A Rapid Method for Presumptive Identification of *Xanthomonas campestris* pv. *dieffenbachiae* and Other Xanthomonads

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ABSTRACT

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Two selective media were developed for the isolation and preliminary identification of *Xanthomonas campestris* pv. *dieffenbachiae*. The principal carbon sources were cellobiose and starch in one medium and esculin and trehalose in the other. The media were identical with respect to antibiotics and growth factors. In tests with 151 identified strains of bacteria and 46 bacterial epiphytes isolated from aroids, both media permitted the growth of *X. c.* pv. *dieffenbachiae* and other pathovars of *X. campestris* but inhibited growth of most other bacteria. The identity of the xanthomonads was rapidly confirmed by use of *Xanthomonas*-specific monoclonal antibodies in an enzyme-linked immunosorbent assay.

Bacterial blight of aroids, caused by *Xanthomonas campestris* pv. *dieffenbachiae* (McCulloch & Pirone) Dye, affects a broad range of ornamental and edible aroids, including *Anthurium, Aglaonema, Syngonium, Dieffenbachia, Epipremnum, Xanthosoma,* and taro (3). The disease was first described in 1939 on dieffenbachia (11). In Hawaii it was first observed in 1971 on anthurium on the island of Kauai (8) and eventually was found on many aroids grown on all of the Hawaiian islands (3,12). Progress has been made in controlling the disease by sanitation, and some control has been achieved through treatment with antibiotics (12). Nevertheless, bacterial blight continues to be a major problem in production of ornamental aroids in Hawaii and anthurium flowers in the French Antilles (Guadeloupe and Martinique) (13). It also has been reported in Venezuela (7), California (5), and Jamaica (F. Young, *unpublished*).

To improve current control measures, more information is needed about the ecology of the pathogen. Such information will be difficult to obtain without a reliable method for rapid, unequivocal detection of the pathogen in mixed populations of microbes. The symptoms of bacterial blight of aroids are easily confused with those of other diseases and with symptoms of injury or nutritional deficiency. Thus, to identify the disease in plants, the presence of the pathogen must be confirmed.

Strains of *X. c.* pv. *dieffenbachiae* are readily isolated from fresh lesions, but with older lesions recovery is often precluded by mixed populations of saprophytic bacteria that grow rapidly on many media. Furthermore, about 35% of 435 pathogenic strains of *X. c.* pv. *dieffenbachiae* tested failed to utilize starch (3) and would not grow on starch-based selective media developed for other xanthomonads (4,14,15).

Our method for developing an isolation medium for *X. c.* pv. *dieffenbachiae* consisted of reducing the complex carbon and nitrogen sources to prevent growth of many saprophytes, adding a short-chain carbon source that

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supported growth of the target organism, and inhibiting most nontarget organisms with selected antibiotics.

We report the development and evaluation of two selective media for the recovery and presumptive identification of *X. c.* pv. *dieffenbachiae*. To confirm the identity of the presumptive colonies of *Xanthomonas*, *Xanthomonas*-specific monoclonal antibodies (1) were used in an enzyme-linked immunosorbent assay (ELISA).

MATERIALS AND METHODS

Bacterial strains. Strains used to evaluate the selectivity of the isolation media are listed in Table 1. Strains of *X. c.* pv. *dieffenbachiae* were isolated from a wide range of aroid hosts collected from numerous nurseries in Hawaii. Strains of *Xanthomonas* and other genera representative of common plant- and soil-inhabiting bacteria and 46 epiphytic bacteria isolated directly from aroids were included in the tests.

Development of selective media. The cellobiose-starch (CS) medium was prepared by heating the following components in 800 ml of distilled water: cellobiose (5 g), $MgSO_4 \cdot 7H_2O$ (0.1 g), K_2HPO_4 (0.8 g), KH_2PO_4 (0.8 g), and agar (15 g). Potato starch (10 g) was added separately to 200 ml of distilled water and then brought to a boil. The thickened starch was added directly to the heated 800-ml cellobiose solution. The medium was stirred on a hot plate until the starch was dissolved, and 1.5 ml of a 1% aqueous solution of methyl green was added. The medium was autoclaved, and then filter-sterilized solutions of the following were added: cycloheximide (150 mg), cephalexin (50 mg), trimethoprim (30 mg), pyridoxine (1 mg), and D-methionine (3 mg). Triphenyltetrazolium chloride (TZC) was added at 0.001% to help distinguish xanthomonads from nonxanthomonads by the differential production of a red pigment in colonies of these bacteria. The final pH was 6.8.

The esculin-trehalose (ET) medium was prepared by adding the following ingredients to 1 L of distilled water: esculin (1 g), trehalose (0.5 g), $FeCl_3 \cdot 6H_2O$ (0.5 g), NaCl (5 g), $MgSO_4 \cdot 7H_2O$ (0.2 g), K_2HPO_4 (1 g), and agar (15 g). The pH was adjusted to 6.8, and the mixture was autoclaved immediately to avoid darkening of the medium upon standing. After autoclaving, filter-sterilized antibiotics and other components were added in the same amounts described for the CS medium. The final pH was 6.5.

Evaluation of media. Bacterial strains were dilution-streaked on TZC medium (9) that was modified by eliminating casein hydrolysate and reducing the final concentration of TZC to 0.001%. After 48 hr at 29 C, the plates were examined for uniformity of colony types, a check

Table 1. Bacterial strains used to evaluate selectivity of media

Species	Representative strains[a]	Source[b]
Agrobacterium rhizogenes	TR108	15
A. tumefaciens	UCBPP388	13
Bacillus subtilis	A890	1
Clavibacter michiganensis subsp. *insidiosum*	ATCC 10253	15
C. m. subsp. *michiganensis*	QR75, QR76	6
C. m. subsp. *sepedonicus*	ATCC 9850	15
Curtobacterium flaccumfaciens pv. *flaccumfaciens*	ATCC 6887	2
C. f. pv. *poinsettiae*	ATCC 9682	15
Enterobacter aerogenes	ATCC 13048	2
E. cloacae	ATCC 13047, WA-10, WT-2, YPV-5B, PP-1	2,10
Erwinia carotovora subsp. *atroseptica*	EA153	15
E. c. subsp. *carotovora*	EC153	15
E. chrysanthemi	EC176	15
E. herbicola	EH-1	1
Escherichia coli	2073	11
Proteus vulgaris	ATCC 13315	2
Pseudomonas aeruginosa	ATCC 27853	2
P. cichorii	M12.2, C330-1, C330-2, C397-1, C408-2L, C411-3a, C413-1, C413-2, C460-3a, C460-4a, C546-2, C546-3, C606-2	1
P. fluorescens	A1075-1, A811-1, A404, 2a, 7a, 9b, 18b, 18c, 19b, C335-1, C395-2b, C508-1, TB2-3, TB3-3	1
P. solanacearum	K60	14
P. syringae pv. *phaseolicola*	HB36	13
P. viridilivida	ATCC 190486	2
Serratia marcescens	A1078	8
Xanthomonas albilineans	LS2	3
X. campestris		
pv. *armoraciae* (syn. *X. raphani*)	XC123, PDCC 1404	15, 17
pv. *begoniae*	A915, X45, XB9	1,4,15
pv. *campestris*	A88, A249, A342, EEXC, OK2, PHW, XC131, XC149, RR68, X54	1,4,6, 7,15,16
pv. *dieffenbachiae*	B-1, B-39A, B-40, B58-2, B66-B-3, B-89, D1.21,D4.1,D14.1,D16.1,D17.2, D26.12, D27.22, D28, D30, D36.3, D37.22, D41, D46.15, D47, D54, D57.1, D57.2, D61.11, D68, D69.1, D70, D78.3, D84, D93, D161, D182, D183, D184, D194, D227, D238, D273, D291, D299, D300, D301, D302, D303, D312, D313, D315, D316, D317, D318, M10, X29, X31, X42, X159, X163, X174, 15b, 16a, 19a, 3bt, 3ct, 42, 45	1,4,10
pv. *hederae*	X24	4
pv. *incanae*	PDDC 574	17
pv. *manihotis*	XM105	15
pv. *oryzae*	XO3	9
pv. *pelargonii*	X38	4
pv. *phaseoli*	A584-1	1
pv. *urticae*	X19, X32	4
pv. *vesicatoria*	A77-1	1
pv. *vitians*	A674, A680-3b, A782-3T, A782-4T, A1057-2, XV164, ATCC 19320, 10TB7, 10TB10-1	1,5,8
Epiphytic bacteria isolated from aroids	8a, 11a, 11c, 12a, 13b, 13c, 14a, 14b, 15a, 21a, 22a, 26a, 28a, 40a, 40c, 1at, 1bt, 1ct, 3at, 41, 43, 44, 46, 47, 48, 49, 50, QR13, D24, D29-4b, D113, D200, D292, D314, Dd-18, X3, X117, C391-4a, C503-1a, S8-2, TB5-2, TB6-2, 4at, A592-5a, Sz, Rz	1

[a] The hosts and geographic origins of strains are recorded at the Department of Plant Pathology, University of Hawaii.

[b] 1 = Local isolation; 2 = American Type Culture Collection, Rockville, MD; 3 = R. Birch, Hawaii; 4 = A. R. Chase, Florida; 5 = J. J. Cho, Hawaii; 6 = E. Echandi, North Carolina; 7 = M. Goto, Japan; 8 = T. Hori, Hawaii; 9 = P. Y. Hsieh, Taiwan; 10 = W. T. Nishijima, Hawaii; 11 = S. S. Patil, Hawaii; 12 = K. Pohronezney, Florida; 13 = M. N. Schroth, California; 14 = L. Sequeira, Wisconsin; 15 = M. P. Starr, International Collection of Phytopathogenic Bacteria, Davis, CA; 16 = P. H. Williams, Wisconsin; 17 = J. M. Young, New Zealand.

of the purity of the strains. Colonies were removed from the TZC medium, suspended in sterile distilled water, and diluted with water to approximately 2×10^8 colony-forming units (cfu) per milliliter (10). These diluted cell suspensions (10 μl) were spotted onto representative plates of FS (15), CS, and ET.

The plates were incubated at 29 C and were observed for 7 days. Bacteria that grew on FS and CS were rated for starch hydrolysis and for type of colony (e.g., large, mucoid colony 7 mm or more in diameter, flat colony 5–7 mm in diameter, a very thin film of growth, or no growth). Growth on ET was described as a large, black zone of esculin hydrolysis extending at least 7–10 mm around the colony, a dark brown zone 5–7 mm in diameter surrounding the colony, a light brown zone less than 5 mm in diameter but still easily recognized as esculin hydrolysis, or no zone. To evaluate the survival of strains on the media after 2 wk at 29 C, samples of the colonies were removed from plates at that time and restreaked onto the modified TZC.

Reactivity with monoclonal antibodies. Two monoclonal antibodies, X1 and X11, that can be used to differentiate *Xanthomonas* from other genera of bacteria (1), were tested with all bacterial strains using ELISA. In preparation for the ELISA, strains were grown for 48 hr on yeast extract-glycerol agar (2). Cells were harvested into a 0.01 M phosphate-buffered (pH 6.8) saline (NaCl, 8.5 g/L) solution containing 0.5% formalin and stored at 4 C. Cells were later washed three times in saline and suspended in 0.05 M carbonate-bicarbonate buffer (pH 9.6). This cell suspension was diluted until the absorbance was 0.1 at A_{600}. This stock suspension was diluted 1:1 in the carbonate-bicarbonate buffer, and 100 μl was placed in microtiter plates (Dynatech Laboratories, Inc., Chantilly, VA). Microtiter plates were air-dried in a forced-air incubator at 37 C. Monoclonal antibodies X1 and X11 were diluted 1:2,000 for the ELISA procedures described by Yuen et al (15).

Efficiency of recovery. The CS, ET, FS, and modified TZC media were compared for recovery of 10 strains of xanthomonads and 10 other bacteria from dilute cell suspensions. The suspensions were prepared from 48-hr cultures of each strain and were diluted with saline to 0.1 optical density at 600 nm. A 10-fold dilution series was made, and 0.1 ml of the 10^{-5} dilution was spread onto the plates with a glass rod. Dilution plates were made in triplicate. Colonies were counted after a 4-day incubation, and percentage recovery was calculated with reference to growth on peptone-glucose agar (10 g of peptone, 5 g of glucose, and 15 g of agar).

The efficiency of CS and ET for recovery of populations of *X. c.* pv. *dieffenbachiae* and other bacteria directly from tissue samples was tested with plants inoculated with known concentrations of bacteria. Two strains of *X. c.* pv. *dieffenbachiae,* B-89 and D182, which were equally aggressive on dieffenbachia but differed in starch utilization (B-89 = +, D182 = −), were grown on yeast-glucose agar (10 g of yeast extract, 20 g of glucose, and 15 g of agar) for 48 hr. Colonies were suspended in saline and diluted as described above.

Subsamples of the dilutions from 10^{-1} to 10^{-9} were injected into leaves of *Dieffenbachia compacta* 'Exotica' with a 26-gauge hypodermic needle. Three adjacent leaves were infiltrated on each plant, starting from the flag leaf. Separate plants were used for each dilution. Each leaf contained two infiltrated areas, each about 5 cm^2 in area. Leaf disks (4 mm in diameter) were excised from the infiltrated zones. The volume of infiltrate injected per leaf disk was calculated from the difference in weight between 10 infiltrated and 10 noninfiltrated leaf disks from each of 10 leaves. Populations in the cell suspensions were calculated from three plate counts on TZC of 0.1 ml of the 10^{-5} dilution.

After infiltration, leaves were rinsed with water. A 4-mm cork borer was used to remove one disk from one of the two infiltrated zones on each of the three leaves. Disks were separately ground in 400 μl of sterile saline with a glass rod. Samples of 100 μl were removed from the suspension and spread onto separate plates of CS and ET, and 10 μl was spotted onto CS and ET plates to evaluate the spot plate method. Leaves were bagged in plastic for 24 hr to maintain high humidity, and plants were then placed on benches in the glasshouse for 10 days. Symptom development was recorded, and samples were removed from the three previously unsampled infiltrated zones per plant.

For controls, two separate dieffenbachia plants were injected with either sterile saline or a cell suspension (about 10^5 cfu/μl saline) of *Erwinia herbicola,* a common plant saprophyte. The controls were treated in the same manner as the xanthomonad-injected plants.

RESULTS

Growth on selective media. After 48 hr, 50% of the strains of *X. c.* pv. *dieffenbachiae* were unable to grow or produced thin, filmlike colonies on FS medium, whereas on CS medium 92% produced colonies at least 5–7 mm in diameter; four strains required 72 hr, and a fifth, D301, took 6 days to reach this size (Table 2). Strain D301 lacked the yellow xanthomonadin pigment and appeared white, resembling *X. c.* pv. *manihotis,* which also grew slowly on CS.

On ET medium all strains of *X. c.* pv. *dieffenbachiae* produced light brown to dark brown zones 5 mm or more in diameter within 48 hr (Table 2). All xanthomonads except *X. albilineans* and *X. c.* pv. *oryzae* grew and produced similar pigment. All strains except those that did not grow were recovered from CS and ET after 2 wk of incubation at 29 C.

Although most of the nonxanthomonads failed to grow on CS and ET, seven strains of *Pseudomonas fluorescens* grew slowly on CS medium and produced small colonies not exceeding 7 mm in diameter within 2 days. Two of the five strains of *Enterobacter cloacae,* ATCC 13047 and WA-10, did not produce visible colonies until day 4,

Table 2. Growth[a] of plant-pathogenic and epiphytic bacteria on three selective media and confirmation with two *Xanthomonas*-specific monoclonal antibodies

Species	No. of strains tested	Culture medium			ELISA[e]	
		FS[b]	CS[c]	ET[d]	X1	X11
Xanthomonas campestris						
pv. *dieffenbachiae*	64	±	+	+	+	+
pv. *armoraciae*, *begoniae*, *campestris*, *hederae*, *incanae*, *manihotis*, *phaseoli*, or *urticae*	21	+	+	+	+	+
pv. *pelargonii*, *vesicatoria*, or *vitians*	11	−	+	+	+	+
pv. *oryzae*	1	−	−	−	+	+
X. albilineans	1	−	−	−	+	+
Pseudomonas fluorescens	14	±	±	−	−	−
Enterobacter cloacae	2	−	+	−	−	−
Agrobacterium rhizogenes or *A. tumefaciens*	2	−	−	+	−	−
Leaf epiphytes	9	±	±	±	−	−
All other strains tested	72	−	−	−	−	−

[a] + Indicates all strains produced visible colonies within 48 hr; ± indicates 10–50% of the strains did not grow; − indicates no growth.
[b] Fieldhouse-Sasser medium (15).
[c] Cellobiose-starch medium.
[d] Esculin-trehalose medium.
[e] The enzyme-linked immunosorbent assay was performed using monoclonal antibodies, X1 and X11, as previously described (1).

but by day 7 these colonies were large and mucoid. The other three strains did not grow on either medium.

Five leaf epiphytes grew on ET medium but not at all on CS (Table 2). Four epiphytes grew on both media, although on CS they produced dry colonies that were easily distinguished from the mucoid types produced by xanthomonads. These four epiphytes resembled xanthomonads in that they were gram-negative rods, had oxidative metabolism, and failed to reduce nitrates. However, three produced cytochrome c oxidase, and all four were negative for proteolysis of litmus milk (unlike *X. campestris*). They also did not produce xanthomonadin pigments, they were nonpathogenic on *Syngonium* and dieffenbachia, and they did not react with *Xanthomonas*-specific monoclonal antibodies X1 and X11.

Recovery efficiency. The efficiency of CS and ET for recovery of *X. c.* pv. *dieffenbachiae* from the cell suspensions ranged from 28 to 130% (Table 3). Four of the nine epiphytes that grew on CS or ET in the spot plate test did not produce colonies on either medium in this test. Five strains developed small, dry colonies on CS. In contrast, all strains of *X. c.* pv. *dieffenbachiae* formed colonies on both media within 4 days.

For plant inoculations the average volume of inoculum was calculated to be 0.86 μl per disk. At the first dilution of inoculum, the number of colony-forming units infiltrated per disk was calculated for strain B-89 as follows:

$$(1.94 \times 10^4 \text{ cfu}/\mu\text{l})\ (0.86\ \mu\text{l/disk}) = 1.67 \times 10^4 \text{ cfu/disk}.$$

Likewise, for strain D182, 9.46×10^3 cfu were injected per leaf disk at the first dilution of inoculum (Table 4).

In all isolations of *X. c.* pv. *dieffenbachiae* from plant tissue, the dilution and spot plate methods appeared comparable (Table 4). If colonies were recovered by the dilution plate method, they also were recovered by the spot plate method except in two cases. Moreover, colony counts were very close to those calculated for the inoculum injected. After 10 days of incubation, bacterial numbers in inoculated tissues exceeded 1,000 cfu/ml at all levels of inoculum (*data not shown*). The populations recovered on the dilution plates again were consistent with those observed on the spot plates.

Strain B-89 could not be recovered at the 2-hr sampling from leaves inoculated with 2 cfu of the pathogen (Table 4), but high cell numbers were recovered from two of the three leaf disks after 10 days. The CS medium was not as efficient as the ET medium for recovery of the non-starch-hydrolyzing strain, D182. Although no bacteria were recovered from tissue inoculated with an estimated 1 cfu per disk and sampled 2 hr after inoculation, lesions developed, and large populations were recovered from all three samples 10 days after inoculation. Bacterial populations of both strains rose to very high levels in all cases where the estimated inoculum was greater than 1 or 2 cfu per disk. No bacteria resembling *Xanthomonas* were recovered from saline controls or from controls inoculated with *E. herbicola,* although the latter survived epiphytically and was

Table 3. Percentage recovery of *Xanthomonas campestris* and other bacteria on four selective media relative to recovery on peptone-glucose agar[a]

Strain	TZC[b] (%)	FS[c] (%)	CS[d] (%)	ET[e] (%)
Xanthomonas campestris				
pv. *dieffenbachiae*				
D1.21	86	0	80	95
D182	97	0	82	96
D184	97	0	28	124
B-89	120	66	57	125
X29	109	105	76	130
D273	74	93	61	110
D318	161	68	62	108
pv. *pelargonii*				
X38	101	0	95	118
pv. *vesicatoria*				
A77-1	94	0	66	95
pv. *vitians*				
XV164	112	0	49	66
Pseudomonas fluorescens				
A1075-1	160	127	100	0
Leaf epiphytes				
8a	93	0	0	0
48	102	0	0	0
13b	122	0	0	0
14b	50	0	0	0
3at	120	0	58[f]	97
46	101	80	89[f]	0
13c	92	72	74[f]	121
41	93	77	86[f]	96
43	109	114	112[f]	108

[a] Each value is the average of three replicates.
[b] Triphenyltetrazolium chloride medium.
[c] Fieldhouse-Sasser medium (15).
[d] Cellobiose-starch medium.
[e] Esculin-trehalose medium.
[f] Colonies were dry, in contrast to mucoid colonies of *X. c.* pv. *dieffenbachiae.*

Table 4. Recovery by dilution plate or spot plate (SP) analysis of *Xanthomonas campestris* pv. *dieffenbachiae* strain B-89 or D182 (starch hydrolysis + and −, respectively) from leaves of dieffenbachia 2 hr after inoculation

Strain	Inoculum (dilution no.)[a]	Cellobiose-starch cfu[b]	Cellobiose-starch SP[c]	Esculin-trehalose cfu[b]	Esculin-trehalose SP[c]	No. of bacteria injected per leaf disk[d]
B-89	10^{-1}	TMC[e]	+	TMC	+	1.7×10^4
	10^{-2}	385	+	523	+	1.7×10^3
	10^{-3}	25	+	56	+	1.7×10^2
	10^{-4}	5	+	16	+	1.7×10^1
	10^{-5}	−	−	−	−	2
	10^{-6}	−	−	−	−	0
D182	10^{-1}	153	+	TMC	+	9.5×10^3
	10^{-2}	11[f]	+[g]	500	+	9.5×10^2
	10^{-3}	−	−	40	+	9.5×10^1
	10^{-4}	1[f]	−	11	+	9
	10^{-5}	−	−	−	−	1
	10^{-6}	−	−	−	−	0

[a] A 10-fold dilution series of the bacterial suspension was made, and samples of each suspension were injected into dieffenbachia leaves.
[b] Colony-forming units on dilution plates. Actual colony counts were multiplied by 4 because only 100 μl of the 400-μl sample was plated. All numbers are averages of three replicates.
[c] + Indicates growth from 10 μl spotted onto plates; − indicates no growth.
[d] The number of cells in the inoculum was determined by viable plate counts, as described in Materials and Methods.
[e] TMC = too many to count.
[f] Recovery from two of three replicates.
[g] Recovery from only one of the three replicates.

recovered on TZC from all samples 10 days after inoculation. Infiltration zones on the dieffenbachia leaves were water-soaked and discolored after 10 days in the glasshouse at all levels of inoculum, including the 10^{-5} dilution, for both strains of *X. c.* pv. *dieffenbachiae*. Symptoms were not observed on any of the control leaves.

DISCUSSION

The addition of cellobiose to a starch-based medium (FS) provides a carbon source for non-starch-hydrolyzing strains of *X. c.* pv. *dieffenbachiae* that do not grow on media used for many other *X. campestris* pathovars (4,14,15). This modification makes CS medium useful for preliminary detection of *X. c.* pv. *dieffenbachiae*. Other pathovars that cannot hydrolyze starch (*pelargonii, vesicatoria,* and *vitians*) also grow on CS medium.

Removing nitrate and yeast extract improves the selectivity of the medium. Nitrogen is supplied in the form of D-methionine in an amount that permits growth of xanthomonads but limits growth of many other bacteria. This reduces the number of contaminants growing on both CS and ET media.

The ET medium differs from most esculin-based media. Broths used for testing esculin hydrolysis usually contain yeast extract and ferric ammonium citrate or ferric citrate. In ET medium the yeast extract was removed, and the ferric ammonium citrate or ferric citrate was replaced by ferric chloride to further reduce carbon sources for growth of nontarget organisms. Thus, certain epiphytes and plant pathogens, such as *P. cichorii,* which normally hydrolyze esculin in the presence of yeast extract or citrates, no longer grew. Growth of *X. c.* pv. *dieffenbachiae* in the absence of yeast extract was enhanced by adding trehalose (0.5 g/L). Similar results were achieved by substituting cellobiose (0.5 g/L) for trehalose, but esculin hydrolysis was delayed.

The growth of nontarget bacteria on semiselective media may pose problems in identification of the target bacteria unless the latter are easily distinguished by colony morphology. *P. fluorescens* and some unidentified leaf epiphytes grew on CS medium but produced colony types that were distinctly different from those of *Xanthomonas.* Colonies of *Enterobacter cloacae* and some other leaf epiphytes could be mistaken for xanthomonads on CS, but they failed to appear on ET. Thus, simultaneous use of the two media enables rapid isolation and presumptive identification of *X. c.* pv. *dieffenbachiae* from plant tissues. A small sample (10 µl) of plant extract can be easily spotted onto the two media and observed for growth within 2–3 days. (*Enterobacter cloacae* produced very little or no growth until the fourth day.) In addition, the appearance of dark zones around colonies on ET helps confirm the presumptive identification of xanthomonads.

Four of the 46 epiphytes tested produced visible colonies within 3 days when about 10^4 cfu were spotted onto CS or ET but not until 4 days when 3 cfu were spotted in 10 µl. These colonies did not resemble those of *Xanthomonas* sp. on CS medium. Moreover, these bacteria did not resemble xanthomonads in physiological tests and did not react with *Xanthomonas*-specific monoclonal antibodies X1 or X11.

The CS and ET media allowed recovery of non-starch-hydrolyzing strains of *X. c.* pv. *dieffenbachiae,* although three such strains grew more slowly, with colonies not clearly visible until day 4, in contrast to 48 hr for the starch utilizers. Strains of pathovars *pelargonii, vesicatoria,* and *vitians* also grew slowly. Although some of these strains took as long as 6 days to form visible colonies, all eventually did.

For rapid assay of leaf samples, small disks of tissue may be ground in 400 µl of saline and 10-µl samples of the resulting suspension may be spotted onto CS and ET media. In the tests reported here, the dilution plate and spot plate methods appeared equally effective for recovery of low populations from inoculated plant tissues. Two hours after inoculation, populations recovered were very similar to those applied. In all but two cases where the pathogen was detected by the dilution plating method, it was also detected by the spot plate method.

Monoclonal antibodies X1 and X11 may be used to enhance the presumptive identification. These antibodies react with any xanthomonad and cannot be used to distinguish *X. c.* pv. *dieffenbachiae* from other xanthomonads. Saprophytic xanthomonads (6) probably would grow on CS and ET and thus could not be distinguished from pathogenic strains. Whether saprophytic strains of *Xanthomonas* are prevalent as epiphytes on anthurium leaves remains to be tested. Of 329 xanthomonads recovered from aroids to date, 13 caused no symptoms upon inoculation, and nine of these could be reisolated from the inoculation sites (D. Norman and A. Alvarez, *unpublished*). Recently, new monoclonal antibodies have been generated that react with and differentiate among strains of *X. c.* pv. *dieffenbachiae* (3). These can be substituted for X1 and X11 in tests requiring such specificity.

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LITERATURE CITED

1. Alvarez, A. M., Benedict, A. A., and Mizumoto, C. Y. 1985. Identification of xanthomonads and grouping of strains of *Xanthomonas campestris* pv. *campestris* with monoclonal antibodies. Phytopathology 75:722-728.
2. Alvarez, A. M., Buddenhagen, I. W., Buddenhagen, E. S., and Domen, H. Y. 1978. Bacterial blight of onion, a new disease caused by *Xanthomonas* sp. Phytopathology 68:1132-1136.
3. Bonner, R. L., Alvarez, A. M., Berestecky, J. M., and Benedict, A. A. 1987. Monoclonal antibodies used to characterize *Xanthomonas campestris* pv. *dieffenbachiae.* (Abstr.) Phytopathology 77:1725.
4. Chun, W. W. C., and Alvarez, A. M. 1983. A starch-methionine medium for isolation of *Xanthomonas campestris* pv. *campestris* from plant debris in soil. Plant Dis. 67:632-635.
5. Cooksey, D. A. 1985. Xanthomonas blight of *Anthurium andraeanum* in California. Plant Dis. 69:727.
6. Gitaitis, R. D., Sasser, M. J., Beaver, R. W., McInnes, T. B., and Stall, R. E. 1987. Pectolytic xanthomonads in mixed infections with *Pseudomonas syringae* pv. *syringae, P. syringae* pv. *tomato,* and *Xanthomonas campestris* pv. *vesicatoria* in tomato and pepper transplants. Phytopathology 77:611-615.
7. Guevara, Y. M., and Debrot, E. C. 1985. Bacterial blight of anthurium in Venezuela. Page 25 in: Abstracts of the 6th International Conference on Plant Pathogenic Bacteria, Beltsville, USA, June 2-7. University of Maryland, Beltsville.
8. Hayward, A. C. 1972. A bacterial disease of anthurium in Hawaii. Plant Dis. Rep. 56:904-908.
9. Kelman, A. 1954. The relationship of pathogenicity in *Pseudomonas solanacearum* to colony appearance on a tetrazolium medium. Phytopathology 44:693-695.
10. Kolmer, J. A., Spaulding, E. H., and Robison, H. W. 1959. Approved Laboratory Technic. Appleton-Century-Crofts, New York, pp. 582-583.
11. McCulloch, L., and Pirone, P. P. 1939. Bacterial leaf spot of *Dieffenbachia.* Phytopathology 29:956-962.
12. Nishijima, W. T., and Fujiyama, D. K. 1985. Bacterial blight of anthurium. Hawaii Inst. Trop. Agric. Hum. Resour., Commodity Fact Sheet AN-4(A). 3 pp.
13. Rott, P., and Prior, P. 1987. Un dépérissement bactérien de l'anthurium provoqué par *Xanthomonas campestris* pv. *dieffenbachiae* aux Antilles françaises. Agron. Trop. (Paris) 42:61-68.
14. Schaad, N. W., and White, W. C. 1974. Survival of *Xanthomonas campestris* in soil. Phytopathology 64:1518-1520.
15. Yuen, G. Y., Alvarez, A. M., Benedict, A. A., and Trotter, K. J. 1987. Use of monoclonal antibodies to monitor the dissemination of *Xanthomonas campestris* pv. *campestris.* Phytopathology 77:366-370.