Rapid Detection of *Xanthomonas campestris* pv. *dieffenbachiae* in Anthurium Plants with a Miniplate Enrichment/ELISA System

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ABSTRACT

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A miniplate enrichment/ELISA system was developed for rapid detection and identification of *Xanthomonas campestris* pv. *dieffenbachiae*, causal agent of anthurium blight. Tissue exudates were applied to individual wells of 96-well tissue-culture plates filled with 150 μl of esculin trehalose medium, which promotes growth of *X. c. dieffenbachiae* and turns brown, indicating esculin hydrolysis. Cells were transferred to a 96-well microtiter plate, and identity of *X. c. dieffenbachiae* was confirmed by ELISA using an *X. c. dieffenbachiae*-specific monoclonal antibody (Xcd 108). Sensitivity and specificity of the miniplate enrichment/ELISA were determined using 10-μl subsamples containing end-point dilutions of *X. c. dieffenbachiae* or mixed suspensions containing various ratios of competitive bacteria to *X. c. dieffenbachiae*. The miniplate enrichment/ELISA system is sufficiently sensitive to detect one or more colony-forming unit per well. Monoclonal antibody Xcd 108 reacted with all strains of *X. c. dieffenbachiae* pathogenic to anthurium but not with most nonpathogenic strains isolated from anthurium. When 142 leaf samples with and without symptoms were assayed, the miniplate enrichment/ELISA system gave positive results for every sample from which *X. c. dieffenbachiae* was isolated by standard procedures, but 3.3% of the samples that gave a positive reaction in the miniplate enrichment/ELISA system were negative using standard procedures. When compared with standard isolation procedures, the predictive value of test results for the miniplate enrichment/ELISA system was 97.9%. The miniplate enrichment/ELISA system also was useful for detection of epiphytic populations of *X. c. dieffenbachiae* on leaf surfaces.

Additional keywords: environment, epidemiology

Anthurium blight, caused by *Xanthomonas campestris* pv. *dieffenbachiae* (McCulloch and Pirone) Dye, is a systemic disease of anthurium. It has been devastating, having a major economic impact in Hawaii. As a consequence of the disease, the number of anthurium farms decreased by 24% and flower sales declined by 22.8% from 1984 to 1988 (22). In Hawaii, most anthuriums are grown in Saran cloth houses at a density of approximately 73,500 plants per hectare. The pathogen appears to be spread by wind, rain, and overhead irrigation as well as by tools and clothing (15,16). Strict sanitation is critical for disease management. Diseased plants must be removed from the production site. Chemicals and antibiotics reduce disease incidence and/or severity but do not eliminate disease (2,3). Attempts at eradication have been unsuccessful because infected plants may not develop symptoms.

Pathogen detection and identification are essential for evaluating disease progress in ecological or epidemiological studies. The most commonly employed isolation and identification techniques for bacterial plant pathogens involve the use of semiselective media (7,9,17), usually followed by confirmatory bacteriological tests. However, large-scale use of semiselective media is hindered by cost, available space, and logistics of handling large numbers of samples. Detection of *X. c. dieffenbachiae* in plants is further limited by epiphytic and saprophytic bacteria that overgrow the pathogen even on semiselective media when only low populations of the latter are present in the sample. Thus, a sensitive assay for *X. c. dieffenbachiae* is needed, particularly for the slow-growing non-starch-hydrolyzing strains of this organism that are often encountered on anthurium (12).

Techniques using either DNA hybridization probes (13,18) or ELISA (6,19) have been developed for other bacterial plant pathogens, but sensitivity is limited to $>10^4$ cfu/ml. Sensitivity was increased 10-fold by an enhanced ELISA (8). With use of an immunofluorescence colony (IFC) staining technique, a single colony can be identified with appropriate antibodies (23). Because bacterial colonies bind large amounts of antibody, high titers are required for immunofluorescence. Thus, the cost limits the applicability of IFC to large-scale field sampling. The polymerase chain reaction (PCR) has been used successfully to detect one bacterial cell per sample (5,20,21). Since assays are performed on relatively small samples (10 μl), the sensitivity is approximately 100 cfu/ml.

This research report addresses the development of a two-step assay that involves miniculture followed by ELISA using a pathogen-specific monoclonal antibody (MAb) for identification. This assay provides a way of processing large sample numbers with a level of sensitivity that is comparable to current reports using PCR.

MATERIALS AND METHODS

Monoclonal antibody production. MAbs were produced by immunizing BALB/c mice with *X. c. dieffenbachiae* strain D150 from anthurium, using previously described methods (1). Selected MAbs were tested with 284 *X. c. dieffenbachiae* strains isolated from anthurium and 18 strains from seven other aroids. Additionally, *X. albilineans* (one strain), *X. citri* (six strains), *X. maltophilia* (five strains), *X. oryzae* (five strains), 87 strains from 21 pathovars of *X. campestris*, 44 *Pseudomonas* strains (15 species), 10 *Clavibacter* strains (six species), 10 *Erwinia* strains (four species), two *Agrobacterium* species, two *Rhodococcus fascians* strains, two *Enterobacter cloacae* strains, and one *Curtobacterium flaccumfaciens* strain were tested for reactivity to MAbs.

Binding curves for MAbs with potentially useful specificities were produced using two strains of *X. c. dieffenbachiae* (D150 and D182) that represent two distinct phenotypic populations of the pathogen (those that hydrolyze starch and those that do not). Suspensions of 24-hr-old cultures of both strains were harvested from YGA medium (4), washed three times in saline, suspended in 0.05 M carbonate-bicarbonate (CBC) buffer (pH 9), adjusted spectrophotometrically to $A_{600} = 0.1$ OD (approximately 1×10^8 cfu/ml), and air-dried in a forced-air incubator at 37 C onto wells of ELISA plates (100 μl/well). Wells containing only CBC or CBC plus *Erwinia herbicola* strain Eh-1 were used as negative controls. Twofold dilutions of MAb Xcd 108 were made from 1:1,000–1:128,000. At each dilution, the MAb was applied to eight wells of the microtiter plate for each of the three bacterial strains. Average ELISA readings were calculated, background values were subtracted, and data were plotted.

Serotyping and pathogenicity tests. Using a panel of previously described MAbs (12) and a MAb generated in this study, 114 *Xanthomonas* strains isolated

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from anthurium tissues and aerosols collected with an Andersen sampler on plates of esculin trehalose (ET) medium were serotyped. For pathogenicity tests, strains were transferred to YDC medium (24), grown for 24 hr at 29 C, suspended in saline, and adjusted to approximately 10^8 cfu/ml. Decimal dilutions of bacteria were made in saline to the 10^{-4} dilution and infiltrated into the mesophyll of two 1-cm^2 sections of the top fully expanded leaf of each of two *Anthurium* cv. Kalapana plants using a syringe with a 27-gauge needle. Leaves were bagged for 24 hr, and then plants were returned to the greenhouse for observation of symptoms for 3 wk. If no symptoms developed in 3 wk, strains were reinoculated into leaves of *Dieffenbachia maculata* cv. Compacta in the same manner.

Miniplate enrichment/ELISA. A modified ET medium (17), containing 200 ppm of cycloheximide, was aseptically pipetted into a 96-well, sterile, tissue-culture plate (Falcon 3075) at 150 μl per well. Then, 10 μl of bacterial suspension, tissue exudate, or leaf wash was pipetted into individual wells of the miniplate. Lids of tissue culture plates were raised 5 mm to increase air exchange and to eliminate an edge effect produced by reduced oxygen at center wells. Plates were incubated at 29 C for 4 days. Wells darkened by esculin hydrolysis were recorded by position. Using a multichannel pipet, 150 μl of CBC was added to every well. After 5 min, bacterial cells were uniformly suspended by pipetting 100 μl of the buffer into and out of the wells three or four times, and 100 μl of suspension was transferred to individual wells of an ELISA plate (polyvinyl chloride, Dynatech). Sample positions in 96-well ELISA plates were identical to those in the 96-well miniculture plates. The ELISA plates were dried for 12 hr at 37 C, and ELISA was performed (17), using MAb Xcd 108 at a concentration of 1:1,000.

Sensitivity and specificity of miniplate enrichment/ELISA system. The lower limits of detection for the miniplate enrichment/ELISA system were determined by assaying a twofold dilution series of *X. c. dieffenbachiae* strains D150 and D182 diluted 10- and 100-fold lower than a calculated concentration of 1 cfu/well. The two strains were streaked on YDC medium, harvested, and suspended in saline, and suspensions were adjusted spectrophotometrically to approximately 10^8 cfu/ml. Thirty-four twofold serial dilutions were made, and 10-μl subsamples were pipetted from each dilution into eight wells of the tissue-culture plate. Each series was replicated three times. Miniplates were incubated for 4 days at 29 C, and wells exhibiting esculin hydrolysis were recorded. Plates were inspected with a dissecting microscope to determine if single colonies developed in the wells containing the greatest dilutions of the initial suspension. Cells were harvested from each well, transferred to and dried in microtiter plates at 37 C, and evaluated by ELISA. Assays were done in triplicate and ELISA readings were averaged. Background readings of saline control rows were subtracted from averaged ELISA readings (A_{450}), and net absorbances were plotted. To calculate the number of colony-forming units applied to each well, 10-μl subsamples of appropriate dilutions of each strain were spotted onto triphenyl tretrazolium chloride medium (TZC) (10), modified by Norman and Alvarez (17). Microcolonies were viewed at 40× with a dissecting microscope and counted after 12–24 hr of growth at 29 C.

Sensitivity of the miniplate enrichment/ELISA system was compared with standard ELISA by evaluating a twofold dilution series of the same initial cell suspensions of the two strains by both methods. Eight 100-μl subsamples of each dilution of the bacterial suspension were pipetted into a microtiter plate. Each dilution series was replicated three times and ELISA performed as outlined by Norman and Alvarez (17).

The lower limits of detection for strains D150 and D182 of *X. c. dieffenbachiae* also were determined in mixed populations of competitive bacteria: *E. herbicola* strain Eh-1, *P. fluorescens* strain A811-1, *A. tumefaciens* strain QR-1, and three epiphytic bacterial strains (776, 861, and 868) isolated from anthurium leaves. Although *A. tumefaciens* is not naturally found in Hawaii and is not associated with anthuriums elsewhere, it was used in this study because of its ability to hydrolyze esculin, which might interfere with the assay. The epiphytic strains represent bacteria that may compete with *X. c. dieffenbachiae* during the enrichment step on ET medium. These bacteria were gram-negative, oxidase-negative, and nonfluorescent on KMB medium (11). The Biolog System version GN 3.0 identified them as, or being the closest match with, *X. maltophilia*. Similarity coefficients were 0.346, 0.951, and 0.399 for strains 776, 861, and 868, respectively.

The bacterial strains were grown on YDC medium for 24 hr at 29 C. Cells were harvested, adjusted spectrophotometrically to 10^8 cfu/ml, and diluted in saline in eight 10-fold dilutions. Contaminants were mixed approximately 1:1 with the two *X. c. dieffenbachiae* strains by mixing a 500-μl subsample from each of the nine dilutions with 500 μl, the same dilution of the saprophytic bacterial strain. Then, 10-μl subsamples were pipetted into eight replicate wells from each mixed suspension. A 96-well miniplate was used for each combination of saprophytic bacteria with *X. c. dieffenbachiae*. Eight negative control wells per plate contained 10 μl of the highest concentration of a competitive bacterium mixed with an equal proportion of saline; eight additional negative control wells contained only 10 μl of sterile saline. A one-way analysis of variance followed by Duncan's multiple range test was used to compare means of both controls to determine background absorbance. A separate miniplate containing only the decimal dilution series of *X. c. dieffenbachiae* mixed with equal portions of saline was processed simultaneously to compare readings of pure cultures with those obtained from mixed bacterial suspensions. Colony-forming units applied to each well were calculated for each of the representative bacteria by evaluating five 20-μl subsamples of appropriate dilutions on modified TZC medium. Microcolonies were observed at 40× and recorded after 12–24 hr of growth at 29 C.

To determine whether low populations of *X. c. dieffenbachiae* could be detected by the miniplate enrichment/ELISA system in the presence of high populations of other bacteria, the six aforementioned strains of competitive bacteria and xanthomonads were tested. A 10-fold dilution series (1×10^8 to 0 cfu/ml) was made for each of the competitive bacteria, and at each step, the competitor was diluted 1:1 (v/v) with 10^{-4} dilution of *X. c. dieffenbachiae* (30–90 cfu/well). Eight control wells were made, and viable counts were determined from 20-μl subsamples.

Comparison of miniplate enrichment/ELISA system with standard culturing procedures. Leaf tissue from 142 anthurium plants was collected from the field. Tissue samples (approximately 1 cm^2) were soaked for 2–3 hr in 1 ml of saline. Then, 10 μl from each plant sample (or saline control) was added to individual wells. Miniplates were incubated, and ELISA was performed as previously described. In addition, a loopful from each of the 142 plant extracts was streaked onto modified TZC medium and incubated for 48 hr. Test results of the two methods were evaluated by standard methods (14).

Detection of xanthomonads on leaf surfaces. Miniplates were used in the field to collect sprinkler wash from anthurium leaves. Samples were removed from the leaf surfaces of 288 plants—138 with visible blight symptoms and 150 with no symptoms. Aliquots (20 μl) were pipetted into individual wells of miniplates, which were processed as previously described. To further check the recovery of xanthomonads from wells showing esculin hydrolysis, bacteria were streaked from 33 of the darkened wells onto separate plates of modified TZC medium. Xanthomonads recovered on the TZC medium were identified by serotyping with MAb Xcd 108 and eight other monoclonal antibodies (12) and were also tested for pathogenicity.

RESULTS

Monoclonal antibody production. MAb Xcd 108 (clone No. 167-108-2-1, class IgG) was selected because of its apparent specificity to anthurium strains in initial tests and because its reactivity patterns were different from all previously described MAbs generated to *X. c. dieffenbachiae* (12). Binding curves for *X. c. dieffenbachiae* strains D150 and D182 to MAb Xcd 108 are shown in Figure 1. Nonspecific binding to *E. herbicola* was negligible (Fig. 1). On the basis of these results, a dilution of 1:1,000 of Xcd 108 was chosen for all subsequent work. MAb Xcd 108 reacted with 99% (281/284) of the *X. c. dieffenbachiae* strains tested from anthurium and 44% (8/18) of those from other aroids; it did not react with *X. albilineans, X. citri, X. maltophilia, X. oryzae*, 87 strains from 21 other pathovars of *X. campestris*, or 72 strains of the other eight genera tested.

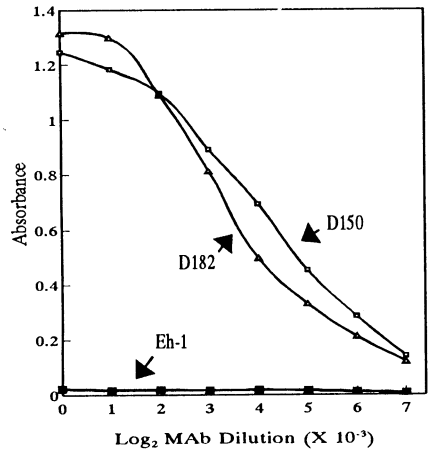

Fig. 1. Binding curves of MAb Xcd 108 to *Xanthomonas campestris* pv. *dieffenbachiae* strains D150 and D182. Bacteria were coated onto microtiter plates (10^8 cfu/ml) and reacted with twofold dilutions (1:1,000–1:128,000) of MAb Xcd 108; absorbance was measured at 450 nm. *Erwinia herbicola* (strain Eh-1) was used as a negative control.

Serotyping and pathogenicity tests. Of the 114 strains isolated from anthurium, 60 were in serotypes 2, 4, 5, and 8, and all 60 reacted with MAb Xcd 108. All but three of the 60 strains (one serotype 2 strain and two serotype 5 strains) were pathogenic on anthurium. The remaining 54 strains were in serotype 12 and produced no symptoms on anthurium over a 3-wk period following inoculation. On *D. maculata* cv. Compacta, 77.8% (42/54) were mildly pathogenic, forming localized water-soaked, chlorotic, and necrotic zones in 3 wk.

Sensitivity and specificity of miniplate enrichment/ELISA system. With standard ELISA, the absorbances decreased rapidly, with end point readings at approximately 4×10^5 and 2.8×10^5 cfu/ml for strains D150 and D182, respectively (Fig. 2). Detection end points were approximately 10^4-fold lower for the miniplate enrichment/ELISA than for standard ELISA. The curves crossed at bacterial concentrations of 5×10^6 to 1×10^7 cfu/ml, indicating that bacterial growth in each miniculture well (and, consequently, the numbers of colony-forming units harvested from each well) had reached a maximum. All wells were observed microscopically, and absorbances from 25 wells are shown in Figure 3. Maximum ELISA values were close to 0.9 OD regardless of the number of colonies observed in individual wells (Fig. 3). Positive ELISA values were obtained from 39 of 48 wells at dilutions calculated to contain approximately 1 cfu/well. In wells in which only single colonies were observed, ELISA values were consistently above 0.2, which was well above the cutoff point for background readings (2 SD above the negative saline control). Thus, 1 cfu/ml was sufficient to attain a maximum reading, and even when greater cell numbers were applied to the wells, the ELISA values did not increase. In contrast, wells containing no visible colonies of *X. c. dieffenbachiae* all had negative ELISA values.

X. c. dieffenbachiae was detected at the lower limits of approximately 1 cfu/well when mixed 1:1 with competitive strains Eh-1 from *E. herbicola*, A811-1 from *P. fluorescens*, and QR-1 from *A. tumefaciens* (Table 1). Similar detection limits were observed when *X. c. dieffenbachiae* was mixed with *X. maltophilia* strains 776 and 861. However, enrichment growth of *X. c. dieffenbachiae* was inhibited when fewer than 10^4 cfu/well of strain D150 were mixed with approxi-

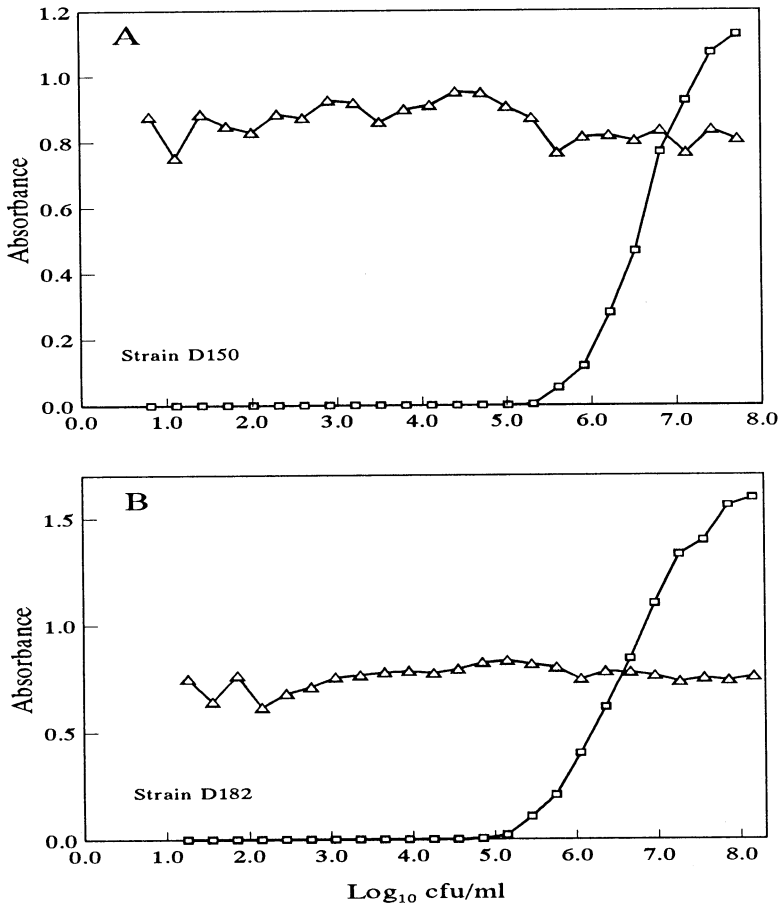

Fig. 2. Comparison of detection end points of standard ELISA (square symbol) and miniplate enrichment/ELISA (triangle) using a twofold dilution series of *Xanthomonas campestris* pv. *dieffenbachiae* **(A)** strain D150 and **(B)** strain D182. MAb Xcd 108 was used at 1:1,000 dilution and absorbances were measured at 450 nm. Background absorbances were subtracted. ELISA values for concentrations lower than 10 cfu/ml are shown in Figure 3.

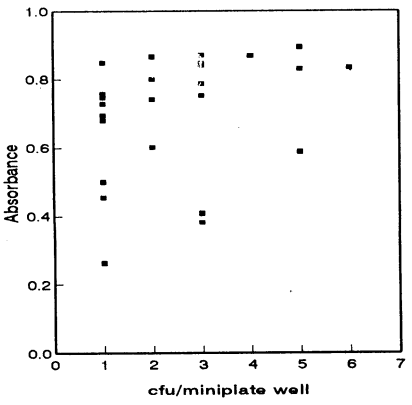

Fig. 3. Absorbances (A_{450}) obtained from ELISA following miniplate enrichment as a function of initial colony-forming units observed in each well at 40×.

mately equivalent numbers of *X. maltophilia* strain 868 (Table 1). Results were similar with the non-starch-hydrolyzing strain D182 except that no inhibition was observed with *X. maltophilia* strain 868.

Average ELISA values (A_{450}) were 0.63 with 55–90 cfu/well of *X. c. dieffenbachiae* and increasing numbers of *E. herbicola* strain Eh-1 or *P. fluorescens* strain A811-1. *A. tumefaciens* strain QR-1 readily hydrolyzed esculin but inhibited enrichment of xanthomonads only at very high numbers (5×10^5 cfu/well) in mixed culture with low numbers of strain D150 (90 cfu/well), or a ratio of 5,586:1. Similarly, low numbers of strain D182 (55 cfu/well) were inhibited only by high numbers of strain QR-1 (3.9×10^5 cfu/well), or a ratio of 7,091:1. ELISA values decreased with increasing numbers of *X. maltophilia* in the mixed population (Fig. 4). However, as the numbers of *X. maltophilia* decreased, inhibition decreased, and *X. c. dieffenbachiae* was detected even when *X. maltophilia* was 1×10^4 cfu/well in a mixed culture containing 50 cfu/well of strain D150 or, similarly, with a mixture of 1×10^5 cfu/well of *X. maltophilia* and 30 cfu/well of strain D182 (Fig. 4).

Table 1. Detection of *Xanthomonas campestris* pv. *dieffenbachiae* in the presence of competitive bacteria by the miniplate enrichment/ELISA system[a]

Strains of bacteria	Log_{10} (cfu/well)	OD[b]
D150:A811-1	5.6:7.7	0.533
	4.6:6.7	0.633
	3.6:5.7	0.656
	2.6:4.7	0.687
	1.6:3.7	0.745
	0.6:2.7	0.789
D150:Eh-1	5.6:5.6	0.850
	4.6:4.6	0.843
	3.6:3.6	0.880
	2.6:2.6	0.883
	1.6:1.6	0.936
	0.6:0.6	0.975
D150:QR-1	5.6:5.5	0.824
	4.6:4.5	1.004
	3.6:3.5	0.920
	2.6:2.5	0.889
	1.6:1.5	0.935
	0.66:0.5	0.919
D150:776	5.97:5.7	0.888
	4.97:4.7	0.889
	3.97:3.7	0.844
	2.97:2.7	0.711
	1.97:1.7	0.711
	0.97:0.7	0.397
D150:861	5.97:6.0	0.764
	4.97:5.0	0.732
	3.97:4.0	0.674
	2.97:3.0	0.664
	1.97:2.0	0.713
	0.97:1.0	0.591
D150:868	5.97:6.0	0.786
	4.97:5.0	0.745
	3.97:4.0	0
	2.97:3.0	0
	1.97:2.0	0
	0.97:1.0	0

[a] Equal volumes from a 10-fold dilution series of *X. c. dieffenbachiae* were mixed with an equivalent dilution of competitive bacteria. D150 = *X. c. dieffenbachiae* (starch hydrolyzing); A811-1 = *Pseudomonas fluorescens*; Eh-1 = *Erwinia herbicola*; QR-1 = *Agrobacterium tumefaciens*; 776, 861, and 868 = *X. maltophilia*.

[b] OD = absorbance (A_{450}), average of positive ELISA wells (minus background readings).

No significant differences in means ($P \leq 0.05$) were found between the two negative controls of the miniplate enrichment/ELISA system. Therefore, the saline row was used as a blanking row to eliminate background absorbance. Two standard deviations of control wells were always <0.1 absorbance.

Comparison of miniplate enrichment/ELISA system with standard culturing procedures. Results were similar with the miniplate enrichment/ELISA system and the standard petri plate culturing method (Table 2). The three samples that were positive by the miniplate enrichment/ELISA but negative by the standard culture indicate that the former procedure may be slightly more sensitive than the latter. Culture plates were often crowded with contaminants, making it difficult to distinguish colonies of *X. c. dieffenbachiae*.

Detection of xanthomonads on leaf surfaces. *X. c. dieffenbachiae* was detected in leaf washes from 23% (66/288) with the miniplate enrichment/ELISA system. Of these, 80% (53/66) were from leaves showing blight symptoms; the remaining were epiphytic populations on asymptomatic plants. Xanthomonads were recovered on modified TZC medium from all the miniplate wells showing esculin hydrolysis. Of the 33 strains cultured and serotyped, 29 reacted in ELISA to MAb Xcd 108 (serotypes 1–8), and all but one were pathogenic to anthurium. Twenty-two of the pathogenic strains were recovered from symptomatic plants and six from asymptomatic plants. The four strains that did not react with MAb Xcd 108 were nonpathogenic.

DISCUSSION

The miniplate enrichment/ELISA system permits detection of a single colony-forming unit per well in the initial sample, making this system approximately 10^4 times more sensitive than a

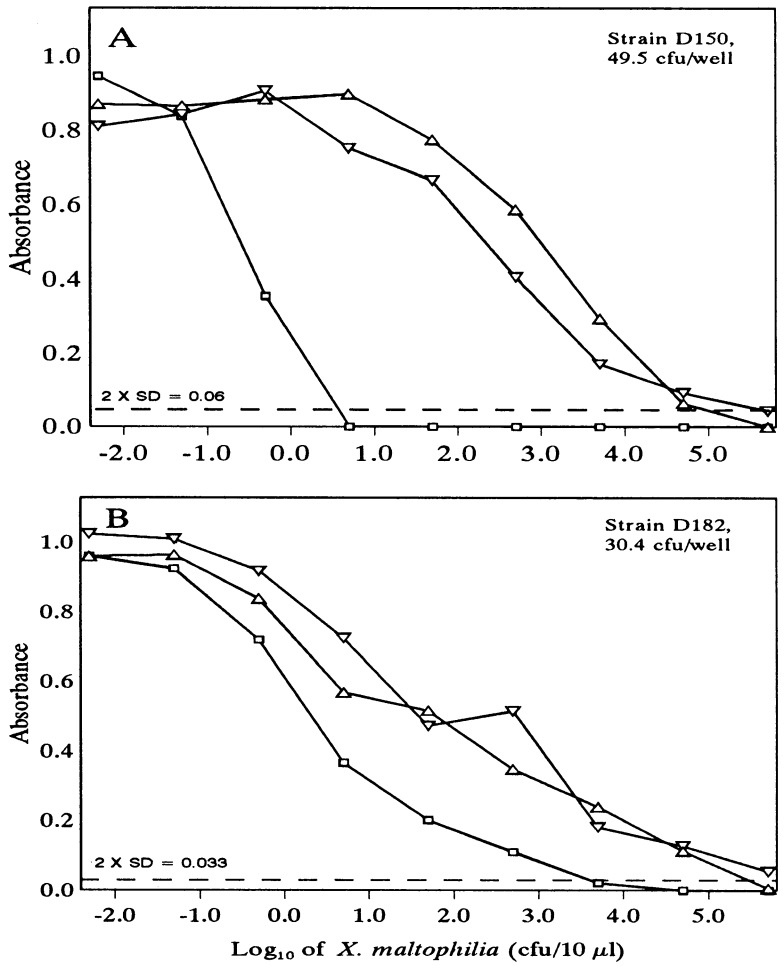

Fig. 4. Detection of *Xanthomonas campestris* pv. *dieffenbachiae* in the presence of *X. maltophilia*. Serially diluted suspensions of three *X. maltophilia* strains—776 (triangle), 861 (inverted triangle), and 868 (square)—were added to a constant number of *X. c. dieffenbachiae* **(A)** strain D150 and **(B)** strain D182. MAb Xcd 108 was used at 1:1,000 dilution and absorbances were measured at 450 nm. Dashed lines indicate 2 SDs of negative control.

Table 2. Detection of *Xanthomonas campestris* pv. *dieffenbachiae* by the miniplate enrichment/ELISA system compared with dilution streaking (standard culture)

Standard culture	Miniplate results[a]		
	Positive	Negative	Total
Positive	89	0	89
Negative	3	50	53
Total	92	50	142

[a]Compared with standard culture: false positive, 3/92 = 3.3%; false negative, 0/50 = 0; total error, 3/142 = 2.1%. Correspondence of miniplate results to standard culture procedure: (89 + 50)/142 × 100 = 97.9%.

standard ELISA. Sensitivity of ELISA is reported at $\geq 10^4$ cfu/ml (6,19). In practice, at least 10^6 cfu/ml are required for reliable assays from field samples. A positive attribute of the enrichment on ET medium is the general absence of interference from other bacteria, such as *E. herbicola* and *P. fluorescens*, which often are isolated from anthurium leaves on nonselective media. Interference occurred when the ratio of *Agrobacterium* to *X. c. dieffenbachiae* was greater than 5,586:1, but this should not pose a significant problem because *Agrobacterium* is not naturally present on anthurium leaves.

Some strains of *X. maltophilia* are likely to pose a problem during enrichment culture because they retard growth of *X. c. dieffenbachiae*. *X. maltophilia* was occasionally isolated from anthurium leaves, but this bacterium does not react with MAb Xcd 108 and thereby can be distinguished from *X. c. dieffenbachiae*. If only internal tissues of plants are being examined for bacteria, surface sterilization by wiping leaves with 70% ethanol effectively eliminates *X. maltophilia* and other surface contaminants (*unpublished*).

Pathogenicity of xanthomonads isolated from anthuriums was usually correlated with reactivity to MAb Xcd 108. All pathogenic strains of *X. c. dieffenbachiae* isolated from anthurium reacted with MAb Xcd 108 (serotypes 1–8), whereas most (95%) nonpathogenic strains did not. Strains in serotypes 9 and 10 are found on aroid hosts other than anthurium (12) but were not encountered in this study. Strains in serotypes 11 and 12 (negative for Xcd 108) are regularly found on anthurium but are not pathogenic to this host. Thus, although many types of xanthomonads and several other genera are amplified in the miniplate enrichment/ELISA system, the anthurium blight pathogen is distinguished from other bacteria by a positive reaction with MAb Xcd 108.

It is interesting that xanthomonads negative for MAb Xcd 108 survived on anthurium leaves without producing symptoms but caused leaf spots on *Dieffenbachia* following inoculation. Host specificity among the natural populations of *X. c. dieffenbachiae* strains in Hawaii was previously described, and current results confirm that monoclonal antibodies are able to distinguish at least some of the host-specific groups (12).

For detection of low populations of the *X. c. dieffenbachiae* on anthurium leaves, the miniplate enrichment/ELISA system is not appreciably more sensitive than standard culturing on semiselective media (17). In the miniplate system, the lowest detectable unit is one viable cell per 10-μl sample, and such limits of sensitivity are well within the ranges currently achieved by PCR for bacterial plant pathogens (5,20,21). To improve detection of bacteria in dilute suspensions, multiple samples can be taken. For example, 96 samples (10 μl each) could be assayed on a single microtiter plate, and if one well is positive, the detection level would have been increased 96-fold over the described assay with little additional material and effort.

The miniculture method reduces the cost of media, eliminates the need for visual identification of colonies, is a measure of viable bacteria, and permits rapid processing of large numbers of samples. For example, samples of leaf wash (10–1,000 μl) can be collected in the field (approximately 100 samples per hour), applied in 10- or 20-μl subsamples to miniculture wells, and incubated upon return to the laboratory. Since purification of bacteria is not needed, processing time is reduced. With the miniplate enrichment/ ELISA system, latent infections of anthurium blight also can be detected in production fields. The method is applicable to detection of other plant pathogens provided that selective media and pathogen-specific antibodies are used for identification.

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