−	−
E. chrysanthemi		544	H. P. Maas Geesteranus	−	+	+	−	−
Escherichia coli		HB101		−	+	+	−	−

the initial test panel (Table 1), but it was obtained from an *Eco*RI digest of total cloned DNA and might represent a group of different fragments of similar size. Therefore, DNA from the 0.8-kb band was subcloned, and 10 clones were selected and cross-hybridized to determine whether the band consisted of a homogenous group of fragments. Since all 10 subclones cross-hybridized with one another, the band probably represented a single sequence. One clone was selected randomly and used as the *E. c. atroseptica* probe in further evaluations. After selecting the single clone, the specificity of the probe was tested against the original test panel of 40 *E. carotovora* strains and an *E. coli* strain in order to confirm previous results. Radio- and digoxigenin-labeled probes were compared on two identical filters. After a 4-h exposure with two intensifying screens at −80 C, the results with ^{32}P were comparable to a 1-h exposure at room temperature using digoxigenin and AMPPD (Fig. 2). Subsequent experiments were exclusively done with digoxigenin-labeled probe.

The resolution of discrete bands from digests of the *E. c. atroseptica* library could be due to amplification of clones containing small inserts that impart a selective advantage or to cloning a repetitive sequence. Repetitive sequences may be released by digestion from various fragments cloned in *E. coli* strains such as DH5α, which are deficient in DNA modification and recombination systems. To determine whether the probe represented a repetitive DNA sequence, genomic DNA from *E. c. atroseptica* was digested with *Eco*RI, vacuum blotted to Zeta-Probe, and probed with digoxigenin-labeled probe. The multiple banding pattern that resulted clearly demonstrates that the fragment used as probe is a repetitive DNA sequence (Fig. 3).

Repetitive DNA sequences are known to exist in both eukaryote and prokaryote genomes (18). Repetitive palindromic sequences, for example, occupied as much as 1% of the genomes of *E. coli* and *Salmonella typhimurium*, which are enteric bacteria closely related to the plant pathogenic erwinias (16). Some of the repetitive DNA elements in eubacteria are species specific and, therefore, useful as taxonomic markers and specific detection probes. Repetitive DNA sequences have already been used for identification of plant-associated bacteria. For example, a repetitive DNA sequence linked to the nodulation gene in *Bradyrhizobium japonicum* was useful for discriminating strains of a single serocluster from other nitrogen-fixing bacteria (15). Similarly, the presence of a repetitive DNA sequence in *Xanthomonas campestris* pv. *oryzae* differentiated this pathovar from other *X. campestris* pathovars (9). In this case, approximately 81 copies of the repetitive sequence were dispersed throughout the genome.

The *E. c. atroseptica* probe labeled with digoxigenin proved to be specific for the subspecies when it was tested against 82 strains of *E. c. atroseptica*, *E. c. carotovora*, *E. c. betavasculorum*, and *E. carotovora* of unknown subspecies (Table 2). Unlike the monoclonal antibody probe for *E. c. atroseptica* serogroup I, which reacts only with serogroup I and XXII strains (4), the DNA probe detected all strains of the subspecies. Only one non-*E. c. atroseptica* strain reacted with the DNA probe. This strain, isolated from cauliflower by B. Lund (ARC Food Research Institute, Norwich, England), was similar to *E. c. atroseptica* in that it produced acid from α-methyl glucoside but was unlike the blackleg bacterium in that it did not produce reducing substances from sucrose and grew at 36 C. In taxonomic studies, this strain grouped with other strains from cauliflower in a separate phenon from other pectolytic erwinias (6,17).

Strong positive signals were obtained in both experiments with digoxigenin-labeled probe for periderm enrichments to which as few as 10 cfu/ml of *E. c. atroseptica* had been added. The signals recorded on film and quantified as gray values demonstrated a clear difference between positive and negative results (Fig. 4). Positive signals with corrected gray values of >90 units were clearly distinguishable from negative signals barely visible as dots on the film but giving gray values of up to 30 units. Samples to which <10 cfu/ml or no *E. c. atroseptica* cells had been added gave signals of <30 units despite extensive growth of saprophytic bacteria as was evident from the high level of turbidity in the enrichment medium. The latter observation provided evidence

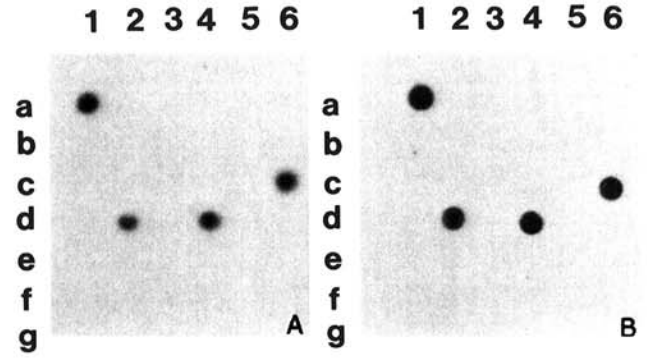

Fig. 2. Comparison of dot blot hybridization with band 5 labeled with **A,** ^{32}P-labeled or **B,** digoxigenin to *Erwinia carotovora* subsp. *atroseptica* on a panel of 36 *E. c. carotovora* and four *E. c. atroseptica* strains representing serogroups I–XL. Bacterial cells were lysed and fixed onto the filter in numerical order by serogroup number as listed in Table 1. Strains at positions a1, c6, d2, and d4 are *E. c. atroseptica* strains 31 (serogroup I), 6 (serogroup XVIII), 196 (serogroup XX), and 198 (serogroup XXII), respectively.

Fig. 3. Southern blot of genomic DNA from **A,** *Erwinia carotovora* subsp. *atroseptica* strain 31, serogroup I and **B,** *Erwinia carotovora* strain 196, serogroup XX cut with *Eco*RI and probed with digoxigenin labeled band 5 probe.

TABLE 2. Reaction of various strains of pectolytic erwinia with monoclonal antibody 4F6 in ELISA (given as absorbance at 405 nm) and hybridization with a digoxigenin-labeled DNA probe (band 5) to *E. carotovora* subsp. *atroseptica*

Strain	Serogroup	Host	Location	Reaction with	
				4F6	Band 5
E. c. atroseptica					
1	XVIII	potato	British Columbia	0.002	+
3	I	potato	British Columbia	0.559	+
6	XVIII	potato	British Columbia	0.007	+
15	I	potato	British Columbia	0.567	+
17	I	potato	British Columbia	0.232	+
19	I	?	British Columbia	0.274	+
20	I	?	British Columbia	0.341	+
30	I	?	Arizona	0.691	+
32	XVIII	potato	Wisconsin	0.007	+
34	XX	potato	Wisconsin	0.013	+
37	XVIII	potato	Wisconsin	0.008	+
39	XVIII	potato	Wisconsin	0.009	+
40	XVIII	potato	Wisconsin	0.007	+
41	I	potato	Wisconsin	0.661	+
42	I	potato	Wisconsin	0.652	+
46	I	potato	Wisconsin	0.610	+
58	XX	potato	England	0.011	+
125	I	potato	Wisconsin	0.678	+
180	?	?	Netherlands	0.574	+
191	I	potato	British Columbia	0.553	+
195	I	potato	British Columbia	0.077	+
196	XX	potato	British Columbia	0.013	+
199	XX	potato	Scotland	0.009	+
203	XX	potato	British Columbia	0.067	+
205	XXII	potato	British Columbia	0.785	+
266	XX	potato	Scotland	0.014	+
296	XX	potato	Scotland	0.006	+
E. c. carotovora					
9	VI	?	British Columbia	0.012	−
13	IV	onion	British Columbia	0.008	−
14	III	?	British Columbia	0.005	−
23	XV	potato	Netherlands	0.010	−
24	III	potato	Netherlands	0.007	−
25	?	chicory	Netherlands	0.009	−
26	V	potato	Netherlands	0.005	−
27	?	potato	Netherlands	0.007	−
50	XIV	potato	Wisconsin	0.010	−
51	XIV	potato	Wisconsin	0.007	−
59	XIII	potato	Netherlands	0.008	−
60	IX	potato	Netherlands	0.008	−
61	X	potato	Netherlands	0.004	−
62	VIII	potato	Netherlands	0.006	−
63	IX	potato	Netherlands	0.007	−
64	?	potato	Netherlands	0.016	−
65	XIV	potato	Netherlands	0.007	−
66	V	potato	Netherlands	0.009	−
67	XII	potato	North Dakota	0.009	−
68	VII	potato	Wisconsin	0.009	−
69	III	iris	Netherlands	0.007	−
70	III	potato	Netherlands	0.006	−
72	VI	chicory	Netherlands	0.006	−
73	III	potato	Netherlands	0.014	−
74	III	potato	Netherlands	0.007	−
75	III	potato	Netherlands	0.011	−
76	III	potato	Arizona	0.007	−
77	III	potato	Arizona	0.009	−
78	III	potato	Arizona	0.007	−
80	III	potato	Netherlands	0.005	−
81	III	potato	Netherlands	0.009	−
82	III	potato	Netherlands	0.017	−
83	XXIX	potato	Maine	0.009	−
84	V	potato	Netherlands	0.012	−
85	V	potato	Netherlands	0.009	−
86	V	potato	Netherlands	0.010	−
87	V	potato	Netherlands	0.007	−
88	V	potato	Netherlands	0.006	−
91	XXVII	potato	Netherlands	0.008	−
92	XVII	potato	British Columbia	0.015	−
93	III	potato	British Columbia	0.010	−
94	XVI	potato	British Columbia	0.014	−
95	XII	?	Wisconsin	0.009	−

(*continued on next page*)

TABLE 2 (*continued from preceding page*)

Strain	Serogroup	Host	Location	Reaction with 4F6	Reaction with Band 5
96	?	potato	Wisconsin	0.009	−
97	?	potato	Wisconsin	0.006	−
E. c. betavasculorum					
29	?	sugar beet	Colorado	0.002	−
E. carotovora subspecies unknown					
43	?	tomato	Scotland	0.015	−
44	?	tomato	Scotland	0.007	−
45	?	tomato	Florida	0.012	−
53	?	cauliflower	England	0.007	−
55	?	cauliflower	England	0.006	−
56	?	cauliflower	England	0.014	−
57	XXIX	cauliflower	England	0.011	±
341	?	potato	Peru	0.008	−
342	?	potato	Peru	0.012	−

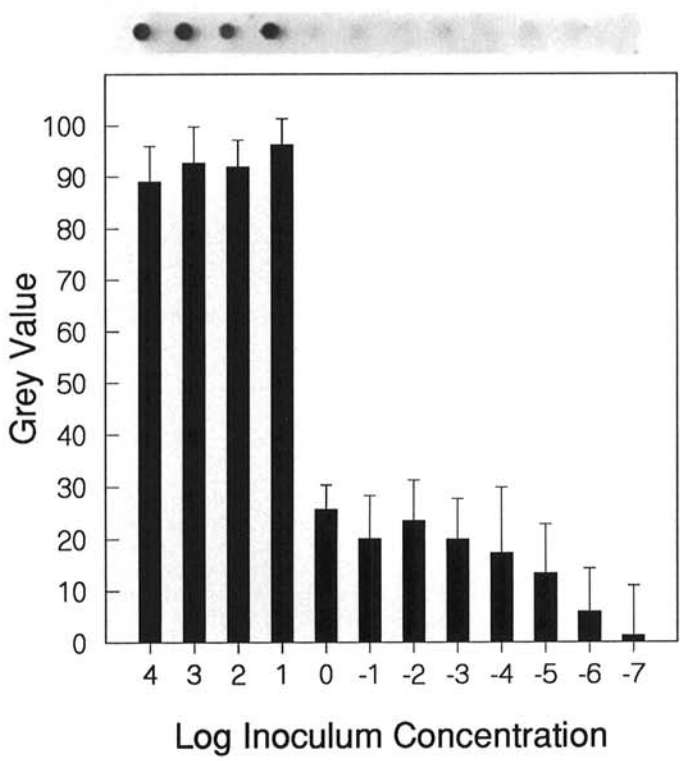

Fig. 4. Intensity of hybridization reaction of digoxigenin-labeled band 5 probe for *Erwinia carotovora* subsp. *atroseptica* to preparations from 48-h enrichments of potato periderm samples to which different concentrations of *Erwinia carotovora* subsp. *atroseptica* strain 31 cells were added prior to incubation. Bacterial cells were added to each sample in a 10-fold dilution series to give a final density of 10^4 colony forming units (cfu)/ml to 10^{-7} cfu/ml prior to incubation. Intensity of hybridization is given as corrected gray values from video images of chemilumograms. Standard errors are based on gray value readings for four separate aliquots from each sample. The video printout above the bar graph of one of the chemilumograms provides a direct comparison of the visual result with the mean gray values.

that soil bacteria did not hybridize with the probe. There was no difference in sensitivity for strains of the different *E. c. atroseptica* serogroups (*data not shown*). Enrichments with added *E. chrysanthemi* or *E. coli* cells gave negative results in both experiments.

By using the general *E. carotovora* probe in combination with the *E. c. atroseptica* probe, it is now possible to index potatoes for both the *carotovora* and *atroseptica* subspecies. In addition, the relatively simple procedure of proteinase K digestion followed by ammonium acetate/ethanol precipitation made it possible to probe crude samples such as tuber tissue enrichments with the DNA probes. By using a microplate rotor and deep well titer plates (Beckman Instruments Inc., Mississauga, ON), which have a capacity of 1 ml, several hundred samples can be processed easily. The number of erwinia on potato tubers may be low, and multiplication of bacteria by enrichment in selective media or amplification of target DNA by the polymerase chain reaction may be required for detection. The success of the nonradioactive detection system as demonstrated in our work makes the DNA procedure practical and economical for laboratories that lack facilities for handling radioisotopes.

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