Detection of *Xanthomonas campestris* pv. *citrumelo* and *X. citri* from Citrus Using Membrane Entrapment Immunofluorescence

R. H. BRLANSKY and R. F. LEE, Professors, Citrus Research and Education Center, University of Florida, IFAS, 700 Experiment Station Road, Lake Alfred, FL 33850, and E. L. CIVEROLO, USDA, ARS, Beltsville, MD 20705

ABSTRACT

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Xanthomonas campestris pv. *citrumelo* (formerly *X. campestris* pv. *citri* strain E), the causal agent of citrus bacterial spot, and *X. citri* (formerly *X. campestris* pv. *citri* strain A), the causal agent of citrus bacterial canker, were easily detected in leaf extracts from symptomatic and asymptomatic citrus leaves and from cultures by immunofluorescence microscopy of bacteria that were trapped on 0.2-μm black polycarbonate membranes. Leaf disks, 5 mm in diameter, were ground in phosphate buffered saline, and the extracts were successively passed through 5.0-μm polycarbonate and 0.2-μm black polycarbonate membranes. The 0.2-μm membranes were washed and subsequently incubated in tetramethyl-rhodamine isothiocyanate (TRITC) labeled immunoglobulin (IgG) prepared against either *X. campestris* pv. *citrumelo* or *X. citri*, or in TRITC-labeled normal serum IgG. The membranes were rinsed, mounted on microscope slides, and viewed with an epifluorescence microscope using a 580–590 nm wavelength filter to detect the presence of fluorescing bacteria.

Citrus bacterial spot (CBS), caused by *Xanthomonas campestris* pv. *citrumelo* (Gabriel) (syn. *X. campestris* pv. *citri* group E) (11), was first reported in 1984 in citrus nurseries in Florida. Citrus bacterial canker disease (CBC), caused by *X. citri* (ex. Hasse) (syn. *X. campestris* pv. *citri*), has occurred since 1986 in citrus groves on Florida's west coast, where the disease was supposedly eradicated in the early 1900s (25). Various forms of CBC occur in South America, Africa, Asia, and the islands of the South Indian Ocean. Recently, forms of CBC have been reported in Mexico (7,23) and North Yemen (10).

In Florida, there has been an effort to identify CBS and CBC and eradicate the diseases by burning plants (25). At the onset of the present eradication effort, the method used for the detection of the diseases was isolation of the bacterium in (pure) culture, the inoculation of susceptible citrus plant material, and, if subsequent development of characteristic canker lesions occurred, the reisolation of the bacterium.

Immunofluorescence has been successfully used for the identification and detection of many bacterial plant pathogens (1,2,5,6,9,16,24). The causal agent of black rot of crucifers, *X. c.* pv. *campestris*, was identified and detected using both direct and indirect immunofluorescence tests on smears from culture plates (24). Aubert et al (1) described an immunofluorescent method in which *X. c.* pv. *citri* was detected and identified in citrus on Reunion Island. The technique was successfully used for detecting bacteria from both symptomatic and asymptomatic plant tissues.

Membrane entrapment techniques, combined with epifluorescence microscopy, have been used to detect bacteria in water (17), foods (4,20,21,22), and intravenous fluids (8). Davis and Dean (5) reported the use of immunofluorescence and membrane entrapment for the diagnosis of the bacterium that causes ratoon stunting disease of sugarcane.

The purpose of this research was to develop and evaluate a rapid and specific membrane entrapment immunofluorescence method for identification and detection of the bacteria causing CBS and CBC in symptomatic and asymptomatic citrus leaf tissues before culturing and pathogenicity tests (18). Such a method would aid regulatory personnel in processing large numbers of samples.

MATERIALS AND METHODS

Bacteria. Bacterial strains used throughout this study are listed in Table 1. These included 25 CBS strains from Florida citrus nurseries, tentatively identified as *X. c.* pv. *citrumelo* (11), 18 strains of *X. citri* strain A, which causes CBC, 10 strains of *X. c.* pv. *aurantifolii* (Gabriel), which causes cancrosis B, C, and D, 16 strains of nonpathogenic *X. campestris* from citrus, 28 strains of other phytopathogenic *X. campestris* pathovars, and 13 strains of other phytopathogenic bacteria. The *X. citri* and *X. c.* pv. *citrumelo* strains from the Florida nurseries were maintained on Wakimoto's medium (3); however, before inoculation of plant material and for in vitro testing, the bacteria were grown at 27 C on nutrient agar supplemented with 0.2% glucose (NGA) (3). All other *Xanthomonas* strains were maintained on NGA or nutrient agar.

Before inoculation of plant material, all *X. c.* pv. *citrumelo* and *X. citri* strains were grown in nutrient broth (Difco) supplemented with 0.2% glucose and 0.5% NaCl (3). The bacterial cells were centrifuged at 10,000 g for 15 min and the cell-containing pellet was resuspended in sterile distilled water and adjusted to an absorbance of 0.1 at 620 nm with a 1.0-cm light path (approximately 10^8 cfu/ml).

Plant material and inoculations. Young foliage of Mexican lime (*Citrus aurantifolia* Swingle) and grapefruit (*C.*

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paradisi Macf.) plants was inoculated with suspensions of either XC62 cancrosis strain A or 084-3048-1 (CBS type strain) as described before (3) by spraying with an atomizer at 25 psi to runoff. The plants were immediately placed in plastic bags for 24–36 hr to maintain high humidity.

Lesions generally appeared 7–10 days after inoculation. Asymptomatic leaf tissues were sampled from leaves with lesions and from leaves without lesions on plants with symptoms.

Symptomatic and asymptomatic leaf disks were collected using a 5-mm (internal diameter) cork borer and were immediately fixed in 3% glutaraldehyde in 0.06 M phosphate buffer, pH 6.8, for 12–18 hr. Afterward, the discs were washed 2–3 times in phosphate-buffered saline (PBS) containing 0.05% NaN_3 and stored at 4 C.

Antisera. Antisera made against strain XC62 (Asiatic canker), strain XC69 (cancrosis B), and strain XC70 (cancrosis C) were those described previously (3). Antisera made against four Florida CBS strains (F1, F2, F3, F4) were prepared in two rabbits each. Immunogen preparations were suspensions of whole bacterial cells in PBS containing 0.2% (V/V) formaldehyde and adjusted to about A_{620} = 1.0 (1 cm light path). Preimmunization blood was collected from each rabbit before the first injection of immunogen. The immunization schedule was as follows: on days one and two, 0.5 and 1.0 ml of cell suspension in PBS was injected intravenously (IV). On day seven, 1.0 ml of cell suspension mixed with 0.5 ml of Freund's incomplete adjuvant was injected intramuscularly. On days 10, 14, and 21, 2.0 ml of cell suspension mixed with adjuvant was injected intramuscularly. Test bleeds were made 1 wk after the last injection. Final production bleeds were made 2 wk after the last injection. The dilution endpoint of each antiserum in individual enzyme-linked immunosorbent assays (ELISA) tests was 1/25,600 against homologous CBS strains. Reactions in indirect ELISA of the heterologous immunogen was the same for all antisera using antisera dilutions of 1/500–1/2,000. Because of these similarities between the antisera, a composite pool of the eight antisera was made by mixing equal volumes of each antiserum produced against Florida CBS strains for use in the immunofluorescence tests described below (hereafter termed FLN antiserum).

Antiserum to *X. campestris* pv. *pruni* was that described by Brlansky et al (2). Antiserum to *X. campestris* pv. *campestris* (strain B-24) was a gift from C. Chang, University of Georgia, Experiment, Georgia. The immunoglobulin G (IgG) of all antisera was isolated using the protein A column procedure (19). Antisera were conjugated to tetramethyl-

Table 1. Reactions of bacterial strains with antisera prepared to *Xanthomonas citri* (XC62) and *Xanthomonas campestris* pv. *Citrumelo* (FLN) in membrane entrapment direct immunofluorescence

Bacteria tested	Strain	Source[b]	Reaction[a] to antisera for: XC62	FLN
X. campestris pv. *citrumelo* (syn. *X. campestris* pv. *citri* group E). Collected from Florida nursery sites and identified as causing citrus bacterial spot	F1/084-3048-1	(2)	–	++
	F2		–	++
	F3/085-3081-1	(2)	–	++
	F4		–	++
	F5/084-3166	(2)	–	++
	F6/X85-0329-1	(2)	–	++
	F14/X85-614	(2)	–	++
	F20/X85-833-1	(2)	–	–
	F49/X85-4600-1	(2)	–	++
	F31/X85-4754-1	(2)	–	++
	F35			
	F54/X85-5436-1	(2)	–	++
	X85-6260	(2)	–	–
	F56/X85-6572-1	(2)	–	–
	F79/X85-7364-1	(2)	–	+
	F80/X85-7364-2	(2)	–	+
	F86/X85/8600-1	(2)	–	–
	X85-11520	(2)	–	–
	F103/X85-12869-1	(2)	–	–
	F119/X85-12869-2	(2)	–	–
	X85-12889	(2)	–	++
	F219/X86-4912	(2)	–	–
	F220/X86-7329-1	(2)	–	–
	F221/X86-7334-1	(2)	–	–
	F222/X86-7774-1	(2)	–	–
	F223/X86-10279-2	(2)	–	+
X. citri (syn. *X. campestris* pv. *citri* group A) causing Asiatic cancrosis on citrus	XC59	(1)	++	–
	XC62	(1)	++	–
	XC63	(1)	++	–
	XC111	(1)	++	–
	XC112	(1)	++	–
	XC113	(1)	++	–
	XC114	(1)	++	–
	XC118	(6)	++	–
	XC124	(1)	++	–
	XC126	(1)	++	–
	F-132	(2)	++	–
	F-134	(2)	++	–
	PH-5	(1)	++	–
	PH-15	(1)	++	–
	PH-28	(1)	++	–
	MI-32	(1)	++	–
	MI-60	(1)	++	–
	MI-62	(1)	++	–
X. campestris pv. *aurantifolii* (syn. *X. campestris* pv. *citri* groups B, C, and D)	XC64 (B strain)	(1)	–	–
	XC69 (B strain)	(1)	–	–
	XC70 (C strain)	(1)	–	–
	XC90 (Mexico)	(1)	–	–
	XC148 (B strain)	(1)	–	–
	T-20 (D-Mexico)	(1)	–	–
	T-21 (D-Mexico)	(1)	–	–
	T-22 (D-Mexico)	(1)	–	–
	T-23 (D-Mexico)	(1)	–	–
	T-24 (D-Mexico)	(1)	–	–
Nonpathogenic *X. campestris* isolates from citrus	B-1	(4)	–	–
	C-1	(4)	–	–
	C-11	(4)	–	–
	C-15	(4)	–	–
	C-315	(4)	–	–

(*continued on next page*)

[a] ++ = Positive reaction, strong fluorescence, cells highly visible.
+ = Weak reaction, low fluorescence, cell shapes visible.
– = No visible fluorescence.

[b] 1 = E. L. Civerolo, USDA, ARS, Beltsville, MD; 2 = J. Miller, Division of Plant Industry, Gainesville, FL; 3 = R. E. Stall, University of Florida, Gainesville, FL; 4 = J. H. Graham, University of Florida, CREC, Lake Alfred, FL; 5 = R. Sonoda, University of Florida, AREC, Ft. Pierce, FL; 6 = Plant Diseases Division Culture Collection of Plant Pathogenic Bacteria, Auckland, New Zealand; 7 = D. Gabriel, University of Florida, Gainesville, FL; 8 = L. W. Timmer, University of Florida, CREC, Lake Alfred, FL; 9 = D. Stuteville, Kansas State University, Manhattan, KS.

Table 1. (*continued from preceding page*)

Bacteria tested	Strain	Source[b]	Reaction[a] to antisera for: XC62	FLN
	GN1-4	(4)	−	−
	GN2-4	(4)	−	+
	HST-S1	(4)	−	−
	IVR4-5	(4)	−	−
	S4-1	(4)	−	−
	S5-1	(4)	−	−
	VN4-3	(4)	−	−
	LA-1	(4)	−	−
	AA1	(3)	−	−
	INA-42	(3)	−	−
	INA-69	(3)	−	−
Other phytopathogenic *X. campestris* isolates;				
X. campestris pv. *alfalfae*	KX-1	(9)	−	−
	ONA	(9)	−	++
	82-1	(3)	−	−
	L676	(7)	−	+
X. c. pv. *begoniae*	083-6167	(2)	−	−
	P87-5102-1	(2)	−	−
	P87-5102-2	(2)	−	−
X. c. pv. *bilvae*	B8600		−	−
X. c. pv. *campestris*	B-24		−	−
	085-763	(2)	−	−
	P85-5902	(2)	−	−
X. c. pv. *dieffenbachiae*	P88-2113	(2)	−	+
	P3074-2	(2)	−	−
X. c. pv. *hederae*	P2863-2	(2)	−	−
	P2944-2	(2)	−	−
X. c. pv. *malvacearum*	P87-4860	(2)	−	−
X. c. pv. *maculifoliigardeniae*	P87-2197-1	(2)	−	−
	P87-2197-2	(2)	−	−
X. c. pv. *pelargonii*	P85-436	(2)	−	−
	P88-2479	(2)	−	−
	P88-3095	(2)	−	−
	P88-2512	(2)	−	−
	P87-2137	(2)	−	−
X. c. pv. *phaseoli*		(7)	−	−
X. c. pv. *poinsettiicola*	P85-2020	(2)	−	−
X. c. pv. *pruni*	P88-2007	(2)	−	−
X. c. pv. *manihotis*	XP-1	(1)	−	−
	CBB-10	(1)	−	−
	CIAT-1105	(1)	−	−
X. c. pv. *vesicatoria*	084-1275	(2)	−	+
	P87-1884	(2)	−	−
Other genera of plant pathogenic bacteria				
Agrobacterium tumefaciens	Chry 9	(3)	−	−
	LBA4013	(3)	−	−
	LBA1050	(3)	−	−
Clavibacter michiganense subsp. *michiganense*	FLA	(3)	−	−
Clavibacter michiganense subsp. *nebraskense*	PF4	(3)	−	−
Erwinia herbicola	EH1	(1)	−	−
Pseudomonas aeruginosa	2284	(3)	−	−
Pseudomonas cichorii	Z1	(3)	−	−
Pseudomonas solanacearum	K60	(3)	−	−
Pseudomonas syringae pv. *tomato*	PT2	(3)	−	−
Pseudomonas syringae pv. *syringae*	P55	(3)	−	−
Pseudomonas viridiflava	PV1	(3)	−	−
Xyella fastidiosa	PWB-1	(8)	−	−

rhodamine isothiocyanate (TRITC) as previously described (2).

For indirect immunofluorescence tests, fluorescein-conjugated goat antirabbit IgG, rhodamine-conjugated goat antirabbit IgG, Texas red conjugated goat antirabbit IgG, and rhodamine conjugated protein A were purchased from Organon Teknika, Cappel Div., Malvern, PA.

Sample preparation and membrane entrapment. Serial dilutions containing 10^8–10^1 cells per ml were made of glutaraldehyde-fixed bacteria from cultures of the bacterial strains described above and were used to test detection sensitivities of the various methods and specificity of the fluorochrome conjugates. Bacterial cell counts were performed with a Batch Counting Chamber (Hausser Scientific, Blue Bell, PA 19422) to verify cell concentrations. Cross-reactivity tests were performed on the same isolates using bacteria from culture diluted to 10^6 cfu/ml and labeled antibodies at 1:20. For both sensitivity and specificity tests, the membrane entrapment immunofluorescence (MEI) procedure was used as described below.

Samples from symptomatic (XC62 and F1 lesions) and asymptomatic leaves were prepared by chopping two 5-mm leaf disks with a razor blade in a few drops of sterile distilled water and placing the resulting extract in a test tube. The extract volume was adjusted to 2 ml with sterile distilled water. The extract was centrifuged in a clinical centrifuge (International Centrifuge Co., Needham, MA, Model CL) for 5 min at ×1000 g. The same procedure was used for plants infected with periwinkle wilt, except that 50–100 mg of stem and petiole tissues were chopped instead of leaf tissue disks.

The resulting supernatant was transferred to a 10-ml syringe, then pushed through a Nucleopore 25-mm Swin-Lok filter assembly with a multiple holder adapter (Nucleopore Corporation, Pleasanton, CA 94566) that contained a 5.0-μm polycarbonate membrane for trapping cellular debris in one holder and a 0.2-μm black polycarbonate membrane for trapping bacteria in the second holder. The 0.2-μm membrane was removed, placed sample side upward in a 35- × 10-mm plastic petri dish, and cut in half with a scalpel. On one half of the membrane, 3–4 drops of a 1:20 dilution of TRITC-labeled IgG specific for either *X. c.* pv. *citrumelo, X. citri* (XC62), XC69, or XC70 were spread on the surface. On the other half, TRITC-labeled normal serum IgG or TRITC-labeled IgG specific for *X. c.* pv. *pruni* was spread. All samples of IgG were diluted in a Tris-BSA-gelatin antibody buffer (20 mM Tris, 0.9% NaCl, pH 8.2 containing 0.1% bovine serum albumin, 1.0% gelatin, and 2.0 mM NaN_3). Incubations in the IgG were for 30–60 min at room temperature and were followed by rinsing in Tris-BSA-gelatin buffer for 5 min.

Indirect antibody labeling was done using a primary unlabeled IgG followed by a fluorescent-labeled goat antirabbit IgG (as described above). Incubation times in primary IgG (diluted 1:20 in Tris-BSA-gelatin antibody buffer) were 30–60 min, followed by washing in Tris-BSA-gelatin antibody buffer for 5 min, incubation in the secondary labeling IgG (diluted 1:20) for 30–60 min, and washing

in Tris-BSA-gelatin antibody buffer for 5 min. The membranes were then mounted on glass microscope slides in Aqua Mount (Lerner Laboratories, New Haven, CT 06513) and viewed with an epifluorescence microscope using a 50-watt mercury lamp. They were viewed in the green excitation range (exciter BP 546, beam splitter FT 580, and barrier LP 590) for rhodamine and TRITC and in the blue excitation range (exciter BP 490, beam splitter FT 510, and barrier LP 520) for FITC. Reactions were rated as follows: ++ = strong fluorescence, cells highly visible; + = weak fluorescence, cell shapes visible; - = no visible fluorescence. All samples were prepared and tested three times.

Field tests. The MEI method was further tested on field samples to determine its effectiveness in detecting bacteria from presumptively infected samples. Samples of leaf spots from five different citrus nurseries were tested as described above for tissue samples. All 5-mm leaf disk samples were fixed in 3% glutaraldehyde as described above so that samples could be removed from quarantined areas. Samples from the west coast of Florida having raised pustules similar to that for CBC (cancrosis A) were obtained in June, 1986, from three home/garden locations and one commercial citrus orchard. All samples were tested using TRITC-labeled FLN antiserum and TRITC-labeled antisera to strains XC62, XC69, and XC70. Controls were done with healthy, greenhouse-grown *C. aurantifolia* leaves, using TRITC-labeled IgG to *X. campestris* pv. *pruni* (2).

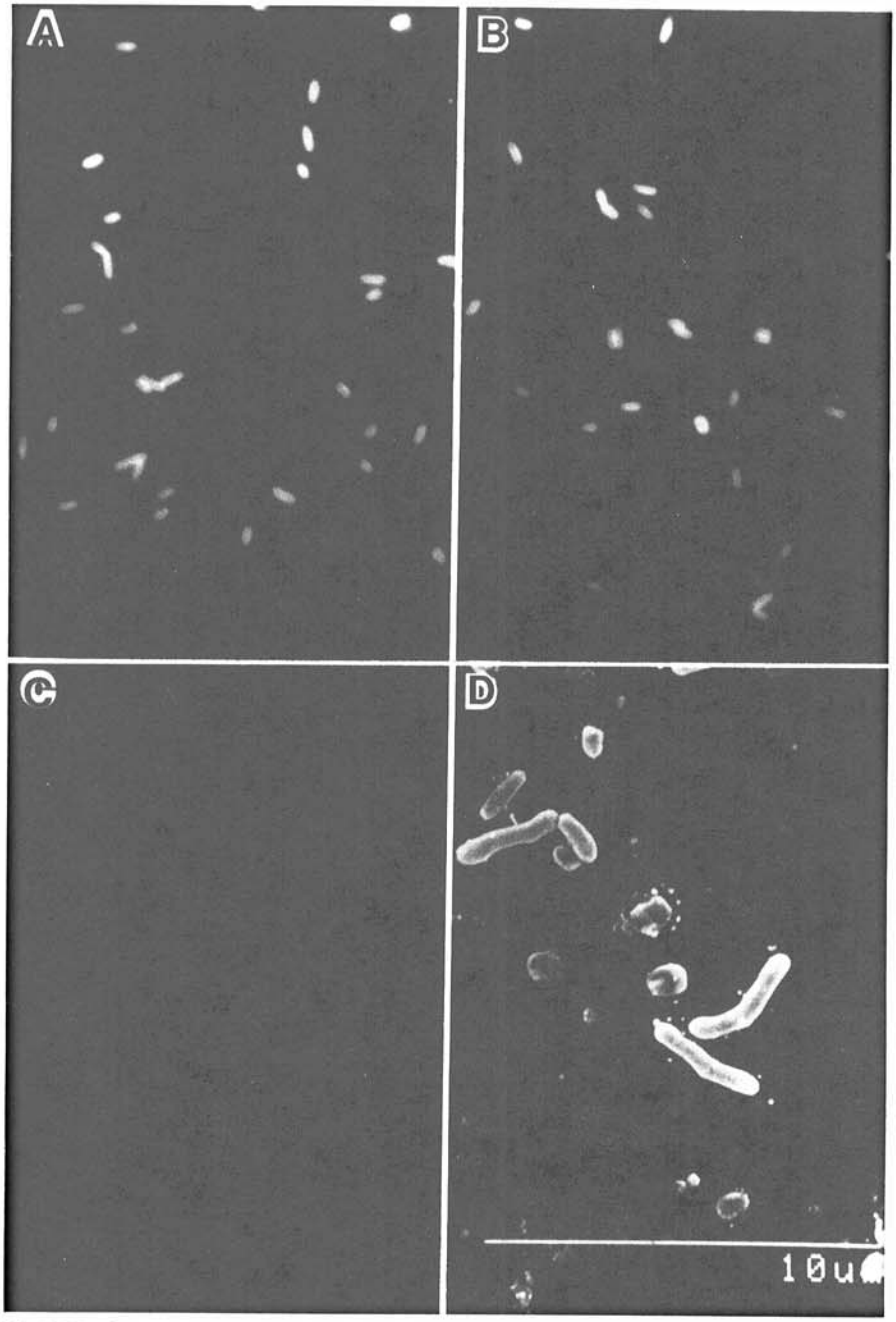

Fig. 1. *Xanthomonas citri* and *X. campestris* pv. *citrumelo* trapped on 0.2-μm black polycarbonate membranes and detected with fluorescing tetramethyl-rhodamine isothiocyanate (TRITC)-labeled antisera specific for the bacterium. **(A)** *X. citri* strain XC62 extracted from two lesions and trapped on the surface of the membrane and detected with TRITC-labeled anti-XC62 IgG = positive reaction. ×1500. **(B)** *X. c.* pv. *citrumelo* strain F1 extracted from two lesions and trapped on the membrane and detected with TRITC-labeled anti-Florida citrus nursery strain (FLN) IgG = positive reaction. ×1500. **(C)** *X. c.* pv. *citrumelo* strain F1 trapped on a polycarbonate membrane and incubated with TRITC-labeled normal serum IgG = negative reaction. ×1500. **(D)** Scanning electron micrograph of the entrapped *X. c.* pv. *citrumelo* strain F1 on the 0.2-μm polycarbonate membrane.

RESULTS

Homologous reactions of bacteria from pure cultures of strains F1, F2, F3, and F4 with FLN antiserum and XC62 with XC62 antiserum were positive and rated ++ (Table 1). Gold-orange fluorescing bacteria were readily seen on the membranes in preparations containing as few as 10^2–10^3 cfu/ml. Heterologous reactions of F1, F2, F3, and F4 with XC62 antiserum and of strain XC62 with FLN antiserum were negative (-). Reactions of strains F1, F2, F3, F4, and XC62 were negative when treated with labeled IgG, raised against *X. c.* pv. *pruni, X. c.* pv. *campestris, X. c.* pv. *aurantifolii* XC69 and XC70, or labeled normal serum IgG. All other bacterial cultures identified as causing CBS reacted negatively with XC62 antisera (Table 1). Eleven of the 25 CBS strains gave strong positive homologous type reactions with the FLN antiserum, whereas three gave only weak reactions (+) and 11 reacted negatively. All Asiatic CBC strains reacted positively with XC62 antiserum, and none reacted with FLN antiserum. None of the *X. c.* pv. *aurantifolii* strains reacted with antiserum to XC62 or FLN (Table 1). Strains XC69 and XC70 did react positively with their homologous antiserum (data not shown in Table 1). When 16 nonpathogenic *X. campestris* isolates were tested, only isolate GN2-4 gave a weak reaction with FLN antiserum. All other *X. campestris* isolates tested did not react with either XC62 or FLN antiserum. Of the 28 phytopathogenic *X. campestris* pathovars tested, weak reactions with FLN antiserum were found with strain P88-2113 of *X. c.* pv. *dieffenbachiae*, with strain 084-1275 of *X. c.* pv. *vesicatoria*, and with *X. c.* pv. *alfalfae* strain L676. *X. c.* pv. *alfalfae* strain ONA showed a positive reaction with FLN antiserum. None of the other pathovars reacted with either XC62 or FLN antiserum. Thirteen other plant pathogenic species were tested and none reacted positively with either antiserum (Table 1).

Both the CBC strain XC62 and the Florida CBS strain F1 were easily de-

tected with their homologous antiserum when two leaf lesions were chopped, diluted, and passed through the membranes (Fig. 1). Bacterial presence on membranes was verified by scanning electron microscopy (Fig. 1). Both bacterial strains were detected by fluorescence only when their homologous IgG was used. Detection of bacteria using a single lesion also was successful.

Bacteria from asymptomatic leaf disk samples from leaves with lesions were also detected. Numbers of bacteria cells were usually lower than those found from lesions.

Indirect labeling methods using either goat antirabbit fluorescent-labeled IgG or fluorescent-labeled protein A also were successful. The brightness of the fluorescence was often variable. Fluorescence levels were acceptable for TRITC- and Texas red-labeled IgG's, however, lower levels of fluorescence occurred with FITC-labeled goat antirabbit IgG and rhodamine-labeled protein A. IgG dilutions of 1:10 did not improve the levels of fluorescence. Fading of the indirect fluorescent labels was also a problem but was reduced by using mounting media containing n-propyl gallate (12).

The MEI technique was successfully used on field samples to detect the presence of bacteria causing CBS and CBC (Table 2). Bacteria were detected in symptomatic field samples of CBS from one of five citrus nurseries when TRITC-labeled IgG to *X. c.* pv. *citrumelo* was used but not when XC62 (strain A) IgG was used. No positive reactions were found with any of the control IgG. Samples from dooryards and from one commercial citrus orchard on the west coast of Florida exhibiting typical CBC strain A symptoms were immunofluorescent positive only with XC62 (strain A) labeled IgG. Bacteria were detected from leaf, fruit, and stem samples as well as from nonlesion areas on leaves with symptoms. The results from these 122 samples were similar to results with cultures of bacteria obtained from these locations (Table 1).

Table 2. Reactions of bacteria from field samples with antisera to bacteria causing citrus bacterial leaf spot and citrus bacterial canker disease, strain A

Location[b]	Number of samples per type[c]	Antisera reaction[a]	
		FLN (CBS)	XC62 (CBCD strain A)
CN85-8	(3/L)	++	–
CN85-8	(12/L)	++	–
CN85-9	(10/L)	–	–
CN85-10	(5/L)	–	–
CN85-10-2	(14/L)	–	–
CN85-10-2	(22/L)	–	–
DY86-6	(2/L)	–	++
DY86-6	(12/L)	–	++
DY86-6	(7/F)	–	++
DY86-6	(7/S)	–	++
DY86-6	(2/NL)	–	–
CO 86-6	(10/L)	–	++
CO86-6	(2/NL)	–	++
CO86-6	(3/S)	–	++
DY86-10-1	(2/L)	–	++
CN86-11-1	(2/L)	–	–
RL-86	(25/L)	–	–

[a] ++ = Positive reaction; – = negative reaction.
[b] CN = citrus nursery; DY = door yard; CO = commercial orchard; RL = rough lemon samples showing leaf spot.
[c] L = leaf sample w/lesion; NL = nonlesion-leaf sample; F = fruit sample w/lesion; S = stem sample w/lesion.

DISCUSSION

In this study, the MEI technique was successfully used to rapidly detect and identify the bacteria that cause both CBS and CBC. The technique was simple, easy to use, and allowed for the identification of bacteria from both culture and infected plant tissues. Bacteria were detected at levels as low as 10^2-10^3 cfu/ml. The specificity of the two antisera against CBS and CBC bacteria was shown. A number of the bacterial strains identified as causing CBS only gave weak or negative reactions using the FLN antisera. This also was found in testing field samples from nurseries presumed infected with CBS, where samples from only one of five nurseries reacted positively with FLN antisera. The bacteria which cause CBS symptoms are obviously a very heterogenous group. Some of these CBS strains have been identified in pathogenicity tests as different from the original CBS strains (F1, F2, F3, F4) (11,15). These strains have been described as weakly aggressive and nonaggressive (15). Gabriel et al (11) also showed that some CBS strains differed in host specificity and had lower pathogenic capacities than strains of CBC. In restriction fragment length polymorphism analyses (11), these CBS strains comprised a heterogeneous group that interrelated with *X. campestris* pathovars *alfalfae, cyamopsidis*, and *dieffenbachiae*. Of other bacteria tested in our study, only four strains cross-reacted with the FLN antiserum. Two of these *X. campestris* pathovars, *dieffenbachiae* and *vesicatoria*, produced only weak reactions, whereas one strain of *X. c.* pv. *alfalfae* produced a positive reaction. It is interesting that this last strain was isolated from Florida.

Gabriel et al (11) also found that previously described *X. c.* pv. *citri* strains (A, B, C, and D) formed two distinct homogeneous groups, the A group and the B, C, D group. In this study, we also found that A strains of CBC were easily distinguished from B, C, and D strains using XC62 antiserum.

The successful use of the MEI technique for the identification of CBS and CBC bacteria from symptomatic and asymptomatic citrus tissues provides an additional quick method that, when combined with culturing and pathogenicity tests, gives a reliable way to identify CBS and CBC. Recently, the MEI technique has been used in conjunction with DNA-DNA hybridization probe analysis on leaf washings to determine the spread of CBS strains in simulated nursery and grove situations (13) and in a central Florida nursery epidemic (14). The MEI proved to be a sensitive, specific, and convenient method for the field-sample processing of CBS epidemics (13).

Additional specific antisera are being developed for use with MEI since many weakly aggressive or nonaggressive CBS strains are weakly or nonreactive with FLN antisera. It appears that the MEI technique should also be useful for detection of many plant pathogenic bacteria where specific antisera are available. The technique is currently being used for the detection of a leaf spotting *Xanthomonas* of rice in Texas and *Xylella fastidiosa* from various plant species (L. Barnes, *personal communication*).

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