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Detection of *Xanthomonas campestris* pv. *pelargonii* in geranium and greenhouse nutrient solution by serological and PCR techniques

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Abstract

Specificity of a new monoclonal antibody, 2H5, to *Xanthomonas campestris* pv. *pelargonii*, causal agent of geranium bacterial blight, was determined by enzyme-linked immunosorbent assay (ELISA) and immunofluorescence tests on 14 strains of *X. c. pelargonii*, 12 strains of other *X. campestris* pathovars, 3 strains of other *Xanthomonas* spp., 3 strains of other plant pathogens, and 43 saprophytic bacteria isolated from geranium. *X. c. pelargonii* was detected in tissue from symptomatic and asymptomatic geraniums sampled from commercial growers, and artificially inoculated plants, by monoclonal antibody-based tests. The intensity of response in ELISA was only moderately correlated (r = 0.56) with symptom severity, while symptom severity was not correlated (r = 0.16) with the number of fluorescing cells in immunofluorescence. The bimodal frequency distribution of ELISA and immunofluorescence results served to validate arbitrarily chosen positive/negative threshold values. Most positive ELISA and immunofluorescence test results were confirmed by the polymerase chain reaction (PCR) using published primers (Manulis et al., 1994. Appl. Environ. Microbiol 60, 4094–4099). In contrast to plant tissue, the bacterium was detected in greenhouse nutrient solution with greater sensitivity by immunofluorescence and PCR than by ELISA. Sensitivity of detection was enhanced 100-fold by concentration of the bacteria by centrifugation.

Abbreviations: GNS – greenhouse nutrient solution; ifu – immunofluorescing units; PBS – phosphate buffered saline; SDW – sterile distilled water.

Introduction

Bacterial blight, caused by *Xanthomonas campestris* pv. *pelargonii* (Brown) Dye (syn. *X. hortorum* pv. *pelargonii* (Brown) Vauterin et al.), is a serious disease of geranium (Strider, 1985). Symptoms consist of water-soaked lesions on the underside of leaves followed by wilting or marginal flaccidity accompanied by interveinal necrosis. Stem rot occurs when the bacterium moves into the stem through vascular tissue. Accurate diagnosis of bacterial blight by visual inspection is hampered by similar symptoms caused by other pathogens or mechanical injury.

Under conditions unfavourable to disease development, the pathogen survives on plant surfaces and within plants without causing symptoms (Digat, 1978; Digat, 1987). Cuttings from latently infected or contaminated plants used for propagation effectively disseminate the disease. Since there are no chemical or other control methods available, accurate and sensitive detection of *X. c. pelargonii* is necessary to permit prompt elimination of infected plants and cuttings.

Potted geraniums are often grown on water-tight tables irrigated by an ebb-and-flow system in which greenhouse nutrient solution (GNS) is pumped from tanks to flood the tables for a short period of time,

and then drained to the ground. To reduce fertilizer use and minimize ground water pollution, some growers in Europe and Canada recirculate GNS. In recirculating systems GNS is collected at the end of the tables and reused rather than drained away. Plant pathogens such as *X. c. pelargonii* surviving in recirculated GNS could spread to healthy plants by water splash as well as cause infection via root wounds. Low populations of *X. campestris* pv. *begoniae* have already been found to be disseminated in an ebb-and-flow system used for potted begonias (Atmatjidou et al., 1991). Since *X. c. pelargonii* could cause severe losses if spread in recirculated GNS, detection of this bacterium in GNS is important.

X. c. pelargonii is usually detected by isolation on a non-selective or selective medium, and identified by biochemical tests and bioassays (Schaad, 1988; Kado and Heskett, 1970; McGuire et al., 1986). Because these methods are time consuming and laborious, serological assays based on polyclonal and monoclonal antibodies (Anderson and Nameth, 1990; Benedict et al., 1990; Digat, 1978; Tuinier and Stephens, 1989) and DNA-based molecular techniques (Berthier et al., 1993; DeParasis and Roth, 1990; Lazo and Gabriel, 1987) have been developed to characterize and detect various *Xanthomonas* spp. Recently, Manulis et al. (1994) published oligonucleotide primer sequences that amplify a specific DNA fragment of *X. c. pelargonii* in the polymerase chain reaction (PCR).

In this study we produced polyclonal and monoclonal antibodies specific for *X. c. pelargonii* to compare detection of the pathogen by enzyme-linked immunosorbent assay (ELISA) and by immunofluorescence. These serological tests were further compared to the PCR procedure of Manulis et al. (1994) for detecting *X. c. pelargonii* in plant samples and GNS.

Materials and methods

Bacterial strains

Cultures of *X. c. pelargonii* and *X. c. begoniae* were obtained from the LMG Culture Collection (Laboratorium voor Microbiologie, Rijksuniversiteit Gent, Belgium). Local strains of *X. c. pelargonii* and strains of other bacterial phytopathogens were obtained from the Diagnostic Laboratory, British Columbia Ministry of Agriculture, Fisheries and Food, Abbotsford, B.C., Canada, from the collection at the Pacific Agriculture Research Centre, Vancouver, B.C., Canada, or isolated from diseased geranium plants (Table 1). The bacterial strains were cultured and maintained on yeast extract-dextrose-carbonate (YDC) medium containing 10 g yeast extract, 20 g dextrose, and 20 g $CaCO_3$ per litre. They were grown at 23 °C. Saprophytic bacteria were isolated from diseased and healthy geranium plants by incubating plant tissue in sterile distilled water (SDW) overnight and streaking the resulting suspension on YDC medium. Cultures were stored on Protect bacterial preservers (STC Technical Service Consultants Ltd., Heywood Lanes, UK) at –80 °C.

Antibody production

Reference strain LMG 7314 of *X. c. pelargonii*, used for immunizations, was grown on nutrient agar and the cells were harvested by flooding the cultures with 0.01 M phosphate buffered (pH 7.2) saline (PBS). The cells were washed once in PBS, fixed in 1% glutaraldehyde for 4 h, and again washed three times with PBS and stored at 4 °C. Glutaraldehyde-fixed cells in PBS (OD_{660} = 0.5) were mixed 1:1 with Freund's incomplete adjuvant (Gibco BRL, Gaithersburg, MD) and 100 μl of the emulsion injected into three BALB/C mice. The mice were injected subcutaneously five times at 21 day intervals. A final booster intraperitoneal injection without adjuvant was given four days prior to harvesting the spleen. Splenocytes were fused with FOX-NY myeloma cells using PEG 4000 and dimethyl sulphoxide as fusogens. Hybridoma cells were propagated in Dulbecco's modified Eagle Medium (DMEM) under aminopterin selection according to standard procedures (Jordan, 1990). Reactivity of antibodies produced by hybridoma clones were tested by indirect ELISA against *X. c. pelargonii, X. c. begoniae, Xanthomonas maltophilia*, and *Erwinia carotovora* subsp. *carotovora* in the initial screening step. A clone producing antibodies that reacted strongly in ELISA and did not cross-react with the heterologous pathovar and species was selected for further work after cloning twice by limiting dilution. Monoclonal antibody was produced in DMEM in spinner flasks and concentrated 100-fold by ammonium sulphate precipitation. Isotype was determined with a commercial typing kit (Behring Diagnostics, La Jolla, CA).

Glutaraldehyde-fixed cells were also used as immunogen to produce polyclonal antiserum in a New Zealand white rabbit. Five, 1 ml injections were given intramuscularly with Freund's incomplete adjuvant at 1:1 at seven day intervals. Seven days after the final injection, the blood was collected and the serum

Table 1. Bacterial strains tested by ELISA, immunofluorescence (IF), and PCR targeting *Xanthomonas campestris* pv. *pelargonii*

Bacterial species and pathovar	Strain[a]	ELISA	IF	PCR
Xanthomonas campestris pv. *pelargonii*	LMG 819	+	+	+
	LMG 820	+	+	+
	LMG 7192	+	+	+
	LMG 7312	+	nt[b]	+
	LMG 7314	+	+	+
	LMG 7357	+	+	+
	BC1	+	+	+
	BC2	+	+	+
	BC3	+	+	+
	BC4	+	nt	+
	BC5	+	nt	+
	BC6	+	+	+
	ATCC 8721	+	nt	nt
	Pg730	+	nt	nt
Xanthomonas campestris pv. *begoniae*	LMG 551	–	–	–
	LMG 7189a	–	–	–
	LMG 7189b	–	–	–
	LMG 7193	–	–	–
	LMG 7303	–	–	–
	LMG 7306	–	–	–
	89-035	–	nt	nt
Xanthomonas campestris pv. *campestris*	Cp670	–	–	nt
	Cp907	–	nt	nt
Xanthomonas campestris pv. *pruni*	ATCC 10016	–	nt	nt
Xanthomonas campestris pv. *vesicatoria*	Vs715	–	nt	nt
	Vs834	–	nt	nt
Xanthomonas fragariae	Fg38	–	nt	nt
Xanthomonas maltophilia	Wal	–	–	–
	Kok	–	nt	–
Erwinia carotovora subsp. *carotovora*	Br1	–	nt	–
	Br2	–	nt	nt
Clavibacter michiganensis subsp. *sepedonicus*	R14	–	nt	nt
3 unidentified isolates (series 1)[c]		– (all)	– (all)	nt
40 unidentified isolates (series 2)[c]		– (all)	nt	nt

[a] Strain designations prefixed LMG from Laboratorium voor Microbiologie, Gent, Belgium; and ATCC from American Type Culture Collection, Beltsville, MD. All other strains in laboratory collection or isolated during this study.
[b] not tested.
[c] Geranium isolates selected from YDC isolation medium on the basis of differing colony morphologies. Series 1 and 2 are from two different commercial greenhouses.

separated from coagulated blood components by centrifugation. Immunoglobulins were precipitated with ammonium sulphate at 50% of saturation, resuspended in 1/2 strength PBS and dialysed against 1/2 strength PBS. The immunoglobulin fraction was separated on a DEAE cellulose ion exchange column.

ELISA and immunofluorescence procedures

Specificity of the serological procedures was tested against bacteria grown on YDC and suspended in SDW to about 10^8 colony forming units (cfu) ml^{-1} by indirect double antibody sandwich ELISA (McLaughlin and Chen, 1990) and immunofluorescence (De Boer, 1990). For ELISA, the polyclonal antibody, diluted 1:400, was coated onto wells of Immulon 2 microtitre plates (Dynatech Laboratories, Inc., Chantilly, VA) to capture bacterial antigens. Suspensions of pure culture were loaded directly into coated plates but for plant samples, 50 μl of sample buffer (2% polyvinylpyrrolidone, 0.2% skim milk powder, and 0.05% Tween in PBS) was loaded into each well before adding 50 μl of sample to minimize background reading. Hybridoma culture fluid was used directly, or when concentrated from batch culture was diluted 1:1000, and used as the detecting antibody. Final absorbance values >0.1 were considered positive on the basis of preliminary experiments in which samples from uninoculated healthy plants consistently had absorbance values <0.05 whereas samples from inoculated plants had absorbances >0.2.

Pure cultures suspended at ca. 10^8 cfu ml^{-1} and plant extracts were tested undiluted and at 1:10, 1:100, and 1:1000 dilutions in immunofluorescence. The selected monoclonal antibody was evaluated by the indirect immunofluorescence procedure using an anti-mouse antibody conjugated with indocarbocyanine (Cy3) (Bio/Can Scientific, Mississauga, ON). Counts of bacterial cells were recorded as the logarithm of one plus the product of the number of immunofluorescing units (ifu) per microscope field at 1000x magnification and the dilution factor (referred to as $\log_{10}$ ifu in the text). Preparations with $\log_{10}$ ifu ≥ 0.7 (i.e. ≥ 5 ifu per field in undiluted preparations) were considered positive.

PCR procedure

PCR was conducted with a thermocycler (Model 480, Perkin Elmer Corp. Norwalk, CT) according to the procedure of Manulis et al. (1994) using the 18-mer oligonucleotide primers (5′-GAGTGTCCAGTGGCAAGC and 5′- GTTGCTGC-CTCTTCCTGC purchased from Nucleic Acid-Protein Service Unit, UBC, Vancouver, BC) for specific amplification of *X. c. pelargonii* DNA. The PCR reaction mix for plant samples was modified from that of Manulis et al. (1994). The 25 μl PCR reactions contained 2.5 mM $MgCl_2$, 0.2 μM of each primer, 0.25 U Ultratherm (Bio/Can), 50 μM of each deoxynucleoside triphosphate, 2.5 μl of 10$\times$ reaction buffer, and 0.25 μl blotto (10 g of skim milk powder in 100 ml of distilled water) under two drops of mineral oil. PCR on GNS samples were done without blotto.

Inoculated plant samples

Disease-free *Pelargonium* zonal-hybrid geraniums (variety Picasso) were grown in the greenhouse at 22 °C and inoculated with suspensions of *X. c. pelargonii* isolates LMG 7314, BC1, BC2, BC3 and *X. maltophilia* at 10^8 cfu ml^{-1} from 3-day-old cultures. To inoculate plants, a small incision was made in the lower stem with a sterile scalpel and 0.2–0.3 ml of bacterial suspension injected into the incision. Each isolate was injected into five plants and control plants were injected with SDW. Disease symptoms were rated four days after inoculation and categorized as follows: 1 – no symptoms; 2 – small lesions on the margin and/or surface of leaf; 3 – large interveinal lesions on leaf; 4 – chlorosis or discoloration of leaf variously accompanied by the other symptoms; 5 – leaf totally necrotic. Seven days after inoculation two leaves per plant, one with and one without symptoms, were collected and placed in small, separate plastic bags (10 cm $\times$ 7.5 cm) with 2–4 ml SDW depending on sample size. The plant tissue was lightly crushed by tapping the tissue in sample bags with a hammer and then shaken overnight at 24 °C.

The fluid from sample bags was tested directly in ELISA and by immunofluorescence. For PCR, 20 μl of 10X lysing buffer (100 mM Tris-HCl, 10 mM ethylene diamine tetraacetate, 10% sodium dodecyl sulphate, and 10% polyvinylpyrrolidone) was added to 200 μl aliquots of sample fluid from the plastic bags and incubated for 1 hour at 55 °C. A half volume of 7.5 M ammonium acetate was then added to the samples to precipitate protein and other cellular debris which was removed by centrifugation at 17,000 g for 15 min. One volume of isopropanol was added to the supernatant and the DNA fraction collected after centrifugation at 17,000 g for 30 min. The pellets were washed with 70%

ethanol and further purified with an ELUQUICK DNA purification kit (Schleicher and Schuell, Keene, NH) as described by the manufacturer. One μl of the DNA fraction eluted from the ELUQUICK column was used per PCR reaction.

Commercial plant samples

Symptomatic and asymptomatic geranium tissue samples were collected from six different greenhouse growers. Samples consisted of leaves or the lower 1-cm portion of stem cuttings. Symptoms were rated as above on the individual leaves or on remaining leaves on the stem cuttings. Symptomatic plant samples included plant tissue with bacterial blight-, *Botrytis* wilt-, and geranium rust-like symptoms as well as natural senescence. Samples were processed and tested in the same way as the samples from inoculated plants.

Greenhouse nutrient solution

GNS containing per litre 230 mg potassium nitrate, 160 mg monopotassium phosphate, 160 g potassium chloride, 250 mg magnesium sulphate, 900 mg calcium nitrate, and 21 mg Saanichton minor elements (Green Valley Fertilizers, Langley, BC), was inoculated at 10:1 with an *X. c. pelargonii* suspension prepared from a 4-day-old culture in 50 ml polypropylene tubes. Five concentrations of bacteria in a 100-fold dilution series were used for inocula; the highest concentration being 1.14×10^9 cfu ml^{-1} determined by plate count on YDC medium. After incubation for 7 days at 23 °C each concentration was tested in triplicate by ELISA, immunofluorescence, and PCR. To detect *X. c. pelargonii*, 25 ml from each tube was centrifuged at 4000 g, and another 25 ml filtered through a 0.2 μm filter. The resulting pellets and filter retentates were resuspended by shaking in 10 ml GNS for one hour. Original and concentrated suspensions were tested in ELISA immediately but additional aliquots were stored at –20 °C for PCR and separately with iodine preservative (10 μl ml^{-1}) at 4 °C for immunofluorescence. The experiment was performed twice.

Results

Specificity of serological tests

Hybridoma 2H5 was selected for further study because it produced an antibody that gave a strong signal in ELISA and did not react with the heterologous pathovar and species in the initial screen. The monoclonal antibody (McAb 2H5) produced by this clone was of the IgG_1 isotype. Subsequent tests with McAb 2H5 on pure cultures of 14 strains of *X. c. pelargonii*, 12 strains of other *X. campestris* pathovars, and 49 other bacteria including plant pathogens and saprophytes confirmed its specificity for *X. c. pelargonii* in ELISA (Table 1). Absorbance values in ELISA for *X. c. pelargonii* strains were 1.5–2.0 whereas absorbances for heterologous bacteria were <0.05. McAb 2H5 was also specific for *X. c. pelargonii* in immunofluorescence; fluorescing bacterial cells were only found in tests with *X. c. pelargonii* cultures and not with cultures of other bacteria tested (Table 1).

Identification by PCR

PCR with DNA extracts from pure cultures confirmed the specificity of the primers from Manulis et al. (1994) as only DNA from *X. c. pelargonii* isolates produced the specific 1.2 Kb fragment (Table 1). Some *X. c. begoniae* isolates produced some very faint bands but none of these were in the 1.2 Kb region.

Inoculated plant samples

The plants which were inoculated with *X. c. pelargonii* developed symptoms after three days. The first symptoms appeared as marginal wilting of basal leaves which later became necrotic. The disease progressed quickly towards the top leaves with isolates BC1 and BC3, and the plants eventually died. However, disease progression was slow with isolates LMG 7314 and BC2. Although plants inoculated with these isolates became stunted, they regenerated and survived for at least three more months until they were discarded. At sampling, seven days after inoculation, only the lower leaves were wilted. The uninoculated controls and the plants inoculated with *X. maltophilia* remained symptomless.

Both wilting lower leaves and symptomless upper leaves on inoculated plants were positive in ELISA with absorbance values of 0.108–2.449, whereas leaves from uninoculated and those inoculated with *X. maltophilia* were negative in ELISA with absorbances of 0.042–0.092 (Figure 1A). Leaves with each symptom type gave a wide range of ELISA values but leaves with the most severe symptoms tended to give higher ELISA readings (Figure 2A). The overall correlation ($r = 0.56$) between symptom type and ELISA reading,

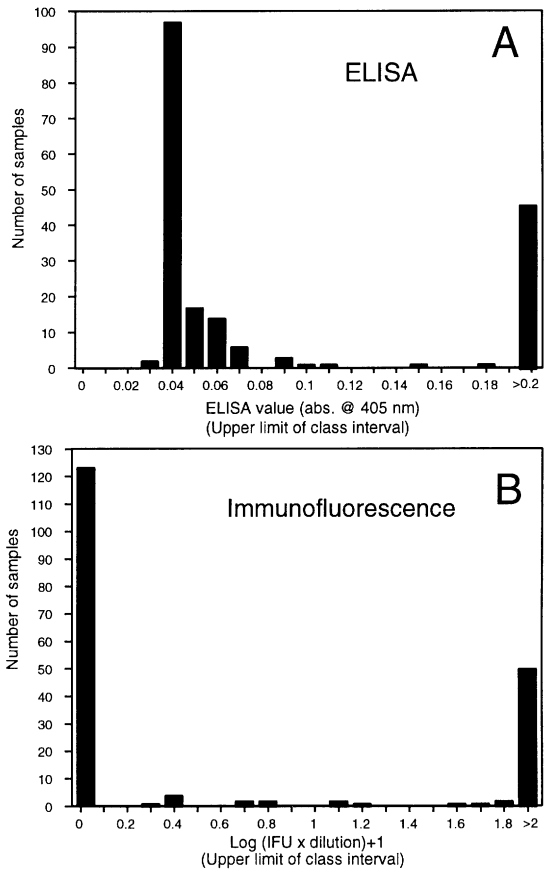

Figure 1. Frequency distribution of ELISA (A) and immunofluorescence (B) results for tests on 189 geranium leaves from two greenhouses in which bacterial blight (*Xanthomonas campestris* pv. *pelargonii*) was present.

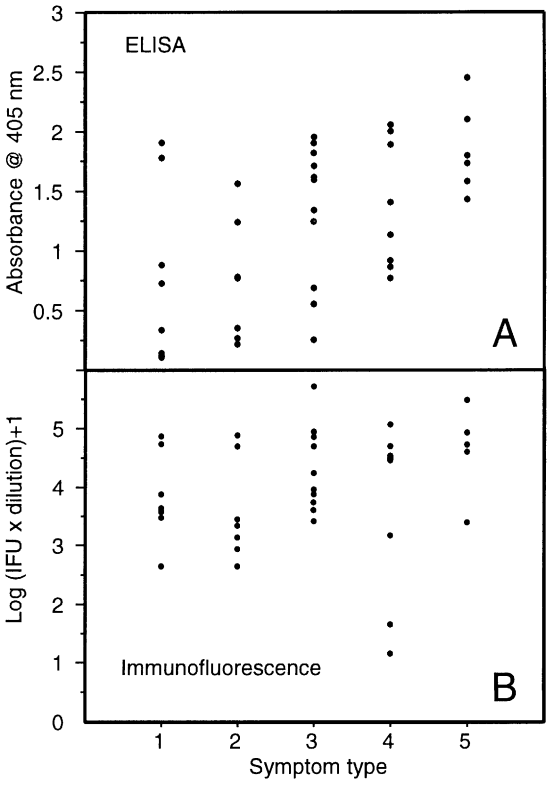

Figure 2. ELISA (A) and immunofluorescence (B) results (based on replicate tests) of individual geranium leaf samples from plants inoculated with *Xanthomonas campestris* pv. *pelargonii* and with varying symptoms of bacterial blight (1 = symptomless; 5 = leaf entirely necrotic; see text for further details).

however, was low. Immunofluorescence values ranged from 1.15 – 5.71 $\log_{10}$ ifu for leaf samples from plants inoculated with *X. c. pelargonii* (Figure 1B). There was essentially no correlation (r = 0.16) of cell counts with severity of symptoms (Figure 2B). Fluorescing bacterial cells were not observed in preparations from control and *X. maltophilia*-inoculated plants. Both the ELISA and immunofluorescence results essentially fit a bimodal frequency distribution (Figure 1).

Commercial plant samples

Disease symptoms on plants from commercial greenhouses were highly variable and the incidence of bacterial blight detection differed among samples from the different greenhouses (Table 2). Of the 882 plant samples that were tested from commercial greenhouses, 9.6% were positive for bacterial blight by ELISA. While all the samples from one greenhouse with a known bacterial blight problem tested positive, all the samples from two other greenhouses without disease problems were negative. The incidence of positive tests on samples from other greenhouses in which bacterial blight had been diagnosed previously by isolation, varied from 11.7–49.3%. All of the positive ELISA results were confirmed by a positive result in immunofluorescence (Table 2). Four samples that were negative in ELISA had fluorescing cells in immunofluorescence; for three of these, immunofluorescence counts were close to the positive/negative threshold. Of the 93 samples tested by all three methods, only 80% of the samples that were positive by both ELISA and immunofluorescence were positive by PCR (Table 2).

Table 2. Reaction of geranium leaf samples with different symptoms in ELISA, immunofluorescence (IF), and PCR tests for detecting *Xanthomonas campestris* pv. *pelargonii*

Grower	Leaf symptoms[a]	No. of samples	Number of positive samples		
			ELISA	IF	PCR
A	1	2	2	2	2
	2	6	6	6	5
	3	5	5	5	5
	4	2	2	2	2
	5	3	3	3	3
B	1	39	14	15	13
	2	19	16	16	12
	3	9	5	5	2
	4	1	0	0	0
	5	7	2	2	0
C	1	41	0	1	nt[b]
	2	14	5	5	nt
	3	20	4	4	nt
	4	19	2	3	nt
	5	17	2	3	nt
D	1	3	0	0	nt
	2	16	9	9	nt
	3	16	4	4	nt
	4	7	1	1	nt
	5	6	3	3	nt
E	1	342[c]	0	0	nt
F	1	540[c]	0	nt	nt

[a] 1 = no symptoms; 5 = leaf necrotic (see text for further details).
[b] not tested.
[c] Tested in composites of 10.

Greenhouse nutrient solution

Viable counts of *X. c. pelargonii* mixed into GNS decreased about 10-fold within 24 h of inoculation, remained constant for about five days, and then decreased logarithmically to undetectable levels after 25 days (data not shown). Immunofluorescence was more sensitive than ELISA for detecting *X. c. pelargonii* in GNS in both replications of the experiment. The result of one of the experiments is given in Table 3. Bacterial populations down to 10^5 cfu ml^{-1} were detected by immunofluorescence whereas sensitivity of ELISA was 10^9 cfu ml^{-1}. Sensitivity of PCR was about 100-fold less than immunofluorescence. Concentration of the bacterial fraction by centrifugation increased sensitivity of immunofluorescence and PCR 100-fold, but did not increase sensitivity of ELISA. Concentration of bacteria by filtration was unsuccessful.

Discussion

Polyclonal antisera have been used previously in ELISA and immunofluorescence to detect *X. c. pelargonii* on geranium plants (Anderson and Nameth, 1990; Digat, 1978). These methods have been useful for indexing propagative material for phytosanitary certification in Europe (Digat, 1987). Inconsistency among batches of polyclonal antisera and cross-reactivity with saprophytic bacteria such as *X. maltophilia* (S. Chittaranjan, unpubl.), however, are problematic. In contrast, monoclonal antibodies generated from the same hybridoma cell line are uniform and a monoclonal antibody to the lipopolysaccharide antigen of *X. c. pelargonii* was highly pathovar specific (Benedict et al., 1990). The lipopolysaccharide is both an integral component of the outer cell membrane and is present as a soluble component in the bacterial matrix. It is likely that McAb 2H5 also reacted with the lipopolysaccharide antigen because on whole, fixed cells lipopolysaccharide is highly immunogenic, and the monoclonal antibody reacted with a cell wall antigen in immunofluorescence and probably with a soluble antigen in ELISA.

Although the monoclonal antibody produced by Benedict et al. (1990) was as specific for *X. c. pelargonii* as McAb 2H5, it was not tested in their study on plant material for diagnostic and indexing purposes. In our study McAb 2H5 detected the pathogen in symptomatic and asymptomatic plant samples by both ELISA and immunofluorescence, and with few exceptions gave consistent results (Figure 1, Table 2). Samples with absorbance values >0.1 in ELISA also had >0.7 $\log_{10}$ ifu in immunofluorescence. However, because the same antigenic determinant was detected by the monoclonal antibody in both tests, the two tests did not complement each other in the same way as those developed for *Clavibacter michiganensis* subsp. *sepedonicus*, causal agent of potato ring rot, where different monoclonal antibodies were used to probe two unrelated antigens (De Boer et al., 1994).

Bacterial blight infection (or contamination) was detected by the serological tests and PCR, independent of symptom expression or symptom type (Table 2, Figure 2). As with many bacterial plant diseases, populations of the pathogen exceed detection limits well before symptoms are visible. Thus, there is

Table 3. Comparison of ELISA, immunofluorescence and PCR for detecting *Xanthomonas campestris* pv. *pelargonii* in greenhouse nutrient solution which had been inoculated at five different levels with the geranium bacterial blight pathogen, before and after concentration of the bacterial fraction

Inoculum concentration	ELISA (OD at 405 nm)	Immunofluorescence ($\log_{10}$ ifu)[a]	PCR (intensity of band)[b]
	Prior to concentration of bacterial fraction		
1.14×10^9	0.836±0.014[c]	4.08±0.45[c]	4
1.14×10^7	0.100±0.015	1.51±0.46	1
1.14×10^5	0.094±0.020	0.59±0.51	–
1.14×10^3	0.092±0.013	0	–
1.14×10^1	0.082±0.014	0.25±0.43	–
uninoculated	0.098±0.023	0	–
	After concentration of bacterial fraction by centrifugation		
1.14×10^9	0.402±0.103	4.49±0.39	4
1.14×10^7	0.041±0.006	0.91±0.57	2
1.14×10^5	0.037±0.008	0.94±0.38	1
1.14×10^3	0.036±0.005	0.55±0.64	–
1.14×10^1	0.033±0.004	0.44±0.41	–
uninoculated	0.039±0.008	0	–
	After concentration of bacterial fraction by filtration		
1.14×10^9	0.101±0.012	2.35±0.10	–
1.14×10^7	0.033±0.001	0	–
1.14×10^5	0.032±0.002	0	–
1.14×10^3	0.032±0.003	0	–
1.14×10^1	0.034±0.001	0	–
uninoculated	0.040±0.007	0	–

[a] $\log_{10}$ ifu = $\log_{10}$(number of immunofluorescing cells per field × dilution) + 1.
[b] 4 = strong band; 1 = weak band; – = no band.
[c] ± standard error.

good potential for disease control strategies involving detection of the pathogen by laboratory indexing of symptomless plants and cuttings taken for propagation. Furthermore, the bimodal distribution of ELISA and immunofluorescence results (Figure 1) permitted setting of realistic positive/negative thresholds with a minimum number of samples giving equivocal results (Sutula et al., 1986).

Most of the ELISA and immunofluorescence positive commercial samples were confirmed by PCR (Table 2). Unidentified components in geranium tissue were inhibitory to PCR (unpublished data) but addition of skim milk to the PCR mix strongly attenuated the inhibition (De Boer et al., 1995). Nevertheless, some serologically positive samples were negative in PCR, probably because of excess inhibitory compounds in the extract. Furthermore, the PCR test was designed with pure cultures and sensitivity may not have been optimal for plant samples. In any case, the usefulness of PCR in diagnosis and indexing tests will be limited by the requirement for a laborious and costly extraction procedure unless a more efficient extraction protocol can be designed. We did find PCR to be useful as a confirmatory test for samples that gave a positive response in ELISA and immunofluorescence.

Sensitivity of ELISA for detecting *X. c. pelargonii* in GNS was particularly poor (Table 3), probably because soluble antigen was not generated by metabolically inactive bacteria in a solution lacking a carbon source. Concentration of bacterial cells by centrifugation and filtration decreased sensitivity of ELISA by removing soluble antigen. However, immunofluorescence, which does not rely on soluble antigen or cell viability but on visualization of intact cells, was about 10,000-fold more sensitive than ELISA for detecting *X. c. pelargonii* in GNS. PCR was slightly less sensitive than immunofluorescence. It was not determined whether the lower sensitivity was due to inhibitory components in GNS, DNA degradation in non-viable bacterial cells, or suboptimal PCR conditions.

In summary, we found that ELISA with McAb 2H5 was a reliable and convenient procedure for diagnosing geranium blight and indexing plants for asymptomatic infections or contamination by *X. c. pelargonii*. ELISA results could be confirmed by immunofluorescence or PCR, but PCR was preferred because it is based on a target molecule that is independent of the ELISA test. Immunofluorescence was the most reliable method for detection of the bacterial blight pathogen in GNS and results could generally be confirmed by PCR.

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